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Acta Medica Academica

Journal of Department of Medical Sciences
of Academy of Sciences and Arts of Bosnia and Herzegovina

ISSN 1840-1848 (Print) Volume 48 Number 2 August 2019 ISSN 1840-2879 (Online)

Online First www.ama.ba

Acta Medica Academica

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2019 Vol. 48, No. 2

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SUBSCRIPTION

Acta Medica Academica is published triannually. The annual subscription fee is € 50 outside of Bosnia and Herzegovina.

PUBLISHER CONTACT INFORMATION

Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina. Contact person: Husref Tahirović, E-mail: husref.tahirovic@untz.ba

COVER PHOTO PICTURE

Bogusławą Kecková (1854-1911; official female doctor in Bosnia and Herzegovina). The photo is from the collection of the National Museum, Prague, Czech Republic. Inv. No. 170. 1074.

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PRINT

Dobra knjiga, Sarajevo, BA. Printed on acid-free paper.

CIRCULATION

500 copies.

THE JOURNAL IS INDEXED IN

Medline/PubMed; EBSCOhost; Index Copernicus; CAB Abstract/Global Health Databases; IndexScholar.com; DOAJ; CrossRef; InfoBase Index.

Print and electronic issues of AMA are covered in Scopus and Embase through Medline.

Acta Medica Academica (ISSN 1840-1848) is an international peer-reviewed triannual journal which is printed as a continuation of the journal Works of the Academy of Sciences and Arts of Bosnia and Herzegovina, Department of Medical Sciences, founded in 1953. *Acta Medica Academica* Online (ISSN 1840-2879) offers free access to all articles at www.ama.ba.

Original Article
Acta Medica Academica 2019;48(2):129-139
DOI: 10.5644/ama2006-124.251

Quantitative Analysis of *CCL5* and *ep300* in Periapical Inflammatory Lesions

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Received: 4 January 2019
Accepted: 7 August 2019

Key Words: Chemokine CCL5 ▪ E1A-Associated P300 Protein ▪ Periapical Granuloma ▪ Periapical Periodontitis ▪ Radicular Cyst.

Objectives. *In silico* bioinformatical analysis suggested that the expression of two genes, *CCL5* (C-C Motif Chemokine Receptor 5) and *ep300* (Histone acetyltransferase p300), could be used as potential new biomarkers in differentiation between periapical granulomas and radicular cysts. Thus, we hypothesized that gene expression of *CCL5* and *ep300* in periapical lesions would classify the lesions as either granuloma or cyst. **Materials.** Patient samples (n=122) included 46 periapical granulomas, 38 radicular cysts and 38 healthy gingival samples as controls. Real-time PCR analysis of *CCL5* and *ep300* transcripts was compared to *SDHA* (Succinate dehydrogenase complex, subunit A) as the reference. Clinical parameters (e.g., intensity of inflammation and lesion size) were measured and correlated with *CCL5* and *ep300* expression. ROC (Receiver operating characteristic) and logistic regression analyses were used to establish the diagnostic character of ΔCt values. **Results.** Granulomas and radicular cysts had significantly higher expression of *CCL5* and *ep300* compared to controls (P<0.05). However, no differences were observed when comparing granulomas and radicular cysts. ROC analyses showed that *CCL5* and *ep300* have good diagnostic accuracy, but low accuracy for distinguishing between the lesions. **Conclusions.** This study confirmed that expression of *CCL5* and *ep300* is relevant for the pathogenesis of periapical inflammatory lesions but cannot be used as a distinctive marker between these lesions.

Introduction

Periapical inflammatory lesions are among the most common lesions of inflammatory origin in human jaws (1). They are characterized by destruction of the periapical tissue, resulting in surrounding bone resorption and eventual formation of cysts or granuloma (2). The development of these lesions is triggered by bacterial infection of the root canal (3). The host immune system fights the infection by recruiting different mechanisms, such as T- and B-cell-mediated anti-bacterial responses, which activate a network of regulatory cytokines that are produced by Th1- and Th2-type T-lymphocytes (1).

Many newer studies are concerned with the analysis of inflammatory mediators and biomarkers and their influence on the development of various periapical lesions (4-

13). Pires *et al.* in their study analyzed the expression of a whole range of cytokines in active and inactive periapical lesions (2). Also, Cavalla *et al*. were engaged in understanding the expression of protein profiles in apical periodontitis for identifying potential diagnostic molecular markers in one of their recent studies (5). In everyday clinical practice, nonsurgical therapy is the first choice for treatment of periapical cysts or granulomas. Numerous studies have attempted to find new biomarkers to differentiate these entities by analysing gene or protein expression (14). Besides laboratory investigations, a bioinformatic study was recently conducted to identify genes concerned with differentiation of periapical granulomas and cysts (15). This study recommended two genes, *CCL5* and *ep300*, as the leader genes in differentiation between periapical granulomas and cysts, respectively. However, laboratory confirmation of the role of these genes in inflammatory periapical lesions has not been conducted.

CCL5 is a cytokine that belongs to the chemokine superfamily (16). It is a natural ligand for the CCR5 receptor (C-C Motif Chemokine Receptor 5) and functions as a chemoattractant for T-cells, monocytes (17) and eosinophils (18), as well as an activator of basophils to release histamine (19). *ep300* is a histone acetyltransferase that regulates transcription via chromatin remodelling and is involved in processes of cell proliferation and differentiation (20). This gene has been implicated in bone metabolism through its interactions with RANKL (Receptor activator of nuclear factor kappa-B ligand) protein (21). Also, it has been suggested that *ep300* is involved in regulation of tooth initiation (22).

The present study hypothesized that *CCL5* and *ep300* are molecular markers for differentiation between periapical granulomas and radicular cysts. We also hypothesized that these potentially new biomarkers are correlated with the inflammation intensity and lesion size.

Materials and Methods

Sample

Patient samples consisted of periapical inflammatory lesions, including periapical granulomas (n=50), radicular cysts (n=59), and healthy gingiva samples as controls (n=40). Patients with periapical granulomas or radicular cysts underwent the oral surgery procedure of periapical lesion extirpation, followed by either apicoectomy or routine extraction of the non-vital tooth/remaining root. All the above-mentioned oral surgery procedures were conducted by N.H. After periapical lesion extirpation, the lesion was divided into two parts. One part was immersed in 10% buffered formalin and then embedded in paraffin FFPE (Formalin-Fixed Paraffin-Embedded) for histopathological diagnosis, and the other part was flash frozen in liquid nitrogen and stored in a freezer at -80°C until the moment of RNA isolation and cDNA synthesis.

On the basis of histopathological analysis carried out by the same pathologist [S.R], all samples in the experimental group were classified either as radicular cysts (n=59) or periapical granulomas (n=50). Of the 59 radicular cysts, 29 samples were extirpated during apicoectomy with cystectomy, while 30 radicular cyst samples were collected during the surgical intervention for extraction of the non-vital tooth/residual root with cystectomy. Out of 50 periapical granulomas, 26 samples were collected during apicoectomy and 24 samples were collected by curettage after extraction of the offending tooth or residual root. Out of 59 samples of cysts, in 11 samples the tooth had previously been treated endodontically, while in 48 samples the tooth had not been previously endodontically treated.

Out of 50 samples of periapical granulomas, in 13 samples the tooth had previously been treated endodontically (root canal(s) obturated with permanent filling material), while in the remaining 37 samples the tooth had not been previously treated endodontically (those were the situations where consulting endodontist insisted for endodontic surgery as first therapy option). For patients with periapical lesions a non-endodontically treated tooth, was obturated with permanent root canal filling material immediately before apicoectomy. Control group samples consisted of healthy gingiva, which was removed as excess during third molar extractions (23-25). These samples were characterized as healthy tissues on histopathological examination and as such were included in the control group (n=40).

Inflammation Intensity

FFPE tissue sections were sliced at 3-5 μm, stained with hematoxylin and eosin, and screened by a pathologist who was blinded to the clinical data. Intensity of inflammation was semi-quantitatively determined as follows: 1 is a mild inflammation that is found in 1/3 of the field of view; 2 is an intermediate inflammation where the inflammatory infiltrate is found in 2/3 of the field of view; and 3 is a severe inflammation that is found in more than 2/3 of the field of view.

Lesion Area Measurements

The size of the periapical lesions was based on radio-graphical findings and measured in mm^2 (largest diameter (mm) x largest root to border distance). Lesions with a diameter larger than 10x10 mm were radiographically classified as cysts, while smaller lesions were classified as granulomas. All samples were confirmed histologically.

RNA Isolation and Reverse Transcription

RNA isolation and cDNA synthesis were conducted according to the manufacturer's protocol using a RNeasy Mini Kit (Qiagen, Germantown, MD, USA) and High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA, USA), respectively. The quantity of isolated RNA was measured for each sample with a spectrophotometer by measuring absorbance at 260 nm in ng/μl (BioSpec nano-UV-VIS Spectrophotometer, Shimadzu Scientific Instruments). Besides measuring the absorbance at 260 nm, the absorbance at 280 nm and 230 nm was measured to obtain the applicable ratios A260/280 nm and A260/230 nm, which is important for identification of purity of the isolated RNA. DNase treatment was not used.

Real-time PCR

All qPCR reactions were carried out on Stratagene Mx3005p qPCR (Agilent Technologies, Santa Clara, USA) using SYBR Green chemistry (Life Technologies, Carlsbad, USA). PCR conditions were: 95°C for 20 seconds and then 40 cycles of 95°C (3 seconds) and 60°C (30 seconds), followed by the standard denaturation curve. The sequences of human primers for *ep300* and *CCL5* were obtained from Zimmermann, Acosta, Kohlhase & Bartsch (2007) and Lu et al. (2014), respectively (26, 27).

Primers for *CCL5* were: Forward, CATATTCCTCGGACACCACAC, Reverse, CTTTCGGGTGACAAAGACGAC. Primers for *ep300* were: Forward, CCAGGAGGCAGAGGTTGTAG, Reverse, AGCATCCCACAGGCCTCTAT. Fast SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, California, USA), 200 nmol/l specific primers and 1 μl of cDNA were used in each reaction. Expression data were normalized to the geometric mean of the housekeeping gene *SDHA* (14) .

Ethics Statement

The study protocol was approved by the Ethical Committee of the Faculty of Dental Medicine of the University of Sarajevo (No: 01-4-216-9/2017). All procedures performed in studys involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Statistical Analysis

Delta Ct (ΔCt) values were calculated as the difference between the Ct value of the sample and Ct of the standard. For comparison of *CCL5* and *ep300* ΔCt values, a Pair Wise Fixed Reallocation Randomization Test was performed using REST software (Relative Expression Software Tool) (28). Spearman's coefficient correlation and logistic regression analysis were used to correlate gene expression on periapical granuloma or radicular cyst development. ROC (Receive Operating Characteristic) and AUC (Area Under the Curve) were estimated to evaluate the criteria validity of ΔCt values and to find qPCR analysis diagnostic reliability (sensitivity and specificity), respectively. MedCalc software v. 17.2 (MedCalc Software, Mariakerke, Belgium) was used for these analyses. A significance level of 5% was used to determine statistical differences.

Results

Macroscopic and histopathological examples of analysed samples (radicular cyst and periapical granuloma) are shown in Figure 1. Gene expression analysis of *CCL5* and *ep300* genes was analysed in 50 granulomas, 59 cysts, and 40 control samples of healthy gingiva. After RNA isolation and qPCR reaction, 14 qPCR reactions were unsuccessful and were excluded from the analysis. In addition, 13 samples were excluded from the analysis because they originated from the same patients. Thus, 27 samples were excluded from the final analysis. The remaining 122 samples were statistically analysed (84 periapical inflammatory lesions and 38 controls).

The comparisons were made between granuloma vs. control, cyst vs. control, [granuloma and cyst] vs. control, and cyst vs. granuloma (Figure 2 and Figure 3). A statistically significant increase was observed in the following comparisons: *ep300* expression in [granuloma and cyst] vs. control (ratio=2.475; P=0.001) and *CCL5* expression in [granuloma and cyst] vs. control (ratio=2.113; P=0.002). Comparing granulomas vs. controls, both markers showed a statistically significant increase in expression- *ep300* (expression ratio=2.885; P=0.000) and *CCL5* (expression ratio=2.397; P=0.005). Similar expression profiles were observed when comparing cysts vs. controls, and an increase in expression of *CCL5* (expression ratio=1.904; P=0.008) and *ep300* (expression ratio=2.180; P=0.003) was observed. No significant differences were observed when comparing the expression of *CCL5* and *ep300* in radicular cysts vs. periapical granulomas.

Regarding any potential correlation between *CCL5* and *ep300* expression with the intensity of inflammation, neither of the two markers showed any statistically significant correlation (*CCL5* rho=0.141, P=0.2881; *ep300* rho=-0.00665, P=0.9601). We assumed that the smaller lesions would have higher *CCL5* expression because this gene was originally bioinformatically identified in granulomas. Correlation of *CCL5* expression and lesion surface area in mm^2 was conducted and a statistically significant negative correlation was found between *CCL5*

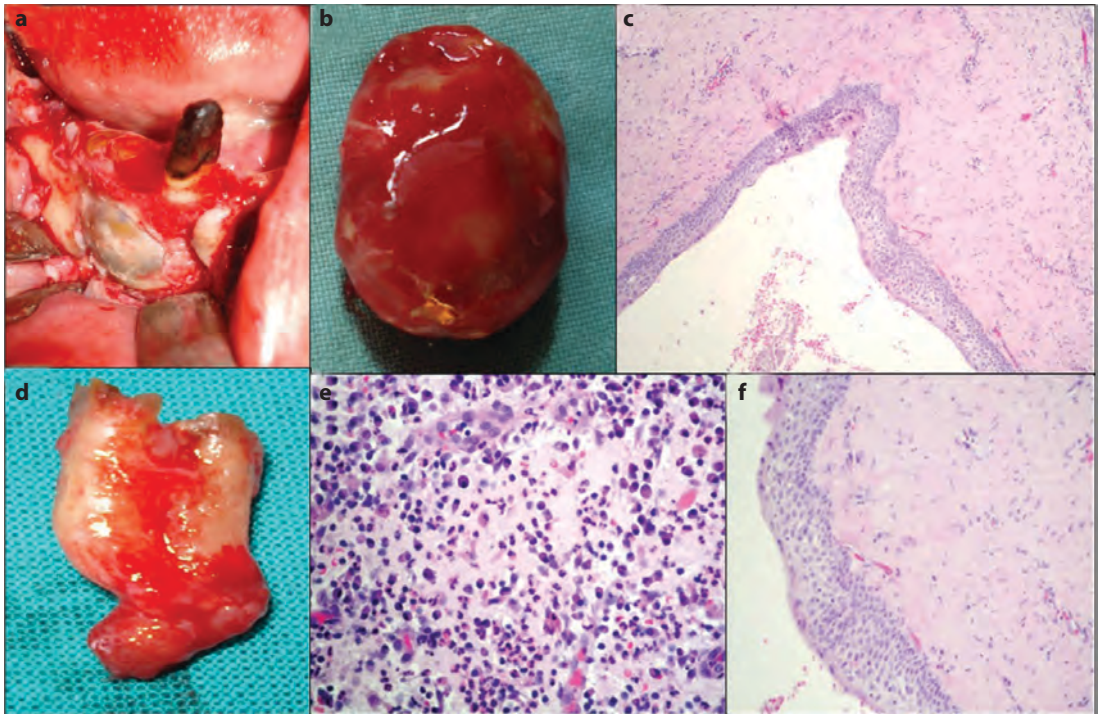

Figure 1. Intraoperative finding of a radicular cyst after raising the mucoperiosteal flap and removal of the thin bone lamella (a). Macro view of an enucleated cyst (b). Radicular cyst microscopic features: connective tissue wall infiltrated by chronic inflammatory cells, epithelial lining-stratified squamous non-keratinized epithelium (HE, X200) (c). Clinical appearance of granuloma on the lower left first molar (d). Chronic non-specific granulation tissue of periapical granuloma (HE, X400) (e). Healthy gingival tissue without signs of inflammation (HE, X250) (f).

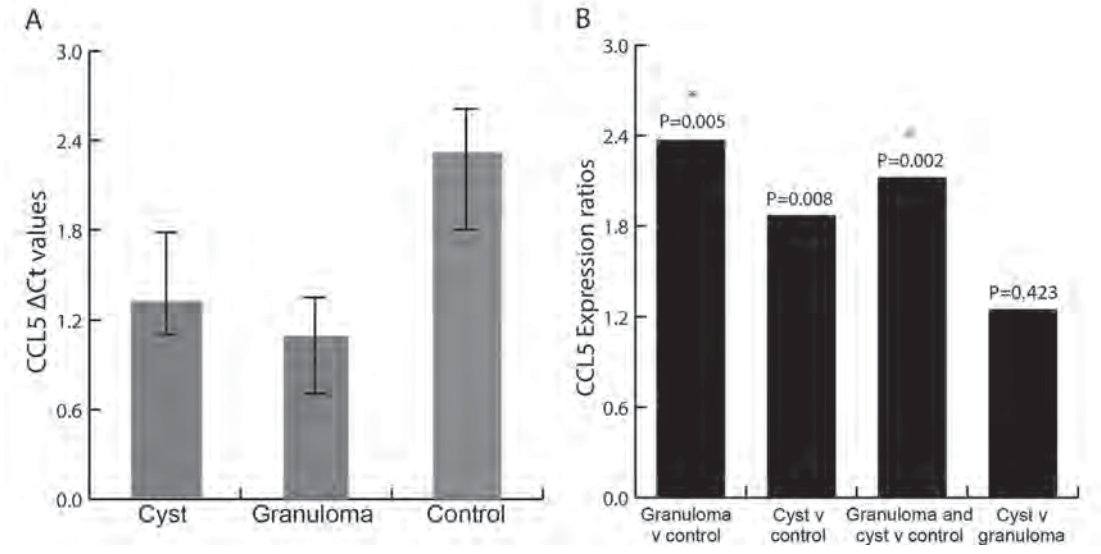

Figure 2. (A) *CCL5* ΔCt values in cysts (n=46), granulomas (n=38) and control (n=38) sample groups. ΔCt for each sample was calculated as the difference between the Ct values of *CCL5* and *SDHA* as the standard. Values are presented as mean ± standard error of the mean. (B) *CCL5* expression ratios between granuloma vs. control, cyst vs. control, granuloma and cyst vs. control and cyst vs. granuloma. *Above indicates a statistically significant difference in expression ratios (P<0.05).

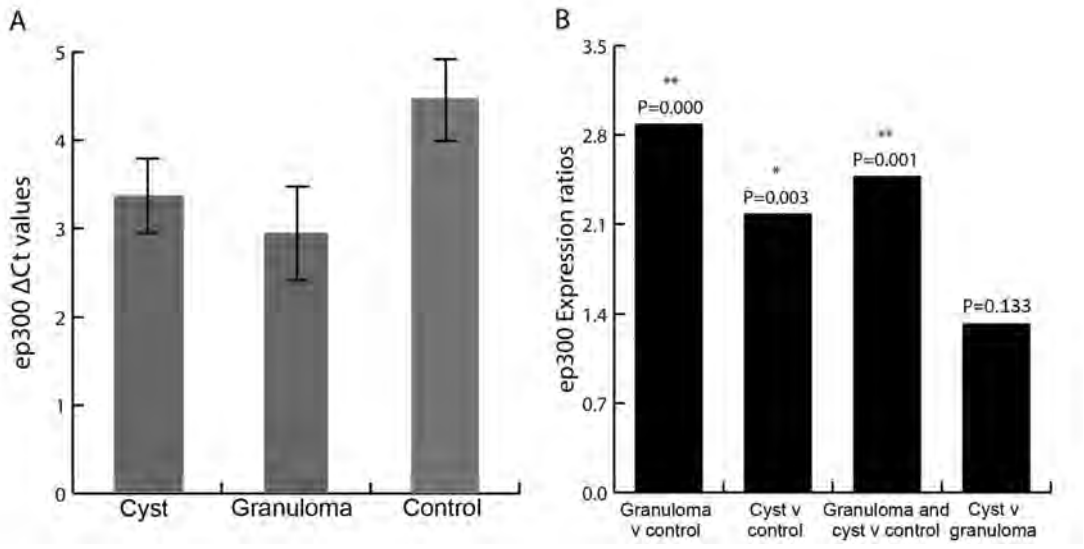

Figure 3. (A) *ep300* ΔCt values in cysts (n=46), granulomas (n=38) and control (n=38) samples. ΔCt for each sample was calculated as the difference between the Ct values of *ep300* and *SDHA* as the standard. Values are presented as mean ± standard error of the mean. (B) *ep300* expression ratios between granuloma vs. control, cyst vs. control, granuloma and cyst vs. control and cyst vs. granuloma. *Above indicates a statistically significant difference in expression ratios (P<0.05). **Above indicates a strong statistical significance in expression ratios (P=0.001).

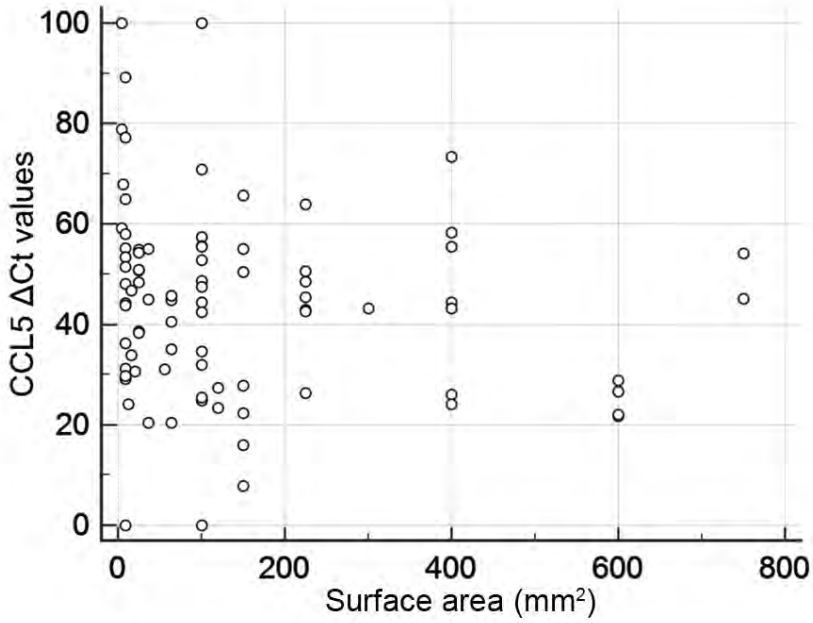

Figure 4. The correlation between ΔCt values for *CCL5* and surface area. The surface area was between 4 and 800 mm^2. Considering that a smaller ΔCt value implies greater gene expression, this result indicates that a greater expression of *CCL5* marker is followed by a smaller surface area.

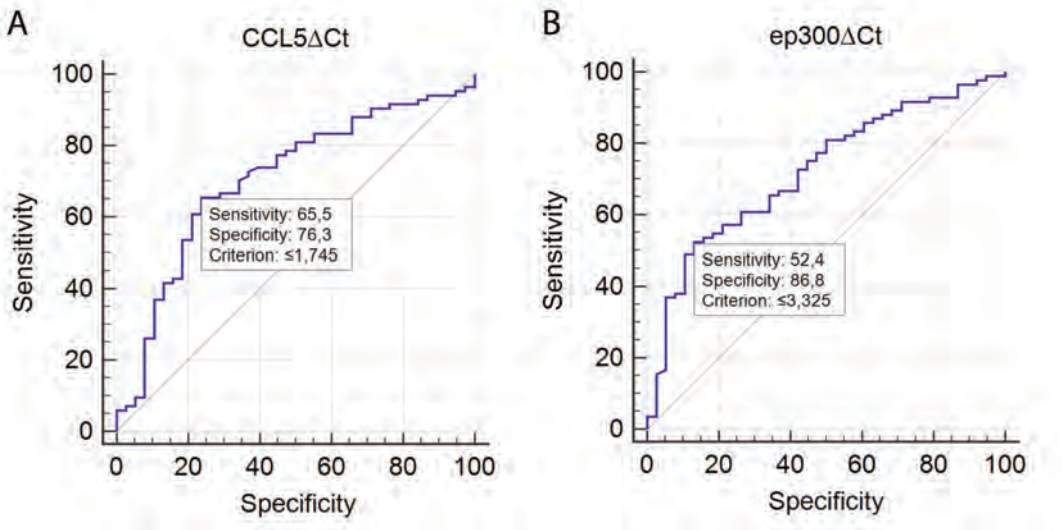

Figure 5. Sensitivity and specificity analysis of qPCR results for *CCL5* and *ep300* markers. A ROC curve (Receiver Operating Characteristic) was used to estimate for (A) *CCL5* and (B) *ep300*. The AUC (Area Under the Curve) was obtained to determine the diagnostic reliability of qPCR analysis.

expression and lesion size, as hypothesized (*CCL5* rho=-0.234, P=0.0320) (Figure 4).

Regarding *ep300*, we assumed that larger lesions would have higher expression of the *ep300* gene because it was bioinformatically identified in cysts. No statistically significant correlation of lesion surface area and expression of *ep300* was found (*ep300* rho=0.00760, P=0.9453).

ROC (Receiver operating characteristic) and logistic regression analyses were used to establish the diagnostic character of ΔCt values. Both *CCL5* and *ep300* genes were diagnostically distinct when comparing the control group and individuals with radicular cysts and periapical granulomas (Figure 5). For *CCL5*, ROC analysis showed good diagnostic accuracy (AUC=0.708; SE=0.0513; 95% CI=0.619–0.787; P=0.0001). The cut-off value was 1.745, maximum sensitivity was 65.48%, and specificity was 76.32%, as shown in Figure 5. The same analysis for *ep300* showed good diagnostic accuracy (AUC=0.722; SE=0.0484; 95% CI=0.634–0.799; P=0.0001). The cut-off value was 3.325, maximum sensitivity was 52.38% and specificity was 86.84%. Neither ROC nor logistic regression analysis indicated any difference between radicular cysts and periapical granulomas (data not shown).

Discussion

One of the new trends in dentistry research is the identification of so-called "leader genes" that are important for the development of various oral diseases. Bioinformatics, which as a theoretical discipline implements information technology in the field of molecular biology, has this indisputable role. Bioinformatics brings together knowledge from various health disciplines and combines this with computer science, biostatistics, engineering and information technologies. By elaborating and analysing data from many databases, this discipline helps in scientific research work, e.g. in setting up research hypotheses etc. Bioinformatics has a special significance for dental research in the field of oral pathology. Today, there is a huge amount of information from this area that is processed by bioinformatic methods.

This is a great help for scientists, especially in finding new ideas for experimental research. Bioinformatics itself is not enough and has many deficiencies, but in combination with experimental research it becomes a significant tool in studying the molecular pathogenesis of the disease. So, we used the research of Poswar *et al.* as a guide in our own research. In other words, bioinformatically identified genes (*CCL5* and *ep300*) were experimentally tested on our own samples (29, 30).

For everyday clinical practice, it is essential to differentiate between periapical granulomas and radicular cysts (3). Therefore, investigation of potential specific markers for differentiation between granulomas and cysts is clinically important.

Poswar *et al.* (2015) conducted a bioinformatics study aimed at identifying potential gene candidates as biomarkers for differentiation between radicular cysts and periapical granulomas (15). The authors named these genes 'leader genes' that could potentially differentiate between these two lesions. For radicular cysts, the suggested leader genes were *TP53* and *ep300*, whereas periapical granulomas were associated with *IL-2RG, CCL2, CCL4*, *CCL5*, *CCR1, CCR3,* and *CCR5* genes. In this study, we examined the expression of *ep300* and *CCL5* in our cohort that consisted of 38 granulomas, 46 cysts and 38 controls. In our study, the control gingival samples were excess material obtained after surgical wisdom tooth removal. Some studies use periodontal ligament as controls, but many studies use normal tissue (healthy gingiva) as the proper control (9, 10, 23, 24, 31). There are studies that use pulp tissue of impacted wisdom teeth as the control (32). There are even studies that use non-oral tissue as control, such as placental tissues (8). Our results showed that periapical lesions have increased expression of both *CCL5* and *ep300* compared to the controls. However, we did not find any differences in *CCL5* and *ep300* expression between granulomas and cysts. We found a significantly negative correlation between *CCL5* expression and lesion size; in other words, the smaller the lesion, the higher the *CCL5* expression.

CCL5 codes for a chemokine, that is 8kDa protein, with a function in the immune system. It is expressed by T lymphocytes (CD4 and CD8) and NK cells. Another name for this gene is RANTES that stands for 'regulated on activation, normal T cell expressed and secreted'. It plays an active role in recruiting leukocytes into inflammatory sites and it induces the proliferation and activation of NK cells. Increased *CCL5* expression has been linked to a wide range of inflammatory disorders and pathologies, such as AIDS (33). Expression of *CCL5* has also been implicated in osteoclast recruitment (34, 35). Additionally, *CCL5* was reported to play a role in the differentiation of multinuclear osteoclast-like cells to active osteoclasts (36). Chemokines are likely to be involved in the expansion of odontogenic cysts.

Several studies have analysed the *CCL5* protein and transcript levels in periapical inflammatory lesions. Marton *et al.* (2000) immunohistochemically detected *CCL5* protein in 6 granulomatous periapical granulomas (37). Silva *et al.* (2005) analysed transcript levels in 15 granulomas, 5 cysts and 3 healthy gingiva controls, where they showed a significantly higher expression of *CCL5* in cysts compared to granulomas (38). In our study, however, we did not detect any difference in *CCL5* expression between granulomas and cysts.

ep300 is an epigenetic transcriptional coactivator that functions as a histone acetyltransferase (22, 39). Its expression is limited to blood cells including myeloid lineage cells, monocytes, T cells (CD4 and CD8), and dendritic cells. It activates HIF1A (hypoxia-inducible factor 1 alpha), which is a master transcriptional regulator of cellular and de-

velopmental response to hypoxia (40-42). The critical regulatory roles of acetyltransferases in inflammation have been reviewed by Ghizzoni, Haisma, Maarsingh & Dekker (2011) (43). Kim *et al.* (2016) showed that *ep300* mediated acetylation of H3K18 in osteoclastogenesis (44). Our study showed for the first time that *ep300* expression was increased in periapical lesions, although no differences were found between different lesion types.

Taking into consideration the fact that radicular cysts are usually larger than granulomas, our aim was to investigate if there were any significant differences in the expression of *ep300* and *CCL5* between these two types of periapical lesions. According to Poswar *et al.* (2015), it was expected that granulomas, as the smaller lesions, would show higher expression of *CCL5,* while cysts would show higher expression of *ep300* (15). Our study confirmed the first hypothesis regarding the *CCL5* expression in smaller lesions. The expression of *ep300* could not be correlated with lesion size.

Conclusion

The results of this study suggest that *CCL5* and *ep300* have a role in periapical lesion pathogenesis. We found that both *CCL5* and *ep300* were diagnostically distinctive when comparing the control group and individuals with periapical lesions, but they could not be used in distinction between granulomas and cysts.

What Is Already Known on this Topic

Most common periapical inflammatory lesions localized in human jaws are periapical granulomas and radicular cysts. These lesions are triggered by bacterial infection of the root canal and have the same origin. The golden standard to differentiate periapical granulomas from radicular cysts is histopathological evaluation. There have been some attempts to find possible biomarkers that could help clinicians to make preclinical diagnoses which could lead to better therapy options. A bioinformatic study (Poswar et al., 2016) has attempted to do this, with the aim of identifying genes that could serve as markers for differentiation between granulomas and cysts. This study recommended two genes, CCL5 and ep300, as leader genes in the differentiation between periapical granulomas and cysts, respectively.

What this Study Adds

According to our knowledge no one has analyzed the expression of of CCL5 and ep300 genes in periapical inflammatory lesions. Poswar et al. conducted a bioinformatical study where they identified potential leader genes for differentiation between most common periapical lesions. For RCs, the suggested leader genes were TP53 and ep300, whereas PGs were associated with IL2RG, CCL2, CCL4, CCL5, CCR1, CCR3, and CCR5 genes. In our study, we tested the results of the above mentioned bioinformatics study where we proved that ep300 cannot be considered diagnostically distinctive for cysts and CCL5 for granulomas. .

Authors' Contributions: Conception and design: NH, AKK and AL; Acquisition, analysis and interpretation of data: AF, SS, NH and AKK; Drafting the article: NH, AKK, AF and SS; Revising it critically for important intellectual content: NH, AKK, AF, SS and AL; Approved final version of the manuscript: NH, AKK, AF, SS and AL.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Acta Medica Academica 2019;48(2):140-146
DOI: 10.5644/ama2006-124.252

Comparative Study of the Efficacy of the Lysozyme, Benzydamine and Chlorhexidine Oral Spray in the Treatment of Acute Tonsillopharyngitis - Results of a Pilot Study

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Received: 29 September 2018
Accepted: 21 February 2019

Key Words: Sore Throat ▪ Antiseptics ▪ Lysozyme.

Objective. Lysozyme is a natural antimicrobial and immunomodulatory enzyme, which is produced as a host response to infectious agents. The objective of this study was to compare the efficacy and safety of lysozyme-based versus benzydamine and chlorhexidine-based oral spray in patients with an acute tonsillopharyngitis associated with a common cold. **Patients and Methods.** A prospective two-arm pilot study (lysozyme/cetylpyridinium/lidocaine spray versus: benzydamine spray—arm 1; chlorhexidine/lidocaine spray—arm 2) was conducted in the primary health care unit. Efficacy was evaluated by the patient's self-assessment of pain, difficulty in swallowing and the throat swelling, by using the visual analog scale (VAS) at baseline and three follow-up visits. Safety was evaluated by the assessment of the frequency and severity of adverse effects. **Results.** Lysozyme-based spray reduced pain faster than benzydamine-based spray and slower than chlorhexidine-based spray. Lysozyme-based and chlorhexidine-based sprays similarly reduced difficulty in swallowing, but were faster than benzydamine-based spray. Similar effects on the reduction of throat swelling were seen in all treated groups. All tested products showed proper safety and were well tolerated, with no serious adverse events reported. **Conclusions.** The lysozyme-based oral spray was shown to be effective and safe in the reduction of pain, difficulty in swallowing and throat swelling in patients with acute tonsillopharyngitis associated with a common cold. Lysozyme-based oral spray (containing natural compound with advantages of influencing immune system and preventing recurrences) had similar activity to benzydamine and chlorhexidine-based oral antiseptic sprays.

Introduction

Acute tonsillopharyngitis is among the most common infections in adults, and it is the second most common reason for visiting a doctor (1). Although it is a self-limiting condition, patients usually seek help for the immediate relief of symptoms such as pain, difficulty in swallowing and the throat swelling.

Viruses are responsible for 85-95% of acute tonsillopharyngitis cases in adults. In contradiction with the European guidelines for the treatment of acute viral tonsillopharyngitis, the antibiotic treatments are still inappropriately prescribed (2). Moreover, acute tonsillopharyngitis is, along with otitis and sinusitis, the leading cause of prescribing antibiotics in the world (2). Unreason-

able prescribing of antibiotics increases the risk of bacterial resistance, a stumbling block in the modern health care system (2, 3). Being the frequent indication for prescription of antibiotics, treatment of acute tonsillopharyngitis significantly contributes to an increase of antimicrobial resistance and treatment costs (4).

In the absence of complications, such as bacterial superinfection, symptoms of acute tonsillopharyngitis can be successfully reduced using local symptomatic treatment (5). Moreover, many over-the-counter (OTC) drugs, such as lysozyme, cetylpyridinium, lidocaine, benzydamine, and chlorhexidine, are available today for the topical treatment of those patients. Oral antiseptics are characterized by direct effects on viral and inflammatory causes, enabling reduction of local pain, rapid onset of action, efficacy and an excellent safety profile in the treatment of acute tonsillopharyngitis (6). Those preparations usually come in the form of sprays, solutions for gargling and lozenges or oriblettes that are applied directly to the mouth or throat mucosa, and are frequently used in the symptomatic treatment of acute uncomplicated cases of tonsillopharyngitis (6-9).

The available data from the comparative clinical trials, mainly placebo-controlled trials, indicate the efficacy and safety of benzydamine-based and chlorhexidine-based oral antiseptics in the topical treatment of acute pharyngitis associated with common cold (7, 8, 10, 11). However, the data regarding the comparison of lysozyme-based versus benzydamine-based and chlorhexidine-based antiseptics in the form of oral spray are limited.

The objective of this pilot study was to compare the efficacy and safety of antiseptics in the form of the oral spray (lysozyme-based versus benzydamine-based spray, and lysozyme-based versus chlorhexidine-based spray) in patients with an acute tonsillopharyngitis associated with a common cold.

Patients and Methods

Study Design and Data Collection

Prospective, two-arm pilot study was conducted in patients with an acute tonsillopharyngitis associated with a common cold. Patients aged 18 years and older with symptoms of acute tonsillopharyngitis which was confirmed at the baseline visit by clinical findings and symptoms on the expanded 21-point Tonsillo-Pharyngitis Assessment (TPA) were recruited in the study in the primary health care unit. Patients were assigned to one of two study arms, i.e. one of two antiseptics. In the first study arm, patients treated with lysozyme/cetylpyridinium/lidocaine oral spray (Lysobact COMPLETE Spray, Bosnalijek JSC Sarajevo, Bosnia and Herzegovina) were compared to patients treated with benzydamine oral spray, while in the second study arm, patients treated with lysozyme/ cetylpyridinium/oral lidocaine spray were compared to patients treated with chlorhexidine/lidocaine oral spray. Concomitant therapy with analgesics, antiinflamatory and antimicrobic drugs were considered exclusion criteria.

Ethics Statement

Medicines & Medical Devices Agency of Bosnia and Herzegovina approved the study. All procedures were made by following the Helsinki Declaration from 1975 and its amendments from 1983.

Evaluation of Efficacy and Safety

Efficacy of local antiseptics was evaluated by analyzing the visual analog scale (VAS) filled out by the patient for the self-assessment of pain, difficulty in swallowing and the throat swelling. Safety was evaluated based on the frequency and severity of the adverse effects of local antiseptics.

Data Collection

The data were collected during the baseline visit and three follow-up visits, at Day 2, Day 3 and Day 5. The data collected at the baseline visit were: general aspect, TPA score (oral temperature, oropharyngeal color, size of tonsils, number of oropharyngeal exanthemas, largest size of anterior cervical lymph nodes, number of anterior cervical lymph nodes, maximum tenderness of some anterior cervical lymph nodes), VAS assessment of pain, difficulty in swallowing and the throat swelling. The data collected at each follow-up visits were: general aspect, VAS assessment of pain, difficulty in swallowing and the throat swelling and the data on the adverse effects.

Statistical Analysis

Normality of quantitative variables distribution was assessed by Kolmogorov-Smirnov test. The significance of the relationship between two categorical variables was analyzed with $\chi 2$ test. The significance of differences between groups for non-normally distributed variables was assessed using Mann-Whitney U test. Friedmans test was used to detect differences in treatments across multiple test attempts and the difference between baseline and follow up visit was tested using Wilcoxon signed-rank test. The statistically significant level was defined as $P<0.05$. Statistical analyses were performed using SPSS 23.0 package (SPSS Inc., Chicago, IL, USA).

Results

In the first study arm, out of 36 patients included, 4 patients were excluded because of the need to include antibiotic or analgesic therapy. Finally, 15 patients were treated with lysozyme-based spray and 17 patients with benzydamine based spray. Demographic characteristics and TPA score of patients in the first study arm are presented in Table 1.

In the paired efficacy analysis (Table 2), a significant reduction in all VAS scores (pain, difficulty in swallowing, throat swelling) for both medicines (lysozyme vs benzydamine based products) (Friedman's test, $P<0.001$) was shown. Both products showed proper safety and were well tolerated, with no serious adverse events reported.

Demographic characteristics and TPA scores of patients in the second study arm are presented in Table 3. In the second study arm, out of 36 patients included, 5 patients were excluded because of the need to include antibiotic or analgesic therapy. Finally, 17 patients were treated with lysozyme-based spray and 14 patients with chlorhexidine-based spray.

In the paired efficacy analysis (Table 4), a significant reduction in all VAS scores (pain, difficulty in swallowing, throat swelling) for both medicines (lysozyme vs chlorhexidine-based products) (Friedman's test, $P<0.001$) was shown. No serious adverse events were recorded, and both products were considered safe and well tolerated.

Table 1. Demographic Characteristics and Tonsillo-Pharyngitis Assessment Scores of Patients Treated with Lysozyme/Cetylpyridinium/Lidocaine Spray and Bezydamine Spray

Characteristics	Treatment		
	Lysozyme/cetylpyridinium/Lidocaine spray	Benzydamine	P
Gender, female [n (%)]	8 (53.3)	11 (64.7)	0.513*
Age, (years, median, min– max)	36 (18–93)	40 (20–75)	0.151*
TPA score median (min–max)	5 (3–10)	6 (1–11)	0.710†

*Chi square test; †Mann-Whitney U test; TPA=Tonsillo-Pharyngitis Assessment.

Table 2. Comparison of Visual Analog Scale Scores of Pain, Difficulty in Swallowing and the Throat Swelling Between Lysozyme/Cetylpyridinium/Lidocaine Spray and Benzydamine Spray at Baseline and Follow-Up Visits

VAS	Visit	Treatment			
		Lysozyme/cetylpyridinium/ lidocaine		Benzydamine	
		Median (min-max)	P*	Median (min-max)	P*
Pain	Baseline	4.0 (1–6)	<0.001	2.2 (0–7)	<0.001
	Day 2	3.0 (1–4)**		2.2 (0–5)	
	Day 3	1.0 (0–3)**		1.4 (0–4)**	
	Day 5	0.5 (0–1)**		1.0 (0–2)**	
Difficulty in swallowing	Baseline	3.2 (1–6)	<0.001	2.3 (0–7)	<0.001
	Day 2	2.5 (1–5)**		2.3 (0–5)	
	Day 3	1.0 (0–3)**		1.1 (0–3)**	
	Day 5	0.0 (0–3)**		0.6 (0–1.5)**	
Throat swelling	Baseline	4.0 (1–7)	<0.001	1.9 (0–6)	<0.001
	Day 2	2.0 (0–6)**		0.7 (0–4)**	
	Day 3	0.50 (0–3)**		0.0 (0–2)**	
	Day 5	0.0 (0–3)**		1.0 (0–1)**	

VAS=Visual analog scale; *Friedman's test; **Wilcoxon test (follow up visit vs. baseline) P<0.05.

Table 3. Demographic Characteristics and Tonsillo-Pharyngitis Assessment (TPA) Scores of Patients Treated with Lysozyme/Cetylpyridinium/Lidocaine Spray and Chlorhexidine/Lidocaine Spray

Characteristics	Treatment		P
	Lysozyme/cetylpyridinium/lidocaine spray	Chlorhexidine/lidocaine spray	
Gender, female, n (%)	12 (70.6)	5 (35.7)	0.052*
Age, years, median, (min– max)	37 (27–68)	53.5 (29–81)	0.003‡
TPA score, median (min–max)	4 (1–16)	6.5 (3–14)	0.092‡

*Chi square test; ‡Mann-Whitney U test. TPA=Tonsillo-Pharyngitis Assessment.

Table 4. Comparison of Visual Analog Scale (VAS) Scores of Pain, Difficulty in Swallowing and the Throat Swelling Between Lysozyme/Cetylpyridinium/Lidocaine Spray and Chlorhexidine/Lidocaine Spray at Baseline and at Follow-Up Visits

VAS	Visit	Treatment			
		Lysozyme/cetylpyridinium/ Lidocaine spray		Chlorhexidine/Lidocaine spray	
		Median (min-max)	P*	Median (min-max)	P*
Pain	Baseline	3.0 (0–7)	<0.001	3.6 (1–6.5)	<0.001
	Day 2	3.0 (0–4.7)		3.25 (1–5.9)***	
	Day 3	1.5 (0–3.3)**		2.0 (0–5)**	
	Day 5	0.0 (0–1.6)**		0.5 (0–2.6)**	
Difficulty in swallowing	Baseline	3.4 (0–6.7)	<0.001	3.35 (1–6)	<0.001
	Day 2	2.8 (0.5–0.5)**		3.0 (1–6)**	
	Day 3	1.3 (0–3)**		1.9 (0–5)**	
	Day 5	0.0 (0–1.6)**		0.5 (0–2.6)**	
Throat swelling	Baseline	2.0 (0–6.3)	<0.001	2.5 (0–7)	<0.001
	Day 2	2.0 (0–3.5)		2.5 (0–5.4)	
	Day 3	0.0 (0–3)**		1.25 (0–5)**	
	Day 5	0.0 (0–1.6)**		0.0 (0–2)**	

VAS=Visual analog scale; *Friedman's test; **Wilcoxon test (follow up visit vs. baseline) P<0.05.

Discussion

To our knowledge, this is the first study to compare lysozyme-based and other antiseptics in the form of oral sprays in patients with acute tonsillopharyngitis associated with a common cold. The lysozyme-based oral spray was shown to be effective and safe in the reduction of pain, difficulty in swallowing and throat swelling.

In the reduction of pain, lysozyme-based oral spray showed the faster onset of action compared to benzydamine-based spray, but delayed onset of action compared to chlorhexidine-based spray. In the reduction of difficulty in swallowing, lysozyme-based oral spray was similar to chlorhexidine-based spray but showed faster onset of action compared to benzydamine-based spray. In the reduction of throat swelling, the similar results were obtained for all treatments (lysozyme, benzydamine and chlorhexidine-based sprays).

Lysozyme, an antiseptic, is also known to have antibiotic and antiviral effects (12). Additionally, as a natural enzyme, it has an overall role in the body justifying the name - "enzyme of the future" (13). Unlike other antiseptics, in addition to its anti-inflammatory activity, lysozyme possess immunomodulatory activity. As an integral component of natural cells of the immune system, it is one of the most critical elements of the local non-specific microbial resistance of the mucosa. This is supported by the fact that the levels of immunoglobulins and lysozyme correlate (13). Also, studies demonstrated the efficacy of lysozyme-based preparations in the local treatment of pharyngitis and tonsillitis in children and adults (10, 14). According to a study with the patient reported outcomes, an oral spray containing the combination of lysozyme-chloride and cetylpyridinium-chloride could quickly, efficiently and safely solve the acute symptoms of tonsillopharyngitis associated with common cold (9). In the treatment of a sore throat, the local symptomatic therapy aims primarily to reduce the pain (6), and the pain reduction is usually the primary outcome of clinical trials testing the efficacy of different treatments for a sore throat (15). However, besides pain reduction, the impact of additional beneficial effects such as anti-inflammatory, immunomodulatory, pro-regenerative should influence the choice of optimal therapy. Therefore, lysozyme based preparations open new perspectives in the treatment of those patients.

Benzydamine is a locally-acting non-steroidal anti-inflammatory drug with local anesthetic and analgesic properties. It is widely used in the symptomatic treatment of pain, irritation and other symptoms of inflammation of the oropharynx (8). Although benzydamine in the form of oral rinse showed the more significant reduction of pain and dysphagia compared to placebo in patients with viral pharyngitis or tonsillitis (16), in our study, in the form of the oral spray, it showed slower efficacy in reduction of difficulties in swallowing when compared to lysozyme-based spray.

In concordance with our results, chlorhexidine-based antiseptic in the form of lozenges was effective in the treatment of a sore throat in patients with upper respiratory infection (17). In the form of an oral spray, chlorhexidine-lidocaine was also as effective and safe as a product based on echinacea and sage (18). In a study that compared the efficacy of benzydamine oral spray and chlorhexidine oral spray for the treatment of streptococcal tonsillopharyngitis, both antiseptics have proven successful as an adjunct to standard antibiotic therapy (7).

Limitations of the Study

The major limitations of this study are the small sample size, no randomization and no

control arm. Larger prospective randomized placebo controlled studies are needed to confirm the obtained results.

Conclusion

The lysozyme-based oral spray was shown to be effective and safe in the reduction of pain, difficulty in swallowing and throat swelling in patients with acute tonsillopharyngitis associated with a common cold. Lysozyme-based oral spray (containing natural compound with advantages of influencing immune system and preventing recurrences) had similar activity to benzydamine and chlorhexidine-based oral antiseptic sprays.

What Is Already Known on this Topic

Bacterial resistance is one of the most significant problems in modern medicine. Although viruses are responsible for 85-95% acute tonsillopharyngitis in adults, antibiotics are often prescribed for this indication contributing to increasing of antimicrobial resistance and treatment costs. Local symptomatic treatment can be employed for reduction of symptoms of acute tonsillopharyngitis not associated with complications such as bacterial superinfection. There are many over-the-counter drugs which can be used for this purpose. Among them, lysozyme based preparations attract attention since it is a natural component of the human immunity (with activity against bacteria, fungi, and viruses and anti-inflammatory effects). There are no data comparing efficacy and safety of lysozyme-based products versus other antiseptics in patients with an acute tonsillopharyngitis associated with a common cold.

What this Study Adds:

This study confirmed that lysozyme/cetylpyridinium/lidocaine oral spray is effective and safe in patients with acute tonsillopharyngitis. Moreover, lysozyme-based oral spray (containing natural compound with advantages of influencing immune system and preventing recurrences) had similar activity to benzydamine and chlorhexidine-based oral antiseptic sprays in the reduction of pain, difficulty in swallowing and throat swelling in patients with acute tonsillopharyngitis associated with a common cold.

Authors' Contributions: Conception and design: MM, BK, AŠ and JDž; Acquisition, analysis and interpretation of data: NGG, ZN, ZS and VČ; Drafting the article: MM; Revising it critically for important intellectual content: UG; Approved final version of the manuscript: NGG, ZN, ZS, AŠ, JDž, UG, BK, VČ and MM.

Conflict of Interest: Authors declare that this study was supported by Bosnalijek d.d. (a company which is the manufacturer of Lysobact COMPLETE Spray).

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Original Article
Acta Medica Academica 2019;48(2):147-158
DOI: 10.5644/ama2006-124.253

Knowledge and Attitudes of Sexually Transmitted Infections Among High School Students in Sarajevo

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Received: 22 April 2019
Accepted: 8 August 2019

Key Words: Knowledge ▪ Behaviour ▪ Awareness ▪ Sexually Transmitted Infections.

Objective. The aim of the study was to evaluate knowledge and behavioural risks related to sexually transmitted infection (STIs) among high school students. **Methods.** The cross sectional study was conducted among students aged 15-18 years old from two high schools in the Sarajevo Canton in the period from October 2017 to March 2018. An anonymous self-administered questionnaire was used. The survey investigated their socio-demographic characteristics, sexual habits and level of knowledge about STIs. The data were analysed using SPSS version 25 and MS Excel 2016. **Results.** In total 278 high school students participated in the study, with a mean age of 17.79±1.026 years, of which 89 (32%) were male, and 189 (68%) were female. There was no significant difference in age in relation to sex distribution (P=0.074). Regarding the number of participants, 234 (84.2%) were from the dental school, while 44 (15.8%) were from a *gimnazija* (grammar high school). There was no significant difference in gender-based distribution by school (P=0.080). Students from the grammar high school had significantly better knowledge about the impact of STI on the foetus (P=0.025) and infected individuals (P=0.001), also about the impact of STI on sterility (P=0.005). **Conclusion.** Our study confirmed the need for implementation of sexual education programs in the final grades of elementary school or in first grades of high school, aimed at improving knowledge of sexually transmitted infections and improving sexual and reproductive health.

Introduction

Sexually transmitted infections (STIs) have been recognized as a major public and social health care problem worldwide (1). Their incidence is constantly increasing, and the young population bears a significant burden from these infections, as well as unwanted pregnancies and births, maternal deaths and abortion (2-4). According to data from the World Health Organization (WHO), more than 1 million STIs are reported every day worldwide (5). Each year, there are an estimated 357 million new infections from one (1) of four (4) curable STIs: chlamydia, gonorrhoea, syphilis and trichomoniasis. The majority of STIs have no or only mild symptoms that may not be recognized as STIs (5).

Generally, sexual and reproductive health care among young people has faced major problems, challenges and dilemmas. Therefore, it is necessary to understand the level of awareness and knowledge about STIs, methods of protection, and sexual practices in this group of individuals. It is important to emphasize youth as the most vulnerable group, with an increase in the birth rate,

especially in adolescents aged less than 15 years old, who primarily live in undeveloped countries with poor social and health care support programs (4). Approximately 70% of patients with STIs are in the age range between 15 and 24 years old. The WHO estimates that one in twenty teenagers contracts an STI during their lifetime (5).

In the context of Bosnia and Herzegovina (BH), the most important factors that contribute to the spread of STIs are the lack of sexual education in the school system, inconsistent and irregular condom use, casual sexual partners, and an inadequate level of knowledge about STI prevention strategies (6), which are predominant in this population. However, a low coverage of vaccine against human papillomavirus (HPV) infection has also been observed in BH due to the insufficient adherence of parents and adolescents to the health care system, for a variety of reasons (belief in the adverse effect of vaccines without scientific medical knowledge, the lack of interest of social and health care workers for recognition of HPV as being an STI, cultural and social aspects).

In this paper, some limitations in relation to the factors that may result in risk-taking behaviours are presented, mainly personal, family or social/school-related (6). Relatively little is known about the socio-demographic factors and sexual behaviour, having in mind the specificity of this population. Although a few studies have been carried out on this topic in BH, the fact is that research of this type needs to be seriously understood and resumed on a high-quality sample in this category, and compared with the available literature (6, 7). In most other countries in Eastern Europe, as in BH, there is lack of data about the prevalence of the most curable STIs in the general population, including youth (8).

Therefore this study was conducted to assess awareness about STIs in a young population, and determine the relationship between socio-demographic factors and knowledge of STIs among high school students in Sarajevo Canton, BH. Furthermore, our goal was to determine correlations between the students' socio-demographic characteristics, sexual behaviour and knowledge about STIs, and compare the results with other relevant studies on the topic.

Material and Methods

Study Population

This was a cross-sectional study design, conducted in Sarajevo Canton, BH. In total, 278 high school students, attending the third and fourth grades at two high schools were included in the study, during the period of October 2017 to March 2018. The multistage sampling method, followed by a simple random sampling technique was used to collect the primary data, with a sample size of 84.2% students from the dental school, and 15.8% from the grammar high school. A semi-structured, self-administered written questionnaire (SAQ) was used for collecting data about knowledge, attitudes and practice in the high school population.

The Questionnaire

For the purpose of this research, a special questionnaire was developed, based on several existing foreign instruments. As a base, the following instruments were used and modified (9-12): the Sexual Activities & Attitudes Questionnaire (Noll at all, 2003), the Teenage Sexual Health and Behaviour Questionnaire (Jomeen and Whithfield, 2010), Survey of Sexual Behaviours (Coulter, 2007) and the Youth Risk Behaviour Survey (Centres for Disease Control and Prevention, 2011). The survey questionnaire consisted of two fundamental parts, including 33 questions about sexual experience, attitudes and beliefs related to sexual relations, use

of condoms as protection, their perception of sexual activity, and a test of reproductive and sexual health.

The first part comprised 15 items: demographic and socio-economic questions (gender, age, the high school that the student attends, grade, municipality of residence, socio-economic status, who they live with, the type of family they live in). The type of family: liberal or conservative was examined to see how students see their personal home environment, because liberal families tend to be more open for conversation about these delicate topics. In some cases students found their parents liberal, for example in relation to cigarettes or going out, but conservative on topics such as sexuality. The next set of questions was about their social life: how often they go out, whether they have a curfew by which they must be at home, also alcohol consumption and smoking cigarettes or using a water pipe to filter smoke, known also as a hookah. The last question was only for girls, regarding their first menstrual period. The second part contained 18 items that we used for testing their knowledge, awareness, attitudes, sexual behaviour and experience, history of STIs, and condom use. The questions were as follows: first sexual intercourse, use of contraceptive methods, number of partners, sexual preferences, and condom usage during the last sexual relationship. Additionally, there were questions about their knowledge of sexually transmitted infections, their causes, the most effective forms of protection, and the consequences. The questionnaire could be answered within 10-15 minutes. The survey procedure was designed to protect the student's privacy, with anonymous participation and participation was not mandatory. The processing and publication of participants' data in this study were strictly in accordance with the Declaration of Helsinki, DoH/Oct2008, including confidentiality and anonymity. Medical doctors, who work at the Department of Microbiology at Medical Faculty, University of Sarajevo presented and explained the meaning of the questionnaire to the students.

Indicators

Socio-economic status was measured by 3-point scales ranging from (1) worse than others in the area to (3) better than others in the area. An indicator of parental control was obtained by categorizing respondents on the basis of where they live and with whom. Respondents living with both parents were coded by a 5-point scale ranging from (1) in a student hostel to (5) with parents. Frequency of condom use was accessed by a 2-point scale ranging from (1) never to (2) always. Responses regarding the number of sexual partners were collapsed into categories: 1-3 partners, 4 or more partners and a "No answer" group.

Answers about the age of the last sexual partner were split into two categories: older than subject or of the same age. Use of condoms during last sexual intercourse was tested with a yes or no question type. Oral sexual intercourse was also tested in the same way. The questions about knowledge of STIs also had only yes and no answers. Knowledge about STIs was tested by a question where the subjects had to mark all the STIs that they know about. These diseases were: Chlamydia, Tuberculosis (TBC), Syphilis, Flu, HIV, Gonorrhoea, and Measles.

We asked about where the subjects obtained their first information about sexual intercourse and possible infections on the basis of eight answers. Also knowledge about the best protection from STIs was tested with the possible answers: oral contraceptives, coil, condom, lubricant. Consequences of STI infection were tested with a question about whom they affect, only the infected persons, or even a pregnancy.

Statistical Analysis

Basic standard methods of descriptive statistics, quantitatively describing or summarizing features of a collection of information were used. Values were represented in frequencies or as a percentage. Chi-square test or Fisher exact test was performed to test the differences in proportions of qualitative variables between groups and Student t-test for testing the differences between quantitative variables. The data were analysed with IBM SPSS Statistics 25.00 (IBM Corporation, Armonk, New York) and MS-Excel. Statistical significance was established with p values less than 0.05.

Results

A total of 278 high school students participated in the study, with a mean age of 17.79±1.03 years. Eighty-nine (32%) were male, while 189 (68%) were female. There was no significant difference in sex distribution in relation to age (P=0.074). Regarding the number of participants, 234 (84.2%) were from the dental school, while 44 (15.8%) were from the grammar high school. There was no significant difference in gender-based distribution by school (P=0.08). The number of questionnaires that were completely answered was 197 of the 278 that were answered (response rate 70.9%). The questions that were not answered by all students were analysed.

The demographic characteristics of the participants were analysed as follows: the municipalities where they lived, socioeconomic status, living situation and family type, frequency of evening outings and curfew limitations. These are shown in Table 1.

No significant demographic differences were found in the characteristics of the respondents based on the school they were attending. The type of family that students came from was similar in both schools. Most of them believed that their family was liberal, but in some situations it was conservative.

The risk factors faced by students from both schools are similar in distribution. The values are shown in Table 2.

Alcohol was regularly consumed by 17.2% of students at the dental school, occasionally by 24.3% of respondents; while 27.3% of the grammar high school students regularly consumed alcohol, with occasional alcohol consumption by 18.2% of respondents. Cigarettes were smoked by 18.0% of the respondents in the dental school, while 22.7% grammar high school students smoked. Some form of drug had been consumed by 2.6% of respondents in the dental school and 4.6% of the respondents in the grammar high school. Hookahs were used by about one-third of the population in both schools. No significant differences in exposure to risk factors were found.

The replies related to menstrual cycle, sexual relations, protection and contraception, the number of partners are shown in Table 3.

The first menstruation, in over 50% of females, was between the ages of 12 and 14 in both schools. The first menstrual period was at under the age of 12 in 24.7% of respondents in the dental school, , and in 34.3% in the grammar high school. There was no significant difference in distribution. 20.9% of the III and IV grade students of the dental school had had sexual relations, and 18.2% of the respondents in the grammar high school. In the dental school 83.3% of the respondents, and 57.1% of the respondents in the grammar high school used some form of protection. There was no significant difference (Fishers exact test P=0.139).

According to the results, the condom is the form of contraception used most often by all high school students. In total, 91.1% respondents used condoms in the dental school, and 71.4% of the respondents in the grammar high school. In the dental school,

Table 1. Demographic Characteristics of High School Students

Variable	Characteristics	Schools				Total	
		Dental School		Gymnasium			
		N	%	N	%	N	%
Gender	Female	154	65.8	35	79.5	189	67.9
	Male	80	34.2	9	20.5	89	32.0
	Total	234	100.0	44	100.0	278	100.0
Age (yr)	Female	18±1		17±2		18±1	
	Male	18±1		18±1		18±1	
Municipalities	Stari Grad	25	11.2	10	23.3	35	13.2
	Centar	33	14.8	12	27.9	45	16.9
	Novo Sarajevo	32	14.4	3	6.9	35	13.2
	Novi Grad	53	23.8	11	25.6	64	24.1
	Ilidža	32	14.4	5	11.6	37	13.9
	Hadžići	6	2.7	0	0.0	6	2.3
	Vogošća	30	13.5	2	4.7	32	12.0
	Ilijaš	10	4.5	0	0.0	10	3.7
	Zenica	1	0.5	0	0.0	1	0.4
	Visoko	1	0.5	0	0.0	1	0.4
	Total	223	100.0	43	100.0	266	100.0
Socio-economic status	Worse	3	1.3	1	2.3	4	1.5
	Average	197	85.3	34	77.3	231	84.0
	Better	31	13.4	9	20.5	40	14.6
	Total	231	100.0	44	100.0	275	100.0
Living with	Parents	216	92.7	41	93.2	257	92.8
	Guardians	7	3.0	1	2.3	8	2.9
	Alone	7	3.0	2	4.6	9	3.3
	Roommate	3	1.3	0	0.0	3	1.1
	Total	233	100.0	44	100.0	277	100.0
Family type	Conservative	29	13.2	6	14.3	35	13.4
	Liberal	93	42.3	18	42.9	111	42.4
	Both types	98	44.5	18	42.9	116	44.3
	Total	220	100.0	42	100.0	262	100.0
Evening outings	Till 3x	72	31.2	16	37.2	88	32.1
	On weekends	80	34.6	10	23.3	90	32.9
	On a daily basis	79	34.2	17	39.5	96	35.0
	Total	231	100.0	43	100.0	274	100.0
Length limitations	Yes	140	60.3	28	65.1	168	61.1
	No	92	39.7	15	34.9	107	38.9
	Total	232	100.0	43	100.0	275	100.0

Table 2. Consumption of Risk Factors

Consumption	Indicators	Schools				Total	
		Dental school		Gymnasium			
		N	%	N	%	N	%
Alcohol	Yes	40	17.2	12	27.3	52	18.8
	No	136	58.4	24	54.6	160	57.8
	Periodically	57	24.5	8	18.2	65	23.5
	Total	233	100.0	44	100.0	277	100.0
Cigarettes	Yes	42	18.0	10	22.7	52	18.8
	No	165	70.8	32	72.7	197	71.1
	Periodically	26	11.2	2	4.6	28	10.1
	Total	233	100.0	44	100.0	277	100.0
Narcotics	Yes	6	2.6	2	4.7	8	2.9
	No	213	91.4	36	83.7	249	90.2
	Tried	14	6.01	5	11.6	19	6.9
	Total	233	100.0	43	100.0	276	100.0
Hookah	Yes	73	31.5	17	39.5	90	32.7
	No	110	47.4	18	41.9	128	46.6
	Periodically	49	21.1	8	18.6	57	20.7
	Total	232	100.0	43	100.0	275	100.0

Table 3. Sexual Behaviour Knowledge Protection and Contraception

Variable	Indicators	Schools				Total	
		Dental school		Gymnasium			
		N	%	N	%	N	%
Menstrual cycles	<12 yr.	38	24.7	12	34.3	50	26.5
	12 do 14 yr.	90	58.4	19	54.3	109	57.7
	>14 years	26	16.9	4	11.4	30	15.9
	Total	154	100.0	35	100.0	189	100.0
Sexual relations	Yes	49	20.9	8	18.2	57	20.5
	No	185	79.1	36	81.8	221	79.5
	Total	234	100.0	44	100.0	278	100.0
Protection	Yes	35	83.3	4	57.1	39	79.6
	No	7	16.7	3	42.9	10	20.4
	Total	42	100.0	7	100.0	49	100.0
Contraception	No	4	8.9	2	28.6	6	12.2
	Condom	41	91.1	5	71.4	46	93.9
	Total	42	100.0	7	100.0	49	100.0
Number of partners	1 to 2 partners	30	63.8	6	85.7	36	66.7
	≥3 partners	17	36.2	1	14.3	18	33.3
	Total	47	100.0	7	100.0	54	100.0
Partners age	Same years	22	46.8	4	57.1	26	48.2
	Older	25	53.3	3	42.9	28	51.8
	Total	47	100.0	7	100.0	54	100.0
Condom use during the last sexual intercourse	Yes	30	65.2	4	57.1	34	64.1
	No	16	34.8	3	42.9	19	35.8
	Total	46	100.0	7	100.0	53	100.0
Oral sex	Yes	45	25.8	8	20.5	53	24.9
	No	129	74.1	31	79.5	160	75.1
	Total	174	100.0	39	100.0	213	100.0

8.9% did not use contraception and in the grammar high school 28.6%.

Most of the respondents in both schools had had 1 or 2 sexual partners. In the dental school, 53.2% of those who had had sexual experience stated that the partner was older, while 57.1% of the grammar high school students had had partners of the same age.

Knowledge about STIs was tested by a questionnaire and the results are presented in Table 4.

Analysing the STI survey results, significant differences in students' knowledge were identified. In the survey questionnaire, only 45.8% of dental school students recognized chlamydia as a causative agent of

Table 4. Knowledge about STIs

Variable	Indicators	Schools				Total	
		Dental school		Gymnasium			
		N	%	N	%	N	%
Knowledge about STIs	Poorly	5	2.3	1	2.3	6	2.3
	Poor	18	8.1	1	2.3	19	7.2
	Neither good nor bad	67	30.3	19	43.2	86	32.4
	Good	96	43.4	16	36.4	112	42.3
	Very good	35	15.8	7	15.9	42	15.8
	Total	221	100.0	44	100.0	265	100.0
Chlamydia marked	Yes	93	45.8	36	87.8*	129	52.9
	No	110	54.2	5	12.2	115	47.1
	Total	203	100.0	41	100.0	244	100.0
TBC marked	Yes	10	4.9	1	2.4	11	4.5
	No	194	95.1	40	97.6	234	95.5
	Total	204	100.0	41	100.0	245	100.0
Syfilis marked	Yes	168	82.4	35	85.4	203	82.9
	No	36	17.7	6	14.6	42	17.1
	Total	204	100.0	41	100.0	245	100.0
Flu marked	Yes	5	2.4	1	2.4	6	2.4
	No	199	97.5	41	97.6	240	97.6
	Total	204	100.0	42	100.0	246	100.0
HIV marked	Yes	194	95.1	40	97.6	234	95.5
	No	10	4.9	1	2.4	11	4.5
	Total	204	100.0	41	100.0	245	100.0
Measles marked	Yes	7	3.4	1	2.4	8	3.3
	No	197	96.6	40	97.6	237	96.7
	Total	204	100.0	41	100.0	245	100.0
Gonorrhea	Yes	134	65.7	25	60.9	159	64.9
	No	70	34.3	16	39.1	86	35.1
	Total	204.00	100.0	41	100.0	245	100.0
Number of correct answers	0 -1 correct answer	21	10.2	2	4.9	23	9.4
	2 correct answers	45	21.9	5	12.2	50	20.3
	3 correct answers	69	33.7	12	29.3	81	32.9
	4 correct answers	70	34.2	22	53.7	92	37.5
	5 - 7 correct answers	0	0	0	0	0	0
	Total	205	100.0	41	100.0	246	100.0

STIs=Sexually transmitted infections.

STIs, in contrast to 87.8% of respondents in the grammar high school. There was a significant difference (Chi-square test=24.14, P<0.001). Knowledge about other possible pathogens was similar, without any significant difference. In total, none of subjects gave more than 4 correct answers.

That a condom is effective protection is believed by 84.8% of respondents in the dental school and 79.5% of the respondents in the grammar high school (Table 5.). Oral contraceptives are seen as effective protection during intercourse by 7.6% of respondents in the dental school and 2.3% of respondents from the grammar high school. From the grammar high school 6.8% of the respondents believed that a coil is an effective method. Students from the grammar high school had significantly better knowledge about the impact of STIs on the foetus (Fisher exact test P=0.025) and infected individuals (Fisher Exact test P=0.001). Also students from the grammar high school had significantly better knowledge about the

Table 5. Attitude and Practice

Variable	Indicators	Schools				Total	
		Dental School		Gymnasium			
		N	%	N	%	N	%
Sexual knowledge	Family member	25	11.8	4	9.3	29	11.4
	School and lectures	111	52.4	16	37.2	127	49.8
	Internet and magazines	26	12.4	7	16.3	33	12.9
	Other (including Proscribed drugs)	13	6.1	1	2.3	14	5.5
	Multiple answers	37	17.3	15	34.9	52	20.4
	Total	212	100.0	43	100.0	255	100.0
Sexual protection	Oral contraception	16	7.6	1	2.3	17	6.7
	Spiral	7	3.3	3	6.8	10	3.9
	Condom	179	84.8	35	79.5	214	83.9
	Lubricant	0	0.0	1	2.3	1	0.4
	Multiple answers	9	4.3	4	9.1	13	5.1
	Total	211	100.0	44	100.0	255	100.0
STI have effect on the fetus	Yes	146	74.1	38	90.5*	184	77.0
	No	51	25.9	4	9.5	55	23.0
	Total	197	100.0	42	100.0	239	100.0
STI have effect only on infected person	Yes	74	37.8	5	11.9	79	33.2
	No	122	62.2	37	88.1*	159	66.8
	Total	196	100.0	42	100.0	238	100.0
All STIs are curable	Yes	7	3.5	2	4.8	9	3.8
	No	190	96.5	40	95.2	230	96.2
	Total	197	100.0	42	100.0	239	100.0
STI can cause sterility	Yes	107	54.3	33	78.6*	140	58.6
	No	90	45.7	9	21.4	99	41.4
	Total	197	100.0	42	100.0	239	100.0
Number of correct answers	0 correct answer	8	3.5	2	4.8	10	3.7
	1 correct answer	86	37.1	11	26.2	97	35.4
	2 correct answer	84	36.2	29	69.0	113	41.2
	3 correct answer	54	23.2	0	0.0	54	19.7
	4 correct answer	0	0.0	0	0.0	0	0.0
	Total	232	100.0	42	100.0	274	100.0

Continuation of Table 5. Attitude and Practice

Variable	Indicators	Schools				Total	
		Dental School		Gymnasium			
		N	%	N	%	N	%
Do you have any of following symptoms	Urethral discharge	0	0.0	0	0.0	0	0.0
	Vaginal discharge	5	2.9	0	0.0	5	2.4
	Problem with urination	0	0.0	1	2.4	1	0.5
	Itching	5	2.7	1	2.4	6	2.9
	Bleeding	3	1.8	0	0.0	3	1.4
	Abdominal pain	1	0.6	1	2.4	2	0.9
	Nothing mentioned above	153	90.5	37	90.2	190	90.5
	Other	2	1.2	1	2.4	3	1.4
	Total	169	100.0	41	100.0	210	100.0
Transmission of HIV by kiss	Yes	18	8.7	5	11.4	23	9.2
	No	138	66.7	34	77.3	172	68.5
	Do not know	51	24.6	5	11.4	56	22.3
	Total	207	100.0	44	100.0	251	100.0
Unwanted pregnancy	Conversation with parents	108	52.2	21	48.8	129	51.6
	Abortion	38	18.4	9	20.9	47	18.8
	Parenthood	39	18.8	3	7.0	42	16.8
	Other	10	4.8	7	16.3	17	6.8
	Multiple answers	12	5.8	3	6.9	15	6.0
	Total	207	100.0	43	100.0	250	100.0
Long lasting relationship	Yes	75	34.2	13	30.2	88	33.6
	No	144	65.8	30	69.8	174	66.4
	Total	219	100.0	43	100.0	262	100.0

STIs=Sexually transmitted infections.

possible impact of STI on sterility (Fisher Exact test P=0.005). In total 23.3% of students in the dental school and 0% in the grammar high school had 3 correct answers about attitudes and practice. Furthermore, 36.2% of students in the dental school and 69.1% in the grammar high school had 2 correct answers. It is interesting that none of the students in either school had 4 correct answers. Answers to questions about unwanted pregnancy showed that around half of the students from both schools would talk with their parents, furthermore 18.8% of students from the dental school would choose parenthood no matter what, while only 7.0% of students in the grammar high school would choose parenthood no matter what. Abortion is an option for 18.8% of students in the dental school and for 20.9% in the grammar high school.

Discussion

Analysis of the results revealed significant demographic differences in the characteristics of high school students, based on the school they were attending. The type of family that students come from is similar in both high schools. Most believe that their family is liberal about topics such as sexuality and sexual education, but some students see their families as conservative. Moreover, no significant differences in risk factors were found. Risk factors in the study conducted by Drago et al., showed that in Italy 79% of responders used alcohol, especially at parties (58%) and during weekends (22%) (13). Alcohol usage and the presence of risk factors is much higher between Italian students than in BH.

Similar results to ours were obtained by Thapa et al. in their study of high school

students in Bajhang, Nepal, where awareness about STIs was 62.3%, but only 38.1% had good knowledge, while 61.9% had poor knowledge about STIs (15) Another study conducted in Bangladesh showed that 70.6% adolescents had awareness about STIs (16). About 19.1% of high school students tested in South Western Nigeria showed poor knowledge, while 74.1% had fair knowledge and 6.9% had good knowledge of sexually transmitted infections (17).

The possible cause of the variations in awareness and knowledge about STIs found in the data collected from the students from the two different high schools in our study is perhaps the difference in the subjects taught at the schools. Across Germany, nearly all students had heard of HIV, but overall knowledge of other STIs was much less satisfactory, with low self-reported knowledge and high levels of ignorance regarding individual STIs (14). Moreover, in our study, recognition of chlamydia as an STI agent was more familiar to students from the grammar high school than the dental school (87.8% vs. 45.8%), which is interesting because the dental students were attending medically oriented courses ($P<0.001$). Results obtained by von Rosen et al. in Germany, showed that more than 46% of participants had never heard of chlamydia and merely 18% knew that chlamydia can be cured. Knowledge and awareness were visibly lower for other STIs, of which the most frequently known infection was hepatitis B. Despite being the bacterial STI with the highest prevalence, chlamydia was the infection with the lowest proportion of participants claiming good knowledge and the lowest rate of awareness (14).

Regarding sexual intercourse, 20.94% of students in the dental school had had sexual intercourse, and 18.18% in the grammar high school. These results are much lower than in an Italian study, where 61% of respondents had already had sexual intercourse with the average age of the first sexual intercourse of 15.5±1.5 years (13).

Regarding preventive measures, 84.83% of the dental school students considered that condoms are effective protection, as well as 79.5% of the grammar high school students. The effectiveness of condoms in preventing STIs is well defined, but consistent condom usage has remained a challenge. Hirsl-Hecej et al. showed that the acceptance of condom use had slightly but significantly increased in comparison to the wave of 1997, and respondents in a 2001 survey among metropolitan high school students in Croatia were more likely to mention the importance of condom use (18, 20). The increase in condom use could be attributed to the increasingly open and informative media addressing adolescent sexuality. According to our results, 91.1% dental students used condom protection against STIs during their latest sexual intercourse, as well as 71.4% from the grammar high school. The decline in condom use among sexually active students is consistent with the findings of other studies, indicating that it decreases with consolidation of adolescent relationships (13, 18, 19). Drago et al. in their study in Italy showed that students were using contraceptive methods much less than in BH: 37.3% used condoms, 6.5% used contraceptive pills, 2.1% both condom and contraceptive pills, while 41% were not currently using contraceptive methods (13). We also examined the dynamics of multiple sexual partners, and students reported mostly 1 or 2 partners (61.2% vs. 85.7%), but this did not reach statistical significance, which is certainly good because there is a smaller chance of contracting STIs with regular partners. Also having a greater number of sex partners was associated with diagnosis of an STI. In addition, a greater number of concurrent partners had a significant correlation with STIs (21). A few students reported having 3 or more sexual partners, which can be explained by the de-

sire to gain a certain reputation among their colleagues. Unfortunately, the limitations of the questionnaire used in the study preclude us from testing this hypothesis.

The next items in our study showed than in a case of unwanted pregnancy around half of the students from both schools would talk with their parents, furthermore 18.8% of students from the dental school would choose parenthood no matter what, while only 7.0% of students in the grammar high school would choose parenthood no matter what. Abortion is an option for 18.8% of students in the dental school and for 20.9% in the grammar high school. Regarding the knowledge and transmission of HIV by kissing there was no significant difference. According to the data from the Croatian Institute of Public Health (20) in 2013 almost 1,300 girls gave birth between 15 and 19 years of age (3.2% of the total number of births, 10.6 births per 1000 adolescents at the age of 15-19) and about 220 pregnancy terminations were recorded at the request of women up to 19 years of age. These results show how important it is to emphasize the prevention of juvenile pregnancies.

Students from the grammar high school in our study showed significantly better knowledge and attitudes about STIs, which is interesting since they are not undergoing medical education. No similar results from other studies were found for comparison. Hirsl-Hecej et al. reported higher levels of knowledge about HIV, which is important considering that during the observed period there was no systematic sex education in their schools (18).

Limitations of the Study

There are several limitations to this study. This paper observed students from two different high schools in Sarajevo Canton, therefore the results may not be generalizable to other high school populations. As has been seen in other studies (14, 15) young people who are sexually active and those who have had STIs appear to be more knowledgeable than those who are not. This lack of information may disguise other important correlates of young people's STI knowledge.

Conclusion

While the majority of high school students are aware about sexually transmitted infections, their knowledge is inadequate. School-based programs remain the main way by which students learn about STIs. Our study confirmed the necessity for implementation of sexual education programs, perhaps in the final grades of elementary school or in the early ones of high school, aimed at improving knowledge of sexually transmitted infections, and increasing sexual and reproductive health.

What Is Already Known on this Topic

Sexually transmitted infections have an important place as a continuous public health problem, both in the world and in our country. However, despite a comprehensive program of prevention and control, there is still very little progress in this field, especially when it comes to middle-income and developing countries.

What this Study Adds

In our study we emphasized the importance of the defined problem, pointed out the necessity of educational programs especially for adolescents as a significant STI population group.

Authors' Contributions: Conceptions and design VS and AM; Acquisition, analysis and interpretation of data: VS, AM, SS and JA; Drafting the article: SS and JA; Revising it critically for important intellectual content: VS and JA; Approved final version of the manuscript: VA, AM, SS and JA.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Scientific Article
Acta Medica Academica 2019;48(2):159-166
DOI: 10.5644/ama2006-124.254

The Presence of Stress, Burnout Syndrome and the Most Important Causes of Working Stress Among Physicians in Primary Health Care – an Observational Study from Banja Luka, Bosnia and Herzegovina

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Received: 3 August 2018
Accepted: 22 April 2019

Key Words: Stress ▪ Burnout Syndrome ▪ the Main Causes of Stress ▪ Primary Care Doctors.

Objective. To investigate the level and causes of stress and the risk of onset of burnout syndrome among physicians employed at the Primary Health Care Centre, Banja Luka. **Subjects and Methods.** Between March 1, 2018, and May 31, 2018 all physicians from the Primary Health Care Centre, Banja Luka were offered the following questionnaires to fill in: a socio-demographic questionnaire, a questionnaire for self-assessment of the level of stress and the Maslach Burnout Inventory for assessment of the risk of burnout syndrome. **Results.** Out of 211 physicians, 85.8% were female. A high level of stress was found in 77.7% of the subjects. Older doctors had higher levels of emotional exhaustion compared to younger doctors with a shorter length of service (r=0.236, P=0.01). Emotional exhaustion was significantly correlated with a high level of depersonalization, a low level of personal accomplishment and a high level of stress (r=0.380, r=-0.174 and r=0.574, P=0.01, P=0.04 and P<0.01, respectively). Depersonalization correlated with a low level of personal accomplishment and stress (r=-0.347 and r=0.283, P<0.01 and P=0.01, respectively), while the level of personal accomplishment was in a negative correlation with stress (r=-0.281, P=0.01). A high stress level was associated with a high degree of emotional exhaustion (OR 56.543; 95% CI 11.35-213.09; P<0.001) as well as lack of personal accomplishment (OR 0.155; 95% CI 0.04-0.50; P=0.003). **Conclusion.** A high level of stress was associated with older age, female gender, as well as with a high degree of emotional exhaustion and a lack of personal accomplishment. Preventive measures are warranted.

Introduction

Burnout syndrome is chronic working stress that manifests in three dimensions (increased emotional exhaustion, an increased level of depersonalization, and a sense of reduced personal accomplishment). This problem often occurs in persons whose profession demands permanent contact with other persons. Health care work is one of the professions with a high risk for development of burnout syndrome (1). The etiopathogenesis of burnout syndrome is complex, but it is generally believed that prolonged "negative stress" is a key factor in its onset. The individual characteristics of each person and the inability to overcome the stress successfully also have an important role. The

negative feelings of persons with a high level of burnout syndrome are correlated with a loss of sense for professional duties, a loss of capability for self-realization, and a loss of personal perspective. The feeling of the absurdity of existence and the loss of interest in anything happening around them, influence all spheres of the life of a person with burnout syndrome. If the person is in this state for a long period of time, they will lose the ability to enjoy life, and the life quality of these persons is significantly reduced (2).

The medical profession is specific, because doctors make a highly emotional contribution to solving the most subtle physical, psychological and social problems of their patients. A doctor's social contacts are not only towards patients but also towards their co-workers, superiors, their patients' parents and relatives, and others (3).

The following are deemed to be the most common causes of stress at work: interpersonal relationships in the workplace (relationships with patients, colleagues, and supervisors), satisfaction or dissatisfaction with work, possible conflict situations at work, insufficient training for performing tasks at work, overwork, unrealized promotion, etc. (4). The results of the majority of studies conducted show that high levels of stress and burnout syndrome are related to younger age, female sex, negative marital status, long working hours, low job satisfaction, financial worries, sleep deprivation, feelings of responsibility, difficulties in collaborating and other possible factors (5-7).

The aims of this study were to investigate the level of stress and the risk of burnout syndrome onset among physicians employed at the Primary Health Care Centre, Banja Luka, and to identify the most common causes of stress at work.

Subjects and Methods

The research was an observational study, conducted by interviewing doctors at the Primary Health Care Centre, Banja Luka. The research was anonymous and voluntarily, and all subjects were informed about the purpose and importance of the research by the authors. All employed doctors were given the following questionnaires: a socio-demographic questionnaire, a questionnaire for self-assessment of the level of stress (8), a questionnaire about the most frequent causes of stress at work, and the Maslach Burnout Inventory (9, 10). The study was conducted in the period from 1st March to 31st May 2018. Participants had enough time to fill in the questionnaires autonomously during their break at work. After interviewing, all the obtained data were entered into an Excel database, and then statistically processed using the SPSS programme (Statistical Package for Social Science software, version 23.0).

Subjects

The study included 211 doctors, out of a total of 246 employed at the Primary Health Care Centre, Banja Luka (response rate 85.77%). Physicians employed in different departments, with various specialities were interviewed: 127 doctors in family medicine, 22 doctors in emergency care, 4 psychiatrists, 4 epidemiologists, 5 physiatrists, 7 gynaecologists, 13 paediatricians, 5 radiologists, 3 specialists in medical biochemistry and 21 dentists. For the sake of statistical processing, the subjects were divided into three groups in relation to age: those younger than 42 years, those aged from 43 to 51 years, and subjects 52 years and older. In relation to length of service, three groups of subjects were formed: subjects with up to 13 years' service, subjects with from 14 to 23 years' service and subjects with 24 years' service or more.

Questionnaires and Measuring

As research tools, we used a socio-demographic questionnaire, a questionnaire for

self-assessment of the level of stress (8) and the Maslach Burnout Inventory (9, 10). The socio-demographic questionnaire included data about gender, age, work place, length of service, marital status and the number of children in the family. The questionnaire for self-assessment of the level of stress contained ten questions, and included four basic factors of overwork (chronic lack of time, exaggerated responsibility, lack of support and exaggerated self-expectations and expectations from colleagues and others). The subjects could answer the questions as follows: almost always (4), often (3), rarely (2) and almost never (1). The total scores were obtained by adding up points, and the maximum score was 40. Subjects whose total score was between 25 and 40 were deemed to have high levels of stress, while subjects whose total score was less than 25 had stress within the normal range.

The original version of the Maslach Burnout Inventory contains 22 questions to which subjects answer as follows: never (0 scores), a few times a year (1), once a month (2), a few times a month (3), once a week (4), a few times a week (5) and daily (6). All the questions were divided into three subscales that serve as indicators for evaluating the level of emotional exhaustion, depersonalization and personal accomplishment. The first subscale, for measuring emotional exhaustion levels, accentuates exaggerated requests directed towards service providers. The second subscale measures the presence of depersonalization that characterizes a negative relationship between service providers and receivers. The third subscale measures levels of personal accomplishment. Emotional exhaustion is evaluated by the answers to nine questions, and the maximum score is 54 (score <17 indicates low, 18-29 moderate, >30 high emotional exhaustion level). Depersonalization is tested by five questions, and the maximum score is 30 (a score<5 indicates low, 6-11 moderate, and >12 high depersonalization levels), and personal accomplishment is evaluated by answers to eight questions. The maximum score is 48 (a score <33 indicates high, 34-39 moderate, >40 low levels of personal accomplishment).

Ethics Statement

Approval for conducting the study was obtained from the Primary Health Care Centre director and the Ethics Committee of the Primary Health Care Centre, Banja Luka. This research was conducted according to the Helsinki Declaration.

Statistical Analysis

Data obtained in this research were statistically processed using the SPSS programme version 23.0. Descriptive analysis, in the form of frequencies and percentages, was used for sample analysis and analysis of answers to each question individually. Continuous variables between groups were compared by the Student's t test for normally distributed values; otherwise the Mann - Whitney U test was used. The relationship of variables was analysed by Pearson's coefficient of linear correlation. Odds ratios (OR) and 95% confidence intervals (CI) assessing the risk of being less stressed were assessed by logistic regression. The following parameters were evaluated in a multivariable model: age, gender, length of service and number of children in family (model 1) as well as all variables from model 1, plus emotional exhaustion, depersonalization and personal accomplishment in model 2. In the analytical methods applied, the level of significance was $P<0.05$.

Results

The research is an observational study that included 211 physicians employed at the

Primary Health Care Centre, Banja Luka. There were 85.8% female subjects. Considering age and length of service, three groups of subjects, that included an approximately equal number of doctors, were formed respectively. The majority of doctors interviewed were married (77.7%), and the largest number of them had two children in their family (30.3%) (Table 1).

The results of the questionnaire for self-assessment of stress levels showed that the largest number of doctors interviewed 164 (77.7%) met the criteria for a high level of stress. The interviewed doctors answered questions about the most frequent causes of stress at their work places. Analysis of the obtained results showed that the most common causes of stress in the interviewed group of doctors were: administrative burden (43.7%), constant changes to legislation (36.4%), overwork with a large number of patients (36.0%) and health care insurance demands (29.9%).

Table 1. Socio-Demographic Data of Subjects

Variables	N	%
Gender		
Male	30	14.2
Female	181	85.8
Age (years)		
<42	72	34.1
From 43 to 51	65	30.8
52 and more	74	35.1
Years of service		
<13	71	33.6
From 14 to 23	69	32.7
24 and more	71	33.7
Marital status		
Married	164	77.7
Unmarried	27	12.8
Divorced	15	7.1
Widowed	5	2.4
Number of children in family		
No children	37	17.5
One child	64	30.3
Two children	95	45.1
Three children	15	7.1

The results from the Maslach Burnout Inventory showed that 20.9% of subjects had a high level of emotional exhaustion, 43.2% a high level of depersonalization, and 36.9% a low level of personal accomplishment (Table 2).

A significant correlation was found between the level of emotional exhaustion and age using Pearson's coefficient of linear correlation, where older doctors had higher levels of emotional exhaustion compared to younger doctors ($r=0.236$, $P<0.01$, Table 3).

Emotional exhaustion was significantly correlated with a high level of depersonalization, a low level of personal accomplishment and a high level of stress ($r=0.380$, $r=-0.174$ and $r=0.574$, $P=0.01$, $P=0.04$ and $P<0.01$, respectively). Depersonalization correlated with a low level of personal accomplishment and a high level of stress ($r=-0.347$ and $r=0.283$, $P<0.01$ and $P=0.01$, respectively), while the level of personal accomplishment was in a negative correlation with a high level of stress ($r=-0.281$, $P=0.01$).

As mentioned above, 47 out of 211 subjects showed a low level of stress (22.3%). In the multivariable logistic regression analysis

Table 2. The Degree of Burnout Syndrome Risk in All Three Dimensions

Degree of burnout	High		Moderate		Low	
	N	%	N	%	N	%
Emotional exhaustion	44	20.9	55	26.1	112	53.0
Depersonalization	91	43.2	71	33.6	49	23.2
Personal accomplishment	66	31.3	67	31.8	78	36.9

Table 3. Correlations of Age and the Number of Children in the Family with the Level of Stress and Components of Burnout Syndrome*

Variables	Age	Children in the family†	Emotional exhaustion	Depersonalization	Personal accomplishment
Children in the family†	0.330‡	-	-	-	-
Emotional exhaustion	0.236‡	0.115*	-	-	-
Depersonalization	0.007	0.018	0.380‡	-	-
Personal accomplishment	0.053	0.013	-0.174§	-0.347‡	-
Stress	0.093	0.035	0.574‡	0.283‡	-0.281‡

*Pearson's coefficient of linear correlation; †Number of children in the family; ‡Correlation is significant at the 0.05 level (2-tailed); §Correlation is significant at the 0.01 level (2-tailed).

Table 4a. Model 1: Multivariable Logistic Regression for Associated Factors of Being "Less Stressed"

Variables	B*	S.E.	Wald	df	OR	95% CI	P-value†
Age	-0.082	0.020	6.360	1	0.921	0.87-0.96	0.023
Gender	1.000	0.420	5.669	1	2.718	1.14-5.95	0.029
Number of children in family	0.021	0.209	0.010	1	1.021	0.74-1.72	0.821

† Statistically significant difference at P<0.05.

Table 4b. Model 2: Multivariable Logistic Regression for Associated Factors of Being "Less Stressed"

Variables	B*	S.E.	Wald	df	OR	95% CI	P-value†
Age	-0.134	0.027	5.990	1	0.874	0.81-0.95	0.003
Gender	1.363	0.570	4.847	1	3.907	1.21-13.74	0.028
Number of children in family	0.075	0.016	0.080	1	1.078	0.64-1.82	0.778
Emotional exhaustion	4.035	0.336	34.841	1	56.543	11.35-213.09	<0.001
Depersonalization	0.771	0.145	0.257	1	2.162	0.61-10.22	0.612
Personal accomplishment	-1.864	0.613	6.981	1	0.155	0.04-0.50	0.003

†Statistically significant difference at P<0.05.

(Table 4a and Table 4b), several factors were independently associated with low levels of stress. Both models showed that younger age and male gender were predictors of "being less stressed". Furthermore, high stress level was associated with high degree of emotional exhaustion (OR 56.543; 95% CI 11.35-213.09; P<0.001) and lack of personal accomplishment (OR 0.155; 95% CI 0.04-0.50; P=0.003).

Discussion

The results of our study showed a high level of stress and burnout syndrome among the doctors interviewed. The most common causes of stress analysed in our study were administrative burden, constant changes of legislation, overwork with a large number of patients and health care insurance demands. The results of other studies also showed a high level of stress in interviewed doctors. A study conducted in Ireland (11) showed that 37% of interns met the criteria for psychological distress. A large percentage (55.4%) of the subjects recruited into this study had high levels of emotional exhaustion, 51.5% had a high level of depersonalization, and 41.6% had a low level of personal accomplishment. The subjects in this study had lower stress levels, but a higher risk for de-

velopment of burnout syndrome than the subjects in our study.

A study conducted in the UK showed that 56.5% oto-rhino-laryngologists were at high risk of developing stress and psychological morbidity, and 28.9% had a high risk for burnout syndrome (12). A study conducted in Germany among 453 hospital residents in medical training, working in 6 different medical specialties, showed that up to 17% of the physicians reported high levels of occupational distress and 9% reported high levels of depressive symptoms (13). In these studies, the subjects reported a high level of stress in a great percentage, but their level of stress was lower than in the subjects in our study.

Although occupational stress is present among doctors worldwide, the results of our study presented significantly higher levels of occupational stress than in other countries. In our conditions, the poor social and economic status of physicians certainly contributes to the presence of a high level of stress. The inadequate income of physicians in relation to their invested effort may also be considered an additional cause of stress. Burnout syndrome is present among physicians all over the world. Physician burn out in the United States has reached epidemic proportions and is rising rapidly, although burnout in other occupations is stable (14).

Large scale research into the prevalence of burnout syndrome at work in family physicians in 12 European countries (Bulgaria, Croatia, France, Greece, Hungary, Italy, Poland, Portugal, Sweden, Spain, Great Britain and Turkey) revealed that 43% examinees had high levels of emotional exhaustion, 35% high levels of depersonalization, 32% low levels of personal satisfaction (15). Our results from the Primary Health Centre Banja Luka have shown that 20.9% of doctors suffer from a high level of emotional exhaustion, 43.2% a high level of depersonalization, and 36.9% a low level of personal accomplishment, which is in accordance with other countries. Research conducted among 123 Canadian family physicians (16) showed that 42.5% physicians have high levels of stress, while research conducted in Italy showed that 35.8% of doctors interviewed had a high level of stress (17). Interestingly enough, our analysis revealed 77.7% of doctors met the criteria for high levels of stress.

Considering the influence of age as a risk factor for burnout syndrome and high levels of stress, the research does not give a single answer. Some authors say that younger age is a risk factor for the onset of burnout syndrome (2) and as doctors gain more experience and grow older, the level of stress decreases and therefore the risk of burnout syndrome itself decreases. Other authors consider that overworked persons and persons exposed to frequent interpersonal conflicts over a long period of time have emotional exhaustion symptoms to a large extent (18). Long-term constant contact with patients and exposure to other risk factors from the working environment may increase the level of stress and the risk of syndrome onset (19). The results of a study conducted among family physicians in the Republika Srpska showed that older age is an important risk factor for a high level of stress and the onset of emotional exhaustion i.e. burnout syndrome (20). Furthermore, we identified female gender, emotional exhaustion and a lack of personal accomplishment as important predictors of a high level of stress.

Burnout syndrome is a common psychological state that may affect human healthcare providers due to their prolonged exposure to job stressors (21). The rates of burnout symptoms can be associated with adverse effects on patients, the healthcare workforce, costs and physicians' health. This problem results in negative impacts on physicians, patients and the healthcare organization and system (22). Stress related anxiety

and burnout may result in increased absenteeism and disability, decreased patient satisfaction and increased rates of medical error (23). These are the main reasons for considering preventive measures for burnout. Numerous authors have investigated strategies for prevention and treatment of persons affected by occupational stress and burnout. The research results are diverse, but most authors show that an individual approach has the best results. The interventions for prevention or alleviation of burnout that are mostly recommended are: shortening of working hours, additional continuous training, stress-management, self-care, communication skills training, etc. (24, 25).

Limitations of the Study

The study included a relatively small number of subjects and was conducted in only one Primary Health Care Centre. To obtain better information about the presence of burnout syndrome throughout the country, further research should include more health care institutions and more subjects.

Conclusion

The research results showed high levels of stress and a high risk for burnout syndrome in doctors in primary health care. It appears that older doctors who have a longer length of service have significantly higher levels of emotional exhaustion compared to younger doctors with a shorter length of service. Observing the results of our research, a high level of stress was associated with older age, female gender, longer length of service, as well as with a high degree of emotional exhaustion and a lack of personal accomplishment. In order to prevent the onset of this syndrome, it is necessary to establish a balance between professional and family obligations, and the ability to deal with stressful situations is of special importance in prevention of burnout syndrome.

What Is Already Known on this Topic

Burnout syndrome is present in different occupations, especially in persons that work with other people. Healthcare work is one of the professions that carries the greatest risk of burnout syndrome. Numerous studies worldwide have shown the high prevalence of burnout syndrome among different profiles of healthcare workers.

What this Study Adds

A small amount of research on this topic has been conducted in Bosnia and Herzegovina, and we hope that this research will contribute to a better insight into the problem of the prevalence of burnout syndrome in primary care physicians in our country.

Acknowledgements: We would like to thank all the physicians employed at the Primary Health Care Centre, Banja Luka, that took part in this research by filling in the questionnaires, and enabling us to conduct this study.

Authors' Contributions: Conception and design: KS, VP and BM; Acquisition, analysis and interpretation of data: KS, VP, BM and BS; Drafting the article: KS, VP, BM and BS; Revising it critically for important intellectual content: KS, VP, BM and BS; Approved final version of the manuscript: KS, VP, BM and BS.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Acta Medica Academica 2019;48(2):167-176
DOI: 10.5644/ama2006-124.255

Screening for Carpal Tunnel Syndrome in Patients on Chronic Hemodialysis

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Received: 29 March 2018
Accepted: 9 March 2019

Key Words: Carpal Tunnel Syndrome ▪ Hemodialysis ▪ Amyloidosis ▪ Screening.

Objectives. To determine the prevalence of carpal tunnel syndrome risk in patients on chronic hemodialysis (HD) using Levine questionnaire for assessment of carpal tunnel syndrome - related symptoms severity and patients' functional status and to examine the relation of the determined risk with the participants' demographic, anthropometric and laboratory data. **Participants and Methods**. This cross-sectional study included 78 chronic HD patients at Department for Nephrology in University Hospital Osijek. All participants filled out the Levine questionnaire to examine the severity of carpal tunnel syndrome - related symptoms and their functional state. The participants' demographic, anthropometric and laboratory data were taken from the medical records and statistically analyzed by SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, SAD). **Results.** Risk for carpal tunnel syndrome was found in 38.5% of the participants. No significant differences between patients with and without the risk were found in sex distribution, underlying kidney disease or vascular access. Patients at risk were older (P=0.044) and had higher body mass index (BMI), (t-test, P=0.019). Participants' age, BMI and predialytic serum urea concentration were independent predictors for carpal tunnel syndrome risk (P=0.033). **Conclusion**. The prevalence of risk for carpal tunnel syndrome among patients on chronic HD was found in more than one third of patients. Older age, higher BMI and higher predialytic serum urea concentration bear a higher risk. Electromyoneurography is thus often indicated in this population to confirm the diagnosis for consecutive surgical treatment.

Introduction

Amyloidosis is a term that refers to a group of diseases characterized by extracellular amyloid accumulation in different tissues and organs, which leads to morphological and functional disturbances. Thirty different types of amyloidosis have been discovered, based on the precursor protein that undergoes a pathological change in conformation and is deposited extracellularly. Suspicion of amyloidosis should be based on clinical findings, while a definitive diagnosis is made based on the findings of tissue or organ biopsy (1, 2). In hemodialysis-associated amyloidosis, the precursor protein is Aβ2-M (β2-microglobulin), which develops as a complication of end-stage kidney disease and long-term hemodialysis. It is a polypeptide that consists of 99 aminoacids which plays a vital role in the immune system because it is a part of the β-chain of type 1 MHC (major histocompatibility complex) molecule (3).

Most of β2-microglobulin is normally excreted through the kidneys (4, 5). Insufficient filtration and catabolism, continuous production and intradialytic production due to membrane incompatibility all lead to an increase in its serum concentration (6). Normal values of serum β2-microglobulin range between 1 and 3 μg/mL, while those values can exceed 100 μg/mL in patients with end-stage kidney disease and chronic hemodialysis patients (7, 8). The pathogenesis of amyloidosis, time needed to develop and its severity is multifactorial and is connected with the duration of chronic kidney disease, duration of hemodialysis treatment, patient age at the start of hemodialysis treatment, bioincompatibility of hemodialysis membranes and many other clinical features (9, 10). Research has shown that deposition of Aβ2-M fibers significantly preceeds the onset of symptoms and clinical findings (10). Disturbances that develop as a consequence of Aβ2-M fiber deposition in the skeletal system are numerous, but the most common one is carpal tunnel syndrome. Hemodialysis-related amyloidosis is one of the most damaging complications of long-term hemodialysis treatment, affecting daily activities and maintenance of quality of life (10).

The most common symptoms of carpal tunnel syndrome are wrist pain, unpleasant tingling, hypoesthesia on the distal end of the median nerve's sensory innervation and a reduction of grip strength. Long-term compression of the median nerve can lead to impaired functionality of the hand as well as hypotrophy, even atrophy of the thenar muscles (11, 12). The prevalence of carpal tunnel syndrome caused by hemodialysis-related amyloidosis correlates with the duration of hemodialysis treatment and, according to the literature, it affects 32-50% patients who have been on hemodialysis for over 10 years. That number jumps to over 80% in patients who have been on hemodialysis for over 30 years. There are certain factors that contribute to the development of carpal tunnel syndrome in patients on chronic hemodialysis other than Aβ2-M fiber deposits, e.g. venous hypertension distally from the vascular access site, increased volume of the extracellular space, thickening of the transverse carpal ligament, etc. (13-16). A definitive diagnosis is made using electrodiagnostic modalities, which determine the median nerve's quality of motor and sensory impulse generation and transfer (12, 13). Treatment of carpal tunnel syndrome can be conservative and surgical. Conservative treatment is recommended for patients with a mild form of the syndrome. For severe cases, surgical decompression of the carpal tunnel is the treatment of choice (13).

Due to the high prevalence of carpal tunnel syndrome among patients on chronic hemodialysis, the aim of this research was to determine the prevalence of carpal tunnel syndrome risk in patients on chronic hemodialysis treated at University Hospital Osijek, using the Levine questionnaire for assessment of carpal tunnel syndrome – related symptoms severity and patients' functional status and to examine the relation of the determined risk with the patients' demographic, anthropometric and laboratory data.

Participants and Methods

This cross-sectional study was conducted at the Department for Nephrology in University Hospital Osijek with the approval of the Department Head and the Ethical Committee for Research at the Faculty of Medicine of Josip Juraj Strossmayer University of Osijek. Of 89 patients with end-stage renal disease on chronic hemodialysis that were at the Department during the research period, 78 of them accepted to participate and were not in an altered state of consciousness, while 11 patients either rejected to participate or were in an altered state of consciousness. All

participants were patients on a fixed regime of hemodialysis, three times a week for an average of four hours. All participants filled out the Levine questionnaire to examine the severity of carpal tunnel syndrome – related symptoms (pain, paresthesias, loss of sensation, weakness, nocturnal symptoms) and their functional status (17). The questionnaire consists of two parts. The symptom severity is determined through eleven multiple choice questions which reflect severity - the first choice being the absence of a symptom, while the fifth, final choice being extreme severity. Functional status is determined by adding up participants' answers on a scale from 1 to 5. Participants answer questions about the difficulty of performing eight specific daily activities (writing, buttoning of clothes, holding a book while reading, gripping of a telephone handle, opening of jars, household chores, carrying of grocery bags, bathing and dressing). Answers range from no difficulty to complete inability to perform a certain activity due to hand/wrist symptoms. All the answers are added up and the final result is a number ranging from a minimum of 19 points to a maximum of 95 points (18).

Demographic (age, sex, year of starting hemodialysis, age at start of hemodialysis, duration of hemodialysis, underlying kidney disease, vascular access type), anthropometric (height, weight, BMI) and laboratory (serum concentration of creatinine, urea, hemoglobin, electrolytes before the start of hemodialysis) data were taken from the medical records of the Department for Nephrology in University Hospital Osijek.

Analysis of laboratory markers was performed at the Department for Clinical Laboratory Diagnostics in University Hospital Osijek. The reference ranges of markers apply for adults. Serum concentrations of the following markers were analyzed:

- Kidney function markers – serum concentration of creatinine in the reference range for women (42-80 μmol/L) and men (49-97 μmol/L), as well as serum concentration of urea in the reference range for both sexes (2.8-8.3 mmol/L);
- Hemoglobin in a reference range for women (119-157 g/L) and men (138-175 g/L);
- Electrolytes – sodium (137-146 mmol/L), potassium (3.9-5.1 mmol/L), calcium (2.14-2.53 mmol/L) and phosphorus (0.79-1.42 mmol/L); reference ranges apply to both sexes.

Quality of hemodialysis was determined using Kt/V, which shows elimination of urea which each hemodialysis. Kt/V by hemodialysis is calculated by using predialytic and postdialytic concentration of blood urea using formulas based on the solution to the Gotch-Sargent urea kinetic model for a single hemodialysis. The Jindal formula was used for the purposes of this research:

$$\frac{Kt}{V} = \left(\frac{urea\,1 - urea\,2}{urea1}\right) \times 4 - 1.2,$$

where urea 1 is the predialytic serum urea concentration and urea 2 is the postdialytic serum urea concentration (19-22).

Ethics Statement

The research followed the ethical guidelines and standards set by the Helsinki declaration and the Croatian Health Care Act. The ethics committee of the Faculty of Medicine at the Josip Juraj Strossmayer University of Osijek gave approval to conduct the research.

Statistical Analysis

Categorical data were descriptively described as absolute and relative frequencies, while numerical data were described as mean and standard deviations, in cases of normal data distribution, while in cases

showing dispersed data distribution, data were described as medians and interquartile ranges. Tests used for difference between groups with and without risk for carpal tunnel syndrome were chi-square test for nominal variables and t-test (for normally distributed variables) and Mann Whitney U test (for dispersed data distribution) for numeric variables. Correlation was determined using the Spearman coefficient of correlation rho. The ability to predict the risk of carpal tunnel syndrome was determined using the multivariate regression (Hosmer-Lemeshow goodness-of-fit) test. Covariates were chosen as characteristics with P<0.100 in the univariate analysis. All P values were two-sided. Statistical significance was at α=0.05. Statistical analysis was performed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA) (23).

Results

Seventy-eight participants on chronic hemodialysis were included in the research, fifty men and twenty-eight women. Median age of participants during the research period was 67 (57-74), while median duration of hemodialysis was 3 years (2-6).

The screening Levine questionnaire determined thirty participants (38.5%) had symptoms that classified them as at risk for carpal tunnel syndrome. Table 1 shows the results of the Levine questionnaire among symptomatic participants and the affected hand. Results of the questionnaire were described as overall score, average symptom severity score and average functional state score.

Table 1. Characteristics of Participants at Risk for Carpal Tunnel Syndrome

Characteristic	Value
Affected hand (N=30)	
Right (N; %)	8 (26.7)
Left (N; %)	8 (26.7)
Both (N; %)	14 (46.7)
Levine questionnaire, total score	31 (24 - 40)*
Average symptom severity score†	1.675 (1.33 – 2.49)*
Average functional status score†	1.12 (1 – 1.75)*

*Median (interquartile range); †Score ranging from 1 to 5.

The most common underlying kidney disease which led to end-stage kidney disease was diabetic nephropathy (N=21), while other common underlying conditions included glomerulonephritides (N=20), interstitial nephritides (N=16) and hypertension (N=14). A statistically significant difference in the distribution of underlying kidney disease among participants with and without carpal tunnel syndrome symptoms was not found (Table 2).

Table 2. Distribution of Underlying Kidney Disease between Participants with and without Risk for Carpal Tunnel Syndrome

Underlying kidney Disease (N; %)	Participants			χ^2	P*
	All (N=78)	At risk (N=30)	Without risk (N=48)		
Diabetic nephropathy	21 (26.9)	10 (33.3)	11 (22.9)	6.73 df = 5†	0.242
Glomerulonephritis	20 (25.6)	5 (16.7)	15 (31.3)		
Interstitial nephritis	16 (20.5)	8 (26.7)	8 (16.7)		
Hypertension	14 (17.9)	5 (16.7)	9 (18.7)		
Polycystic kidney disease	4 (5.1)	0	4 (8.3)		
Other	3 (3.8)	2 (6.7)	1 (2.1)		

*χ^2 test; †Degrees of freedom.

Table 3 (3a and 3b) shows differences in participant characteristics between those with and without symptoms of carpal tunnel syndrome. There were no significant differences in sex distribution between the two groups. There was a larger number of participants who had an arteriovenous fistula as vascular access in the group of participants experiencing symptoms of carpal tunnel syndrome. Participants experiencing symptoms of carpal tunnel syndrome were older (Mann Whitney U test, P=0.044) and heavier (t test, P=0.019) than those who did not experience symptoms. Predialytic serum urea concentration was higher in participants experiencing symptoms of carpal tunnel syndrome, but the difference did not reach statistical significance. There were no statistically significant differences between the two groups in the remaining analyzed characteristics (sex, age at start of hemodialysis treatment, time since starting hemodialysis treatment, vascular access, quality of hemodialysis, predialytic serum concentrations of creatinin, sodium, potassium, calcium and phosophorus).

Table 3a. Differences in Characteristics between Participants with and without Risk for Carpal Tunnel Syndrome

Characteristic	All participants (N=78)	Participants at risk (N=30)	Participants without risk (N=48)	Test value	P
Sex [N (%]; M:F	50 (64.1): 28 (35.9)	18 (60): 12 (40)	32 (66.7): 16 (33.3)	$\chi^2 = 0.36$ df =1¶	0.550*
Age in 2016 (year)	67 (57–74)†	70 (62–81)†	65 (55–73)†	z= - 2.01	0.044‡
Body mass index (kg/m^2)	26.1 (4,7)§	27.6 (4,6)§	25.1 (4,5)§	t=2.4	0.019‖
Age at start of hemodialysis (year)	61.5 (53–70)†	61 (55–75)†	63,5 (52–68)†	z= - 1.35	0.177‡
Time since starting hemodialysis (year)	3 (2–6)†	4 (2–7)†	3 (1–5)†	z= - 1.27	0.206‡
Vascular access [N (%)], rteriovenous fistula:catheter	42 (53.8): 36 (46.2)	19 (63.3):11 (36.7)	23 (47.9):25 (52.1)	χ^2=1.77 df=1	0.184*
Vascular access side [N (%)], right:left	41 (52.5): 37 (47.5)	16 (53.3):14 (46.7)	25 (52.1):23 (47.9)	χ^2=0.01 df=1	0.914*
Hemoglobin (g/l)	101.6 (12.8)§	102.2 (10.3)§	101.2 (14.3)§	t=0.34	0.735‖
Creatinine (μmol/l)	690.8 (192.2)§	687.4 (160.3)§	692.9 (211.3)§	t= - 0.12	0.903‖
Urea (mmol/l)	21.57 (6.18)§	23 (5.45)§	20.7 (6.45)§	t=1.71	0.091‖

*χ^2 test; †Median (Interquartile range); ‡Mann-Whitney U test; §$\bar{x} \pm$ SD; ‖t-test; ¶Degrees of freedom.

Table 3b. Differences in Characteristics between Participants with and Without Risk of Carpal Tunnel Syndrome

Characteristic	Participants			t*	P
	All (N=78); $\bar{x} \pm$SD	At risk (N=30); $\bar{x} \pm$SD	Without risk (N=48); $\bar{x} \pm$SD		
Hemodialysis quality (Kt/V)	1.59 (0.28)	1.57 (0.26)	1.61 (0.3)	- 0.58	0.566
Sodium (mmol/l)	137 (2.5)	137.3 (2.3)	136.8 (2.7)	0.78	0.435
Potassium (mmol/l)	4.84 (0.83)	4.84 (1.07)	4.84 (0.64)	0.02	0.984
Calcium (mmol/l)	2.17 (0.2)	2.17 (0.2)	2.16 (0.2)	0.37	0.713
Phosphorus (mmol/l)	1.68 (0.51)	1.72 (0.48)	1.65 (0.53)	0.54	0.590

*t-test.

Table 4. Predictors of High Scores in the Levine Questionnaire, Multivariate Regression (Hosmer-Lemeshow Goodness-of-Fit), Test (N=78)

Characteristic	Exp (B)	95% CI	P
Age in 2016 (years)	1.069	1.017 – 1.123	0.008
Body mass index (kg/m^2)	1.157	1.031 – 1.3	0.013
Predialytic urea (mmol/l)	1.108	1.007 – 1.219	0.035

Exp(B)=Odds ratio; CI=Confidence interval.

Table 5. Spearman's Correlation Coefficient (P) Between Levine Questionnaire Results and Characteristics of Participants with Carpal Tunnel Syndrome Related Symptoms (N=30)

Characteristic	Total score	Average score			
		Symptom severity		Functional status	
	ρ	P	ρ	P	ρ	P
Age in 2016 (years)	- 0.04	0.850	- 0.03	0.882	0.11	0.544
Body mass index (kg/m^2)	0.25	0.190	0.30	0.103	0.12	0.529
Time since starting hemodialysis*	0.14	0.455	0.01	0.606	0.27	0.146
Age at start of hemodialysis (years)	- 0.09	0.642	- 0.07	0.714	0.03	0.846
Hemodialysis quality (Kt/V)	0.28	0.136	0.29	0.118	0.29	0.116
Creatinine (μmol/L)	- 0.24	0.195	- 0.19	0.311	- 0.30	0.109
Urea (mmol/L)	- 0.28	0.124	- 0.26	0.163	- 0.42	0.022
Hemoglobin (g/L)	- 0.17	0.363	- 0.13	0.463	- 0.22	0.253
Sodium (mmol/L)	0.32	0.087	0.20	0.292	0.48	0.007
Potassium (mmol/L)	- 0.05	0.785	- 0.06	0.754	- 0.12	0.529
Calcium (mmol/L)	0.09	0.641	0.12	0.527	0.08	0.678
Phosphorus (mmol/L)	- 0.22	0.224	- 0.20	0.286	- 0.31	0.093

*Years.

Multivariate regression (Hosmer-Lemeshow goodness-of-fit) test showed that patient age, BMI and predialytic serum urea concentration were independent risk factors for development of carpal tunnel syndrome symptoms according to the screening Levine questionnaire (χ^2= 16.75, P=0.033). With each year of age, risk for development of carpal tunnel syndrome symptoms grew by 6.9%, each kg of body mass increased that risk for 15.7%, while raising the predialytic serum urea concentration raised that risk for 10.8% for each mmol/L (Table 4).

We analyzed the correlations between participant characteristics in the group experiencing carpal tunnel syndrome symptoms with all three Levine questionnaire results – total score, average symptom severity score and average functional status score (Table 5). Among participants experiencing carpal tunnel syndrome symptoms, average functional status score in the Levine questionnaire was higher, the higher the predialytic serum sodium concentration (Spearman correlation coefficient ρ=0.48, P=0.007) and the lower the predialytic serum urea concentration (Spearman correlation coefficient ρ=-0.42, P=0.022). There were no statistically significant differences between the total Levine questionnaire score and analyzed characteristics, as well as between average symptom severity score and analyzed characteristics.

Discussion

Risk for carpal tunnel syndrome according to the screening Levine questionnaire was found in 38.5% of participants, which represents a considerable prevalence. Carpal tunnel syndrome in these patients differs from the one affecting the general population pathogenetically. That, in addition to the fact that carpal tunnel syndrome is the most common complication of hemodialysis-related amyloidosis (5, 24) is why it should be given special attention. Furthermore, many studies have shown that carpal tunnel syndrome prevalence is two times higher than its prevalence in the general population (13, 25, 26).

One of the key risk factors for developing carpal tunnel syndrome in patients on chronic hemodialysis is duration of hemodialysis. A statistically significant link between duration of hemodialysis and risk for development of carpal tunnel syndrome was not found in this research, but the explanation for that finding can potentially be found in the duration of hemodialysis itself – it was relatively short, with the median duration of hemodialysis being 3 years and the interquartile range between 2 and 6 years. Harris and Brown stated that between 32% and 50% of patients on chronic hemodialysis for longer than 10 years are affected by carpal tunnel syndrome, while Otsubo et al. proved in their research that this prevalence jumps up to 80% in patients who have been on chronic hemodialysis for 30 years or even longer (24, 25).

Sixteen (53.3%) of participants who experienced symptoms of carpal tunnel had unilateral symptoms, while 14 (46.6%) of them had bilateral symptoms. The lower average functional status score suggests that participants in the group at risk, despite having symptoms related to carpal tunnel syndrome, did not experience a change in quality of life and an effect on performing daily activities. Among all participants, diabetic nephropahy was the most common underlying kidney disease. The fact that there were no statistically significant links between the risk for carpal tunnel syndrome and diabetic nephropathy suggests that the Levine questionnaire was specific enough not to mix and/or overlap with similar symptoms that participants might be experiencing due to diabetes.

Participants experiencing symptoms of carpal tunnel syndrome were older, P=0.044, and had a higher BMI, P=0.019. Furthermore, while it did not reach statistical significance, predialytic urea concentration was also higher in this group. This was an interesting finding, considering that there were no differences between the two groups in quality of hemodialysis, P=0.566, which was determined using the Kt/V formula, which, in turn, is based on predialytic and postdialytic serum urea concentration (19). Therefore, physiological aging processes, pathological effects of obesity and metabolic syndrome, as well as increased serum urea concetration all have a negative effect on patients on chronic hemodialysis and might contribute to the development of carpal tunnel syndrome as the most common complication of hemodialysis-related amyloidosis. Those results were somewhat expected because other studies have also shown that increased age (26) and a higher BMI (27, 28) carry a higher risk for development of carpal tunnel syndrome. Using the multivariate regression analysis, we showed that the risk for development of carpal tunnel syndrome symptoms increases by 6.9% with each year of life, by 15.7% for every kilogram per square meter and by 10.8% for every mmol/L of predialytic serum urea concentration increase. Using this method, we confirmed the independent influence of the aforementioned risk factors.

Participants in the group that was at risk for carpal tunnel syndrome started hemodialysis treatment earlier in life and have been on hemodialysis longer than participants who did not experience carpal tunnel

syndrome related symptoms. This was another expected result because it reflects the development of clinical symptoms among patients on chronic hemodialysis – it takes a certain period of time for the amlyoid, Aβ2-M in this case, to be deposited in tissues (tissue surrounding the carpal tunnel in this case) in quantities that are large enough to cause manifestations (10).

When examining the differences in vascular access between the two groups of participants, the group at risk for carpal tunnel syndrome had a larger number of arteriovenous fistulas compared to the group without any symptoms of carpal tunnel syndrome. However, there were no statistically significant differences between the two groups based on the vascular access for hemodialysis and its positioning (left/right). Furthermore, there were no statistically significant links between the hand affected by carpal tunnel syndrome symptoms and the hand with the vascular access in the group, which was a useful piece of information because it eliminated the possibility that the cause of existing symptoms (pain, numbness, tingling etc.) screened for by the Levine questionnaire is not carpal tunnel syndrome, but complications of arteriovenous fistulas of catheters (e.g. arterial steal syndrome, venous hypertension) (15, 16).

The only statistically significant correlation found between demographic, anthropometric or laboratory data and test scores was between the average functional status score and predialytic serum urea concentration. This result was somewhat surprising because it showed that the average functional status score was higher, the lower the predialytic serum urea concentration. There is no simple explanation for this finding – the expected correlation would have been opposite, with average functional status score rising with the elevation of predialytic serum urea concentration. It is possible that urea has a two-sided effect, or in other words, that it increases the risk up to a certain point and that in participants who are already symptomatic and at risk for carpal tunnel syndrome it perhaps reflects their nutritional state (29).

Further diagnostic evaluation is needed to confirm the diagnosis, which includes measuring the serum levels of β2-microglobulin and, as a final confirmatory diagnostic method, electromioneurographic conduction study of the median nerve. Since carpal tunnel decompression, as therapy of choice for this disease, is not a complicated or extensive procedure, confirming the diagnosis of carpal tunnel syndrome caused by hemodialysis-associated amyloidosis would remove a source of pain and limitation in daily activities for many patients on chronic hemodialysis.

Limitations of the Study

Our study looked at the prevalence of carpal tunnel syndrome in chronic hemodialysis patients. However, due to the fact that the research was conducted in a single institution, the sample size was not large. Our hope is to expand the research to other institutions providing hemodialysis treatments to get a even more exact prevalence of carpal tunnel syndrome risk. Carpal tunnel syndrome can not be diagnosed based on a questionnaire alone, as was stated in the introduction, which limited our study due to not having information about symptoms or definitive diagnoses based on neurological examinations. Additionally, since this was a cross-sectional study, we could not get longitudinal results that would show questionnaire score progression over the course of a longer period.

Conclusion

Based on the results of our research, we can conclude that the prevalence of patients on

chronic hemodialysis who are at risk for carpal tunnel syndrome is quite high (38.5%) and that old age, high BMI and high predialytic serum urea concentration carry a higher risk for development of carpal tunnel syndrome symptoms. Furthermore, risk for carpal tunnel syndrome was more prevalent in participants who were on a regime of chronic hemodialysis longer and started hemodialysis earlier in life, while it is not affected by the underlying kidney disease. The presence of symptoms examined by the Levine questionnaire was not connected with the ipsilateral site of vascular access. Predialytic serum potassium concentration has a positive, while predialytic serum urea concentration has a negative correlation with the functional status of participants affected by symptoms examined by the questionnaire. Because of such high prevalence of at risk patients, further diagnostic evaluation is indicated to confirm the diagnosis of carpal tunnel syndrome and treat it with surgical decompression.

What Is Already Known on this Topic

Carpal tunnel syndrome is one of the most common complications of hemodialysis-related amyloidosis. Hemodialysis is an effective treatment option for end-stage kidney disease and with a large number of patients on a chronic regime, progressively worsening complications are common and should be addressed and treated.

What this Study Adds

This study sheds light on the prevalence of symptoms of carpal tunnel syndrome among chronic hemodialysis patients. With a third of patients showing symptoms, further diagnostic testing (laboratory measurement of β2-microglobulin and electromioneurography) should be done to confirm the diagnosis and, if necessary, surgical resection performed to alleviate symptoms.

Acknowledgments: This research paper is based on the final graduation paper of the first author, which was presented at the Faculty of Medicine, Josip Juraj Strossmayer University of Osijek in June 2016.

Authors' Contributions: Conception and design: LZ; Acquistion, analysis and interpretation of data: MK and LZ; Drafting the article: MK; Revising it critically for important intellectual content: LZ; Approved final version of the manuscript: MK and LZ.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Original Article
Acta Medica Academica 2019;48(2):177-182
DOI: 10.5644/ama2006-124.256

Retrospective Evaluation of the Diagnostic Accuracy of the Modified Alvarado Scoring System (MASS) in a Croatian Hospital

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Received: 10 December 2018
Accepted: 26 April 2019

Key Words: Acute Appendicitis ▪ Diagnostic Accuracy ▪ Modified Alvarado Scoring System (MASS).

Objective. Diagnosing acute appendicitis (AA) is challenging and this has stimulated surgeons to develop scoring systems that could potentially decrease the rate of misdiagnosis in patients with suspected appendicitis. One of the most widely used today is the Modified Alvarado scoring system (MASS), however its sensitivity and specificity varies a great deal between studies. As a result, we wanted to assess the diagnostic accuracy of MASS retrospectively at our institution to achieve the highest possible value of sensitivity and decrease the number of false negative patients. **Material and Methods.** We retrospectively calculated MASS for all subsequent patients who had undergone an appendectomy at our institution between July 2015 and March 2017. **Results.** In 118 out of 146 operated patients, AA was confirmed intraoperatively. There was a statistically significant difference between the average MASS score in the positive and negative appendectomy groups (6 v. 4, respectively, P<0.001), with a significantly higher number of females among the negative appendectomies (P<0.001). When lowering the cut-off to a value as low as ≥3, the sensitivity of the MASS score increased to 97.45% (95% CI: 92.7 – 99.5), thus obtaining a very low false negative rate of merely 2.55%. **Conclusion.** This retrospective diagnostic accuracy study confirmed the higher average MASS score in the group of patients with confirmed AA diagnosis. A MASS score above the proposed low cut-off value (≥3) can be a useful tool to help surgeons ruling in patients with AA in order to reduce the risk of missing diagnosis.

Introduction

Acute appendicitis (AA) represents the most common indication for emergency abdominal surgery (1). The lifetime risk of developing appendicitis is approximately 7%. When diagnosed early and treated on time, morbidity from acute appendicitis is very low. On the other hand, unrecognized appendicitis with delayed surgical intervention can lead to major complications or even death (2, 3). This is the reason why surgeons usually have a defensive approach when encountering patients with suspected appendicitis, which results in relatively high rate of negative appendectomies (8-28%) (4-6).

Diagnosing appendicitis is not always simple. The typical clinical presentation with migration of pain to the lower right quadrant of the abdomen or localized peritonitis is only found in less than half of the patients with acute appendicitis (7, 8). Besides el-

evated inflammatory parameters, such as leukocytes and CRP, no specific laboratory marker for appendicitis exists yet. An abdominal CT-scan recognizes acute appendicitis with high accuracy, but the high dosage of harmful radiation makes it absolutely unacceptable for routine use in patients suspected of having appendicitis, especially considering the high rate in the younger female population (9, 10).

All these issues have encouraged surgeons to develop a scoring system that would potentially decrease the rate of misdiagnosis in patients with suspected appendicitis. Although a variety of different scoring systems for acute appendicitis have been proposed, the most widely used today is the Alvarado scoring system (11). It was first devised in 1986 for pregnant women, and later it was also used in the general population. This scoring system, with a maximum total score of ten, is based on six clinical and two laboratory parameters, where leukocytosis and right iliac fossa tenderness are considered to be the most important factors, and are therefore assigned two points (Table 1). In many medical centers differential blood count is not part of routine laboratory investigations for patients with abdominal pain. For this reason Kalan et al. proposed a modified Alvarado scoring system (MASS) that uses the same value categories but without the left shift of leukocytes, so the scores range from 0 to 9 (Table 1) (12).

Considering the fact that the sensitivity and specificity of MASS for diagnosing appendicitis at the recommended cut off point of ≥7 varies a great deal between studies (13-18), the present situation at our University Hospital does not include an initial evaluation of patients with suspected acute appendicitis using any of the proposed scoring systems. Our hypothesis includes reversing the original paradigm of the Alvarado scoring system – we propose applying the Alvarado scoring system to evaluate patients who have already been designated to undergo surgery, and so assist surgeons in supporting their initial decision.

Therefore we aimed retrospectively to assess the diagnostic accuracy of the modified Alvarado score in operated patients. Additionally, we wanted to propose a different MASS cut-off point which would be a useful tool to decrease the number of false negatives to the lowest possible number.

Table 1. Alvarado Score and Modified Alvarado Scoring System (MASS) for Diagnosis of Acute Appendicitis (11, 12)

Alvarado Score		MASS	
Symptoms	Point	Symptoms	Point
Migratory right iliac fossa pain	1	Migratory right iliac fossa pain	1
Anorexia	1	Anorexia	1
Nausea/vomiting	1	Nausea/vomiting	1
Signs		Signs	
Tenderness in right illiac fossa	2	Tenderness in right illiac fossa	2
Rebound tenderness	1	Rebound tenderness	1
Elevated temperature (>37,3C)	1	Elevates temperature (>37,3°C)	1
Laboratory findings		Laboratory findings	
Leukocytosis (WBC >10×109/L)	2	Leukocytosis (WBC >10×109/L)	2
Shift to the left of neutrophiles (>70%)	1	-	-
Total	10	Total	9

MASS=Modified Alvarado Scoring system.

Materials and Methods

Subjects

We retrospectively analyzed all adult patients who underwent emergency appendectomy due to clinically suspected acute appendicitis at the University Hospital "Sveti Duh" (Zagreb, Croatia) in the period between July 2015 and March 2017. The decision to operate was made by the senior attending surgeon, based on clinical judgment (physical examination, imaging and blood test). No diagnostic scoring systems were used pre-operatively. The exclusion criterion was the absence of the data needed to calculate the MASS. The Hospital Ethical Committee granted approval for the retrospective analysis of the study data.

Methods

The MASS was retrospectively calculated for all patients based on the clinical signs and laboratory investigations obtained during initial admission in the emergency room.

Statistical Analysis

All clinical variables, except age, are given in absolute number and percentages. Age is given as the median and range, and was tested between groups with the Mann-Whitney test. The clinical and demographic variables were compared with a comparison of the proportions test. The difference between categorical data was tested with Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal MASS score cut-off value for the amended purpose, and to calculate the area under the curve (AUC), sensitivity, and the specificity of the MASS, with a respective 95% confidence interval (CI). Statistical analyses were performed using MedCalc Statistical Software version 16.2.0 (MedCalc Software bvba, Ostend, Belgium). $P<0.05$ was defined as the threshold of significance.

Results

During the study period, from July 2015 to March 2017, 153 patients underwent emergency appendectomy. Seven patients did not have all the relevant medical data to calculate the MASS score, thus leaving 146 patients eligible for inclusion in the study (76 males and 70 females).

In 118 out of 146 operated patients, acute appendicitis was confirmed intra-operatively, yielding a negative appendectomy rate of 19.2% at our Hospital. Sixty-eight patients underwent laparoscopic appendectomy, 75 patients underwent open appendectomy, and in 3 patients conversion from laparoscopic to open procedure was performed.

The study population was divided into two groups, depending on the rejected (negative appendectomies) or confirmed diagnosis of AA. Comparison of the basic demographic characteristics of the study subjects is shown in Table 2.

Among 28 patients with negative appendectomy, 4 cases had pelvic inflammatory disease, 2 cases had mesenterial lymphadenitis, 2 cases had enterocolitis and 1 case each had coecal diverticulitis, sigmoidal diverticulitis, Meckel`s diverticulitis, terminal ileitis, inflamed sigmoidal cancer and epiploic appendicitis. In 14 cases the cause of symptoms was unknown (Table 3).

The retrospectively calculated MASS score for each patient, showed a statistically significant difference between the average MASS score in the positive and negative appendectomy groups (6 v. 4, respectively), $P<0.001$. Although there were significantly more women in the negative appendectomy group (Table 2), no difference was observed in the average MASS score between genders (0.292).

Table 2. Comparison of Basic Demographic Characteristics Between Groups of Patients with Confirmed / Negative Appendectomies Diagnosis of Acute Appendicitis

Patient (N=146)	Appendectomies		P*
	Negative (N=28)	Confirmed (N=118)	
Male, N (ratio)	3 (0.04)	73 (0.96)	-
Female, N (ratio)	25 (0.36)	45 (0.64)	-
P*	<0.001	<0.001	-
Age, years	38.5 (20 – 79)	37 (20 – 85)	0.720

The results are presented as absolute numbers and ratio, except age which is given in median and range (min-max); *P<0.05 is considered statistically significant; The P value corresponds to the difference between the groups in columns, except for age. There was no statistically significant difference in mean age between the patients subdivided according to the postoperative outcome (P=0.720). However, there was a significantly higher number of females among the negative appendectomies (P<0.001), and a significantly higher number of males in the AA group (P<0.001).

Table 3. Causes of Acute Abdominal Pain in the Group of Negative Appendectomy Patients

Negative appendectomy group	
Diagnosis	N
Pelvic inflammatory disease	4
Enterocolitis	2
Lymphadenitis mesenterialis	2
Coecal diverticulitis	1
Sigmoidal diverticulitis	1
Meckel`s diverticulitis	1
Terminal ileitis	1
Inflamed sigmoidal cancer	1
Appendicitis epiploica	1
Unknown	14
Total	28

According to the current recommendations regarding the MASS score cut-off values (≥7), the sensitivity and specificity measured were 44.1% (95% CI: 34.9 – 53.5) and 89.3% (95% CI: 71.8 – 97.7), respectively. However, the ROC curve analysis identified a MASS cut-off value of >5 to achieve the optimum combination of both sensitivity (68.6%, 95% CI: 59.5–76.9) and specificity (71.4%, 95% CI: 51.3 – 86.8) in identifying the patients with AA. The area under the ROC curve revealed good diagnostic accuracy ((AUC) 0.745 (95% CI: 0.666 – 0.814), P<0.001). If the cut-off is lowered to values as low as ≥3, the sensitivity of the MASS score increases to 97.45% (95% CI: 92.7 – 99.5), thus obtaining a very low false negative rate of merely 2.55%.

Discussion

This retrospective diagnostic accuracy study confirmed the higher average MASS score in the group of patients with confirmed AA diagnosis compared to the negative appendectomy group. The ROC analysis identified a lower optimal cut-off value of ≥3 for the amended purpose of the Modified Alvarado Scoring System, thus increasing the sensitivity to a respectable 97.45%.

Our study confirmed the literature data about the difficulties in diagnosing AA in women, especially in childbearing age. There were significantly more women in the negative appendectomy group, however no difference was observed in the average MASS between men and women in that particular group. Perhaps it would be interesting to explore this point through prospective evaluation in female patients only.

When deciding about the optimal cut-off point, the real question is whether this scoring system could assist surgeons reduce the risk of missing diagnosis by identifying patients with suspected acute appendicitis. Considering the variable literature data about the diagnostic accuracy of MASS, it

is not likely that the MASS will become the dominant criteria in management of patients with acute abdominal pain at our Hospital. More likely the final decision will always be in the domain of the attending surgeon. In that light, and considering the results obtained, perhaps it would be wiser to aim at higher sensitivity. To increase the sensitivity of the MASS score further and thus help the attending surgeons in ruling out the true negative patients, we wanted to calculate the measure of diagnostic accuracy at a very low cut-off value of ≥3 MASS points. When applying this proposed lower cut-off value of ≥3 on our retrospective study group, we obtained a very low false negative rate of merely 2.55%.

Conclusion

Although our study has some shortcomings, it represents a valuable insight into the initial official data about the diagnostic accuracy of MASS at one Croatian University Hospital. In conclusion, we propose the use of MASS at a cut-off of ≥3 as a useful tool to help surgeons ruling in patients with AA in order to reduce the risk of missing diagnosis.

What Is Already Known on this Topic

Unrecognized appendicitis with delayed surgical intervention can lead to major complications or even death. Diagnosing appendicitis is not always simple. A variety of different scoring systems for acute appendicitis have been proposed, and the most widely used today is the Modified Alvarado scoring system. The sensitivity and specificity of the Modified Alvarado scoring system for diagnosing appendicitis at a recommended cut off point of ≥7 varies a great deal between studies.

What this Study Adds

We aimed to assess retrospectively the diagnostic accuracy of the modified Alvarado score in operated patients and to propose a different MASS cut-off point for the amended purpose. Following the current recommendations regarding the MASS score cut-off values (≥7), the measured sensitivity and specificity were 44.1% (95% CI: 34.9 – 53.5) and 89.3% (95% CI: 71.8 – 97.7), respectively. We identified a MASS cut-off value of >5 to achieve the optimum combination of both sensitivity (68.6%, 95% CI: 59.5 – 76.9) and specificity (71.4%, 95% CI: 51.3 – 86.8) in identifying the patients with AA. By lowering the cut-off to values as low as ≥3, the sensitivity of the MASS score increased to a respectable 97.45% (95% CI: 92.7 – 99.5), thus obtaining a very low false negative rate of merely 2.55%.

Authors' Contributions: Conception and design: BB and VRB; Acquisition, analysis and interpretation of data: BB, VRB, MB, FR and AMŠ; Drafting the article: BB and VRB; Revising it critically for important intellectual content: AMŠ; Approved final version of the manuscript: BB, VRB, MB, FR and AMŠ.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Acta Medica Academica 2019;48(2):183-192
DOI: 10.5644/ama2006-124.257

Ultrasound Evaluation of Radial Nerve Palsy Associated with Humeral Shaft Fractures to Guide Operative Versus Non-Operative Treatment

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Received: 1 February 2019
Accepted: 9 May 2019

Key Words: Humerus Fracture ▪ Ultrasound ▪ Radial Nerve ▪ Injury.

Objective. To determine the effectiveness of diagnostic ultrasound (US) at evaluating the condition of the radial nerve in the setting of humeral shaft fractures. **Materials and Methods.** An observational study was performed of 18 patients with radial nerve palsy associated with humeral shaft fractures who underwent US examination to assess the condition of the radial nerve. **Results.** Six patients with humeral shaft fractures treated nonoperatively in a functional brace had US findings consistent with contusion or stretch radial nerve injury. Twelve patients ultimately underwent surgery either because US showed an entrapped or lacerated radial nerve, or for other operative indications. There was a 92% concordance (11/12 patients) between US and intraoperative findings with regards to the condition and location of the radial nerve, with the remaining case being complicated by delayed surgical treatment secondary to patient factors. **Conclusion.** Our study demonstrates that US is an effective diagnostic tool in evaluating radial nerve injuries in the setting of humeral shaft fractures and can aid in clinical decision making by differentiating between patients with nerve laceration or entrapment who may benefit from surgery from those with neurapraxia managed nonoperatively.

Introduction

Injuries to the radial nerve in the setting of humeral shaft fractures are among the most common peripheral nerve lesions associated with trauma (1-5). The intimate relationship of the radial nerve with the shaft of the humerus within the spiral groove makes it particularly vulnerable to traction, transection, or entrapment injuries with fractures of the middle and distal third of the humerus (6, 7). Humeral shaft fractures are commonly treated non-operatively owing to Dr. Sarmiento's extensive work and research with the functional brace (8). When there is an associated radial neuropathy, nonoperative management of the humeral shaft fracture and expectant management of the nerve palsy remain the most common treatment recommendation (2, 4, 5, 7). In a systematic review of studies of humeral shaft fractures associated with radial nerve palsy, Shao et al. report a prevalence of radial nerve palsy of 11.8%. They report a spontaneous recovery rate of 70.7% from their meta-analysis of 532 radial nerve palsies in 4517 fractures (3). Other studies have reported spontaneous nerve recovery rates as high as 89% (9).

Delayed return of radial nerve function is commonly evaluated with electromyography (EMG) (3, 10).

Diagnostic peripheral nerve ultrasound (US) has gained popularity due to its cost-effectiveness and non-invasive nature with very low risk (11). This imaging modality not only has the ability to determine if a nerve is intact, but it can reveal the precise location of the nerve in relationship to bone or other structures as well as show inflammatory changes within and about the nerve (11, 12). US evaluation of the radial nerve in the setting of humeral shaft fracture allows for differentiation of nerve injury secondary to contusion or stretch injury versus laceration or entrapment within the fracture site (3, 13-15). If signs of nerve transection, partial-thickness tear or entrapment are identified, a change of management may be indicated with conversion to early exploration and operative fracture fixation to prevent delayed treatment and problematic healing of the fracture with interposed nerve tissue. Our study examines the utility of US evaluation of the radial nerve in cases of humeral shaft fractures associated with radial nerve palsy. We explore the correlation of US findings to the actual state of the nerve observed intraoperatively.

The purpose of this study is to determine the effectiveness of diagnostic US at evaluating the condition of the radial nerve in the setting of humeral shaft fractures and to discuss whether this information may help guide surgeons in their clinical decision making for operative versus nonoperative treatment of these injuries.

Materials and Methods

Institutional Review Board approval was obtained for this observational study. A retrospective review was conducted of patients with humeral shaft fracture who had undergone US evaluation of the radial nerve from 2012-2015. Subsequent eligible patients from January 2015-December 2017 were enrolled in the study prospectively. All patients age 18 years and older who presented to our institution with a humeral shaft fracture and had an initial examination concerning for radial nerve palsy were included in the study. In addition, patients with a humeral shaft fracture that the treating surgeon deemed operative in nature were also eligible for study enrollment for as a control group to obtain US evaluation of presumably normal radial nerves. Operative indications for humeral shaft fractures included open fractures, fractures with associated vascular injury requiring surgical repair, and ipsilateral extremity fractures or polytrauma patients who would undergo surgery for improved mobilization of the extremity. Other relative indications for surgery included patient preference/desire for earlier mobilization, body habitus, and inability to tolerate a brace. Exclusion criteria included any pre-existing compromise to radial nerve function.

All US examinations were performed in the Department of Medical Imaging on Logiq E9 US machine (General Electric Healthcare, Chicago, Illinois, USA) with 6-15 MHz high-resolution multifrequency linear transducer by a musculoskeletal (MSK) trained US technologist and one of four fellowship trained musculoskeletal radiologists. On the US images, the radial nerves were considered normal when they showed a stippled honeycomb appearance on the short axis US images with hypoechoic areas corresponding to the nerve fascicles and surrounding hyperechoic rims corresponding to endoneurium, perineurium, and epineurium. Normal nerves appear hypoechoic with alternating hyperechoic bends on the long axis US images. Completely transected nerves show focal hypoechoic bulbous thickening at their ends consistent with stump neuromas. Partially torn nerves appear hypoechoic and irregular

Figure 1. Intact radial nerve in 18-year-old female with humeral shaft fracture and radial nerve palsy status post all-terrain vehicle (ATV) accident (Patient 4). (A) Photograph of the left upper extremity shows ultrasound probe over the swollen posterior mid arm in the region of the humeral shaft fracture. (B) AP radiograph of the left humerus shows moderately displaced and mildly comminuted oblique humeral shaft fracture. (C) Long axis and (D) short axis grayscale ultrasound (US) images at the posterior aspect of the left mid arm/humerus shows mildly edematous but intact radial nerve (arrows) superficial to the humeral shaft fracture site (block arrows) consistent with neurapraxia. Small portion of the radial nerve shows mildly increased echogenicity (arrowhead in C, between arrows in D) without discontinuity. The patient was treated conservatively in a Sarmiento brace with full radial nerve recovery achieved after 10 weeks.

while contused nerves appear hypoechoic, heterogeneous and thickened. Entrapped nerves dive into the fracture site. Because of significant pain and discomfort in the setting of the acute humeral shaft fractures, all patients were examined in the supine position on the ultrasound stretcher or in the hospital bed with their affected arm at the examiner's side. Figure 1 demonstrates patient positioning, humeral shaft fracture, and the US technique utilized in this study in a patient and representative US images of their intact radial nerve.

The radial nerves were first traced along their short axis using the "elevator" technique for rapid screening of long nerve segments and after that along their long axis throughout the arm to their bifurcation into the superficial and deep branches at the anterior aspect of the elbow proximal to the

supinator muscle. For completeness of the US exam the superficial and deep radial nerve branches were then traced along the patient's forearm using the same examination technique. On a case by case basis, pillows were placed under the patient's back at the examiners side to partially roll patient's body away from the examiner and enable better exposure of the examined upper extremity along the pathway of the radial nerve. Of note, in the non-acute trauma setting, the radial nerve can be examined with the patient in a sitting position.

Seventeen of 18 patients had their US imaging within 24 hours after injury. Patients with US diagnosis of radial nerve entrapment or laceration were treated with open reduction internal fixation (ORIF) of their humeral fracture. Patients were followed in clinic until they showed signs of nerve recovery or were lost to follow up. At the conclusion of the study, patients that had been lost to follow up were contacted via telephone.

Results

Eighteen patients were enrolled in the study (Table 1). The average age of patients was 48 years old (range 18-81), and 56.6% of patients were male. Mechanisms of injury included ground level fall (6), fall from several feet (3), motor vehicle or all-terrain vehicle (ATV) accidents (5), bicycle accidents (2), assault (1), and wrestling (1). Fourteen patients included in the study had a radial nerve palsy, four in the nonoperative bracing group and ten in the operative group. Two patients in the nonoperative group underwent US imaging because their initial clinical exam on presentation was concerning for radial nerve palsy however they were noted to have full recovery of the radial nerve function prior to hospital discharge.

Treatment

Eight patients with no US findings of radial nerve entrapment or laceration were initially treated with a functional brace. Of those eight, two subsequently went on to ORIF, one because of inability to tolerate the brace and one after developing a fracture nonunion. Six patients underwent ORIF due to US showing entrapment or laceration of the radial nerve (Figure 2). Four other patients underwent ORIF for other indications including polytrauma, pathologic fracture, and surgeon judgement.

Ultrasound and Operative Findings

Of the 12 patients who underwent ORIF, US findings were compared to intraoperative assessment of the radial nerve condition. There were six cases where US demonstrated an intact radial nerve, which was concordant with intraoperative findings. These patients served as a control group. This group included one case (Table 1, Patient 18) in which US reported a thickened but continuous nerve perched on the fracture site. Direct visualization during surgery confirmed nerve continuity with the distal fracture fragment tenting the nerve. US accurately diagnosed nerve entrapment or laceration in five cases that were concordant with intraoperative findings. In one patient (Table 1, Patient 8), US showed "a portion of the nerve which is not well seen, highly suggestive for entrapment and partial laceration". Operative findings confirmed partial nerve laceration and entrapment as seen in Figure 2.

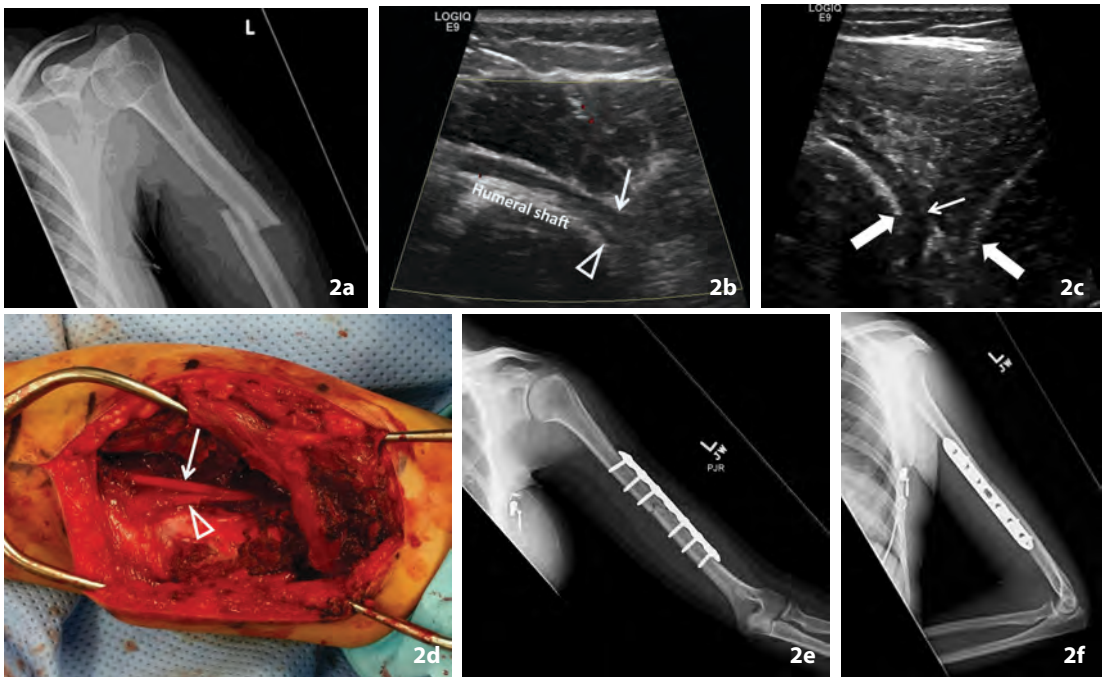

Figure 2. Transverse humeral shaft fracture with associated radial nerve entrapment and partial laceration in a 33-year-old female status post bicycle accident (Patient 8). (A) AP radiograph of the left humerus shows completely displaced mildly overriding humeral shaft fracture. (B) Long axis power Doppler US image at the level of the left humeral shaft fracture shows radial nerve entrapment at the fracture site (arrow) with partial laceration (arrowhead). (C) Long axis grayscale US image shows radial nerve entrapment (arrow) at the humeral shaft fracture site (block arrows). (D) Intraoperative photograph of the posterior aspect of the right arm confirmed radial nerve entrapment (arrow) and partial laceration (arrowhead) at the humeral shaft fracture site which correlates with the US findings. (E) AP and (F) lateral postoperative radiographs of the left humerus obtained two weeks later show a laterally applied low contact dynamic compression plate with 6 cortical screws transfixing the left humeral shaft fracture in anatomic alignment and without hardware complication. The patient achieved full radial nerve recovery at final follow up.

Table 1. Patient Information and Findings

Patients	Age (y)	Sex	Mechanism of injury	Humerus radiographs	Ultrasound findings	Method of treatment	Operative findings
1	38	M	MVC	Transverse midshaft with butterfly fragment	Difficult visualization secondary to large body habitus however no definitive entrapment seen as nerve appears superficial to fracture site	Functional bracing	N/A*
2	31	M	Assaulted with ice pick	Long spiral comminuted fracture	Normal appearance of radial nerve	Functional bracing	N/A
3	37	M	Ground level fall	Transverse midshaft fracture	Stretched and mildly thickened radial nerve with no evidence of entrapment	Functional bracing	N/A
4	18	F	ATV accident	Transverse midshaft fracture	Radial nerve superficial to fracture site without evidence of entrapment	Functional bracing	N/A
5	81	M	Fall from bed	Midshaft spiral fracture	Radial nerve edematous but contiguous with no evidence of transection or entrapment	Functional bracing	N/A
6	21	F	ATV accident	Transverse midshaft fracture	Enlargement and irregularity of the nerve with tenting at the fracture site	Functional bracing	N/A

Continuation of Table 1. Patient Information and Findings

Patients	Age (y)	Sex	Mechanism of injury	Humerus radiographs	Ultrasound findings	Method of treatment	Operative findings
7	32	M	Wrestling	Oblique distal shaft fracture	Entrapment of a short segment of the radial nerve between the proximal aspect of the fracture fragments	ORIF	RN†
8	33	F	Bicycle accident	Transverse midshaft fracture	Radial nerve appears to dive towards and into the fracture site with a portion of the nerve not well-seen, highly concerning for entrapment and partial laceration	ORIF	RN‡
9	33	M	MVC	Oblique midshaft fracture with butterfly fragment	Nonvisualization of the radial nerve at the fracture site concerning for entrapment and transection, with associated thickening of the nerve just distal to the fracture	ORIF Tendon transfers	RN§
10	52	F	Fall from truck	Spiral fracture of distal shaft with butterfly fragment	Findings concerning for radial nerve entrapment at the level of the fracture with mild diffuse enlargement of the visualized nerve	ORIF	RN‖
11	76	F	Ground level fall	Long spiral fracture of mid to distal shaft	Nonvisualization of the radial nerve at the fracture site worrisome for radial nerve entrapment	ORIF	RN¶
12	74	F	Ground level fall	Long spiral fracture of proximal to midshaft	1st US: irregular, enlarged, edematous radial nerve without discontinuity or entrapment 2nd US: abutment and possible impingement of the nerve at the posterior edge of the fixation plate distally	ORIF after unable to tolerate brace	RN**
13	59	F	Ground level fall	Segmental comminuted midshaft fracture	Edematous radial nerve about the fracture site without definitive evidence of entrapment	ORIF after development of nonunion	RN††
14	60	M	Ground level fall	Oblique midshaft fracture with butterfly fragment	1st US: poor visualization of radial nerve suggesting focal entrapment 2nd US: findings consistent with partial entrapment at region of posttraumatic deformity	ORIF Tendon transfers	RN‡‡
15	52	F	Ground level fall	Spiral fracture of distal shaft with butterfly fragment	Thickened edematous nerve without entrapment	ORIF (surgeon judgement)*	RN§§
16	69	M	Fall from bed	Midshaft oblique fracture	Unremarkable appearance of radial nerve	ORIF (pathologic fracture)	RN‡‡
17	68	M	MVC	Transverse distal shaft fracture	Mild nerve edema, no entrapment	ORIF (polytrauma)	RN‖‖
18	23	M	Bicycle accident	Comminuted distal shaft fracture	Radial neve perched on distal fracture fragment with mild thickening distally but no evidence of laceration or entrapment	ORIF (surgeon judgement)*	RN¶¶

ORIF=Open reduction internal fixation; ATV=All-terrain vehicle; MVC; Motor vehicle collision; *Not applicable; †Open reduction internal fixation performed based on surgeon judgement of healing potential of the fracture based on fracture pattern/alignment and/or patient factors; ‡Radial nerve entrapped at the fracture site with a small partial laceration involving approximately 10% of the nerve fascicles; §Radial nerve transected at level of fracture with the distal end frayed and crushed; ‖Radial nerve entrapped within the fracture site anterior to the fracture, transposed in front of the humerus; ¶Radial nerve entrapped over a spike of the distal humeral fracture segment; **Radial nerve identified and protected. Postoperative radial nerve palsy managed expectantly; ††Radial nerve identified in posterior fascia adjacent to triceps, in continuity; ‡‡Radial nerve identified and protected; §§Radial nerve in continuity with minimal bruising in close proximity to the fracture but not entrapped; ‖‖Radial neve visualized adjacent to fracture with evidence of trauma but in continuity; ¶¶Apex of distal fracture fragment tenting and stretching the radial nerve.

In one of the patients (Patient 14) with US findings consistent with radial nerve entrapment, surgery was delayed six weeks due to patient factors and systems issues. This patient did not undergo US examination at his initial presentation to the Emergency Department due to the unavailability of an MSK trained US technologist on a weekend evening. He was initially treated in a Sarmiento brace and instructed to have an US study performed the following week as an outpatient. The ultrasound was performed two weeks later and showed radial nerve entrapment. Despite US findings, this patient didn't follow-up in orthopaedic clinic until five weeks after his injury, at which time radiographs showed varus alignment of the fracture site with minimal callus formation. After that the patient underwent ORIF of the right humeral shaft fracture. Operative note described that the radial nerve was protected during the procedure however the condition of the nerve was not described in the operative report. Postoperatively this patient did not regain radial nerve function and ultimately underwent tendon transfers ten months later.

Based on results of our study, there was a 92% (11/12 patients) concordance between US examination and intraoperative findings with regards to the condition and location of the radial nerve (including the six patients in the control group), with the remaining case being complicated by delayed surgical treatment secondary to patient factors.

Orthopaedic Clinic Follow-up and Clinical Results

There were two patients who did not follow up in orthopaedic clinic. Of the remaining 16 patients, mean follow up time was 13 weeks (range 2-40 weeks). Only 11 patients had greater than four weeks of follow up, all of whom went on to fracture union. Four of the six patients treated nonoperatively had full radial nerve function at their final clinic follow up visit. One patient had partial recovery of radial nerve function at her two week postoperative visit. Upon phone interview at nine months post-injury, she reported full motor recovery with remaining mild paresthesias that she felt were continuing to improve. The last of the six patients treated non-operatively was lost to follow-up.

Of the operatively treated group, six patients reported full radial nerve recovery and two patients reported partial recovery. Two patients underwent tendon transfers after initial fixation of their humeral shaft fracture. Another patient passed away two weeks postoperatively secondary to metastatic esophageal cancer, and the final patient in the operatively treated group did not have clinic or phone follow-up.

Discussion

Humeral shaft fractures associated with radial nerve palsies are a debilitating injury. Several studies have described a high level of disability and poor functional outcomes associated with upper extremity peripheral nerve injuries (16-18). Management of these injuries has traditionally been with expectant care as humeral shaft fractures frequently heal well with functional bracing and the majority of associated nerve palsies recover spontaneously. However, approximately 30% of patients with these injuries may not regain adequate radial nerve function with expectant management (3). Recovery for these patients may be long and cumbersome as radial nerve function is often monitored for 3-6 months before it is determined that the nerve will not recover spontaneously.

Some authors have advocated for more routine early nerve exploration for radial nerve palsies associated with humeral shaft fractures (19, 20). However, as the majority of nerve palsies recover spontaneously, routine nerve exploration would lead to

overtreatment and even potential iatrogenic injury. Diagnostic US can help differentiate patients who would benefit from early nerve exploration, nerve repair, or acute tendon transfers from those patients expected to have spontaneous nerve recovery. Identification of this subset of patients who may benefit from early surgical intervention may help to decrease disability and improve recovery time for these patients.

Shao et al. (3) published a systematic review in 2005 of radial nerve palsy associated with humeral shaft fractures and presented an algorithm to guide treatment of these injuries. The algorithm includes US evaluation within three weeks of injury to assess the status of the radial nerve (3). If the nerve is continuous, loss of function is thought to be secondary to neurapraxia which may be managed conservatively. If the nerve is lacerated or entrapped, early surgical intervention is preferred as nerve function would not be expected to recover spontaneously. Depending on the condition and location of the entrapped and/or injured nerve, operative treatment could include simple extraction of the nerve from within the fracture site, nerve repair, or acute tendon transfers. If the nerve is severely contused or has an irreparable laceration, the likelihood of a prolonged or unpredictable recovery is high. In some cases, the nerve would not be expected to recover at al. In these settings, early tendon transfers may be beneficial in optimizing the patient's functional recovery by restoring active extension of the wrist and digits. For example, pronator teres to extensor carpi radialis brevis is an ideal transfer as it restores wrist extension while sacrificing few if any functional deficits given that the pronator teres muscle maintains its function as a pronator after transfer while also allowing the patient to be able to actively extend their wrist. This eliminates the necessity of prolonged wrist bracing that patients with a wrist drop secondary to radial nerve palsy would typically require in order to prevent a wrist flexion contracture.

In 2001 Bodner et al. conducted a prospective US study in 11 patients with humeral shaft fracture associated with radial nerve palsy (13). In this study the interval between trauma and US study was 1-8 weeks with a mean of 19 days which is different from our study in which 17 of 18 patients had their US studies within 24 hours of injury. It is more difficult to perform an US study in patients with hyperacute trauma with associated marked soft tissue edema and limited range of motion. However, we believe that prompt US examination of patients with acute humeral shaft fractures and associated radial nerve palsy during their initial evaluation in the emergency department may expedite clinical decision making of nonoperative versus operative treatment and avoid delays in surgical treatment.

There may be several challenges to implementing a protocol of routine prompt US evaluation for patients with humeral shaft fractures and associated radial nerve palsy. At our institution, MSK US studies are read by fellowship-trained MSK radiologists with experience in US evaluation of peripheral nerve injuries, however we recognize that not all institutions will have these resources available. Musculoskeletal US trained technologists or fellowship trained MSK radiologists are not routinely available at most institutions on nights and weekends. Patients may be instructed to follow up for an US examination on an outpatient basis, however issues of patient compliance, insurance coverage, etc. may delay evaluation or treatment.

There are several limitations to our study. Because US findings were used to help guide clinical decision making, surgeons were not blinded to the results of the US reports. This may introduce a degree of observer bias as surgeons' descriptions of intraoperative findings of the radial nerve may be influ-

enced by their knowledge of the US findings. However, this limitation could not be avoided because the clinical decision making for nonoperative versus operative treatment was partially based on US findings.

The focus of this study was to evaluate the accuracy of US evaluation of the radial nerve in predicting intraoperative findings with a secondary goal of assessing clinical outcomes. However, our cohort had poor clinic follow-up, and for many patients we relied on phone calls and thus the patients' subjective impression of their arm function and symptoms in reporting of our final outcomes. A potential focus for future studies is to evaluate clinical outcomes to determine whether routine use of US in patients with radial nerve palsies associated with humeral shaft fractures may lead to faster recovery, improved function, and differences in health care costs.

We had a small sample size due to the relative infrequency of this injury. However, to our knowledge this is the largest case series in the literature evaluating US use for humeral shaft fractures associated with radial nerve injuries. We found a high concordance (92%) between US and intraoperative findings describing the state of the radial nerve. Based on results of our study, we routinely perform US examination in patients with humeral shaft fractures associated with radial nerve palsy at our institution at the time of their initial presentation.

Conclusion

Diagnostic US is an effective and efficient method for evaluating the radial nerve in the face of humeral shaft fractures. US evaluation provides a means of separating patients with a radial nerve palsy due to laceration or entrapment where surgical intervention is indicated from those who have a neurapraxia that can be managed nonoperatively. Patients with radial nerve injuries not expected to recover spontaneously may benefit from nerve repair or early tendon transfers. Earlier surgical intervention in this subset of patients may allow for earlier initiation of rehabilitation protocols, faster recovery, and reduced costs.

What Is Already Known on this Topic

The majority of radial nerve palsies associated with humeral shaft fractures may be treated nonoperatively with successful return of nerve function. However, injuries in which the radial nerve is lacerated or entrapped in the fracture site, as opposed to nerve palsies caused by contusion or stretch injuries, would not be expected to recover spontaneously and may require further treatment such as nerve repair or tendon transfers.

What this Study Adds

Our study demonstrates that US is an effective diagnostic tool in evaluating radial nerve injuries in the setting of acute humeral shaft fractures and can aid in clinical decision making by differentiating between patients with nerve laceration or entrapment who may benefit from surgery from those with neurapraxia managed nonoperatively.

Acknowledgements: Melissa Esparza, MD received a grant from the University of Arizona ACGME Resident Leadership Scholarship to support submission, presentation, travel expenses, etc. related to this project. No authors have any proprietary interests in the materials described in this article.

Authors' Contributions: Conception and design: CM, ME, JW and MT; Acquisition, analysis and interpretation of data: CM, ME, KM, JW, MT and LT; Drafting the article: CM and ME; Revising it critically for important intellectual content: ME, JW, MT, LT and CM; Approved final version of the manuscript: ME, JW, MT, LT, CM and KM.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Acta Medica Academica 2019;48(2):193-204
DOI: 10.5644/ama2006-124.258

The Incidence of Smoking Habits and the Degree of Nicotine Dependence in Education Workers

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Received: 5 March 2019
Accepted: 15 August 2019

Key Words: Smoking ▪ Dependence ▪ Fagerstrom Test ▪ Education Worker.

Objective. The aim of this research was to establish the incidence of smoking habits and the level of nicotine dependence in education workers in the Central Bosnia Canton (CBC). **Materials and Methods.** The research was conducted in the 2017/2018 school year. It covered education workers in all elementary (N=53) and high schools (N=23), in the area of the CBC. A total of 857 subjects were included. For the purpose of this research a special questionnaire was designed that included information on smoking, how long people had smoked, age and gender, smoking habits, and a modified Fagerstrom test for assessment of nicotine dependence. **Results.** In relation to smoking status, the subjects were divided into two categories: 646 (75.38%) non-smokers and 211 (24.62%) of those who declared themselves to be active smokers. All subjects were considered in relation to four age groups. The average age of the subjects was 42.14 years, and the average age of the subjects who were smokers was 43.61 years. Of the total number of smokers (n=211), the number of those who believed that the consumption of cigarettes by staff members encourages pupils to smoke was 76 (36.01%). The largest number of subjects (746, 87%) believed that during their time in school or the school yard, pupils are in a situation where they are able to see educational workers smoking. The degree of nicotine dependence in education workers in the CBC was mostly mild (53.55%, N=113) and then moderate (44.54%, N=94) and severe (1.89%, N=4). **Conclusion.** There is a significant number of smokers (24.62%) amongst education workers in schools in the CBC. The level of nicotine dependence in education workers is most often mild (53.55%) then moderate (44.54%) and severe (1.89%).

Introduction

Tobacco smoke is produced by the incomplete combustion of tobacco leaves and contains about 7000 chemical compounds, of which the most harmful are tar compounds, carbon monoxide, and nicotine. Nicotine and tobacco products are related to dependence. Some studies have revealed that tobacco products, and especially cigarettes, are related to levels of dependence equivalent to illegal drugs (1).

In today's modern world, tobacco smoking is a pervasive dependence disease, the most widespread social disease and a top-priority public health problem. Non-infectious diseases (primarily carcinomas, diabetes, cardiovascular diseases and chronic lung diseases) are directly responsible for more than 36 million deaths each year. In view of the fact that tobacco is the greatest risk factor for non-infectious diseases, avoiding tobacco is of crucial important in

reducing premature deaths (2). Tobacco use is still clearly a major public health issue, and it is critically important to identify the risk factors related to starting smoking. Most research dealing with smoking has focused on adolescents, because preventing them starting to smoke is one of the most valuable approaches to reducing the burden of smoking. Moreover, thousands of adolescents try cigarettes every day, and some of them will become adults addicted to smoking (3).

Adolescence is a period of "increased vulnerability" to incitement to use tobacco. Various factors have been identified as initiators of smoking in adolescents. In view of the fact that adolescents spend most of their time in school, teachers can have an important influence, because they interact with pupils almost every day. The use of tobacco by teachers and approval of the use of tobacco amongst this important group of people is likely to increase the probability of smoking by pupils, due to the perception that using tobacco is normative, commonplace and acceptable behaviour (4). This is especially so because teachers are also those responsible for teaching plans and lessons aimed at preventing smoking, and key leaders in creating public opinion relating to school policies on combating tobacco use (5).

Smokers mainly admit the harm they are doing to themselves, and many say they do not enjoy it, but they still smoke. The reason for this is that the nicotine in the cigarettes creates a strong urge to smoke, which undermines and overwhelms concerns about the negative consequences of smoking and the decision not to smoke in those who are trying to stop (6). Dependence on cigarettes stems from the fact that they supply a highly controlled dose of a drug, nicotine, to the brain quickly and in a form which is accessible, available and pleasant (7). At the end of the last century the importance was recognized of nicotine in maintaining the smoking habit, and the difficulties in stopping smoking. This led to the need to create an instrument to measure nicotine dependence (8).

The aim of this research was to establish the incidence of smoking habits and the level of nicotine dependence in education workers in the Central Bosnia Canton (CBC).

Materials and Methods

Area of Research

The CBC is an administrative unit of the Federation of Bosnia and Herzegovina. It is located in the central part of Bosnia and Herzegovina and has an area of 3,189 km^2, with a population in 2015 of 251,714 inhabitants, of whom 58,021 are children aged from 0-18 years. This research included education workers from eleven municipalities of the CBC (9).

Subjects

The research took place during the 2017/2018 school year and 873 permanently employed education workers took part from all elementary schools (N=53) and high schools (N=23) in the eleven municipalities of CBC.

Methods

The research was conducted in the form of a questionnaire. It was voluntary and anonymous. Anonymity was ensured whereby the questionnaire was conducted in the schools and the subjects completed the questionnaire themselves and it did not require any personal details. It was placed into a box prepared in advance. For the needs of this research, a specially structured questionnaire was designed with 20 questions, which contained information on smoking, how long they had smoked, their age and gender, smoking habits, and a modified Fagerstrom

test for assessment of nicotine dependence (8, 10). Fagerstrom's test for nicotine dependence was developed in 1978 and tests dependence on nicotine using the self-assessment method. The questionnaire was distributed to the teachers in person, and the professional staff gave oral instructions about how to complete the questionnaire and the purpose of the research, and were available for any questions. The teachers were assured that the information they gave would remain confidential, so they were encouraged to be honest in their answers. The questionnaire had to be fully completed to be included in the analysis.

For assessment of dependence, we analysed the replies that are an integral part of the Fagerström Tolerance Questionnaire. It consisted of 6 questions with answers supplied. Each answer carried 0, 1, 2 or 3 points, whereby the total score indicated the nicotine dependence of the individual subject. Scores were from 0-11 points. Mild nicotine dependence in a smoker produces a score of 0-3 points, moderate dependence 4-8 points, and more than 9 points indicates severe dependence.

Ethical Aspects of the Research

The Ethics Committee of the Institute for Public Health of the CBC approved the research (no. 1-03-06/18). All participants gave verbal consent to participation in this research. The informed consent of the teacher was presumed when they completed and returned the questionnaire.

Statistical Analysis

Data entry was initiated at the same time as the data were collected. The data in the research were checked, verified and entered into MS Excel 2013. Incomplete and inconsistent data were rejected and not included in the final analysis. In the end, data for 857 subjects were used for analysis. Data analysis was undertaken using SPSS version 17 software. Numerical and percentage distributions were used to present the descriptive data, the hi-square test was used to confirm correlations, and statistical significance was tested at a level of 5% probability.

Results

Of the total number of subjects (N=857) the research included 267 (31.16%) men and 590 (68.84%) women. Regarding the percentages of subjects related to place of residence, 544 (63.48%) subjects were from urban settings, and 313 (36.52%) a rural environment. Of the total number of smokers (N=211), those from a rural setting had a share of 66/313 (21.09%), whilst 145/544 (26.65%) smokers were from an urban environment (χ^2=3.02 i P=0.082) that is, there was no statistically significant difference in the number of smokers in relation to their place of residence. In relation to gender, the proportion of women smokers was 143/590 (24.2%) in comparison to men with 68/267 (25.5%) (χ^2=0.09 (P=0.763). The difference is not statistically significant.

In terms of smoking status, the subjects were divided into two categories: 211 (24.62%) declared themselves to be active smokers, and 646 (75. 38%) non-smokers (Table 1). The non-smokers also included former smokers, 169 of them (19.72). The difference in distribution of smokers and non-smokers in age groups was statistically significant (χ^2=8.60; P=0.035).

The largest number of subjects was in the 31- 40 years age group (35.12%). The average age of the subjects was 42.14 years, where smokers were on average older, 43.61 years, and non-smokers 41.66. The largest number of subjects who were smokers had consumed cigarettes for more than 15 years (42.65%), followed by the group who had smoked for 5-10 years (28.44%), and a

Table 1. Subjects in Relation to Age Group and Smoking Status

Age (years)	Subjects	Smokers	Non-smokers
	N (%)	N (%)	N (%)
21-30	136 (15.87)	22 (10.43)	114 (17.65)
31-40	301 (35.12)	77 (36.49)	224 (34.67)
41-50	205 (23.92)	48 (22.75)	157 (24.30)
51-65	215 (25.09)	64 (30.33)	151(23.37)
Total	857 (100)	211 (100)	646 (100)
Mean ±SD	42.14 ± 10.55	43,61±10,50	41.66 ±10.52

group for 11 – 15 years (18.96%) whilst the fewest smokers were in the group who had smoked for up to 5 years. The average length of smoking was 14.84 years (Table 2).

In relation to the length of work in education, the largest proportion of subjects, 361 (42.12%), stated that they had worked for more than 15 years, 326 (38.04%) subjects had worked for 5-15 years, and 170 (19.84%) subjects had worked in education for less than 5 years.

To the question "To what extent are you responsible for improving live style habits in school children?" the largest proportion of subjects (403, 47.02%) replied that this was not one of their primary tasks, but that they sometimes taught children about health, 352 (41.07%) subjects replied that this was one of their primary tasks, and that they taught children a great deal about health, whilst 102 (11.91%) subjects did not teach children at all about topics related to life style habits. Of the 352 (41.07%) subjects who teach children about health a great deal, 130 smoke (29.26%). A large proportion of subjects (514, 59.98%) stated that they believe consumption of cigarettes by teaching staff encourages pupils to consume them too, whilst 343 (40.02%) subjects replied negatively. In our sample there was a statistically significant difference in the attitude of subjects about how far teaching staff smoking cigarettes encourages pupils to do the same χ^2=34.12 (P=0.052).

There was also a significant difference in the attitude of smokers in comparison to non-smokers. Of the total number of smokers (211), the number of those who believe that consuming cigarettes by teaching staff encourages pupils to smoke too was 76 (36.01%), whilst the number of smokers who do not believe that consumption of cigarettes by teaching staff encourages pupils to consume them too was 135 (63.98%); where χ^2=66.78 (P=0.053).

Table 2. Smoker Subjects in Relation to the Duration of Smoking

Age group (years)	Length of smoking (years)			
	1-5	6-10	11-15	≥15
	N (%)	N (%)	N (%)	N (%)
<30	9 (42.7)	11 (18.3)	2 (5.0)	-
31-40	9 (42.9)	33 (55.30)	20 (50.0)	15 (16.7)
41-50	3 (14.3)	6 (10.30)	13 (32.5)	26 (28.9)
>50	-	10 (16.7)	5 (12.5)	49 (54.4)
Total	21 (100)	60 (100)	40 (100)	90 (100)
	Mean±SD	14.84 ± 7.50 years.		

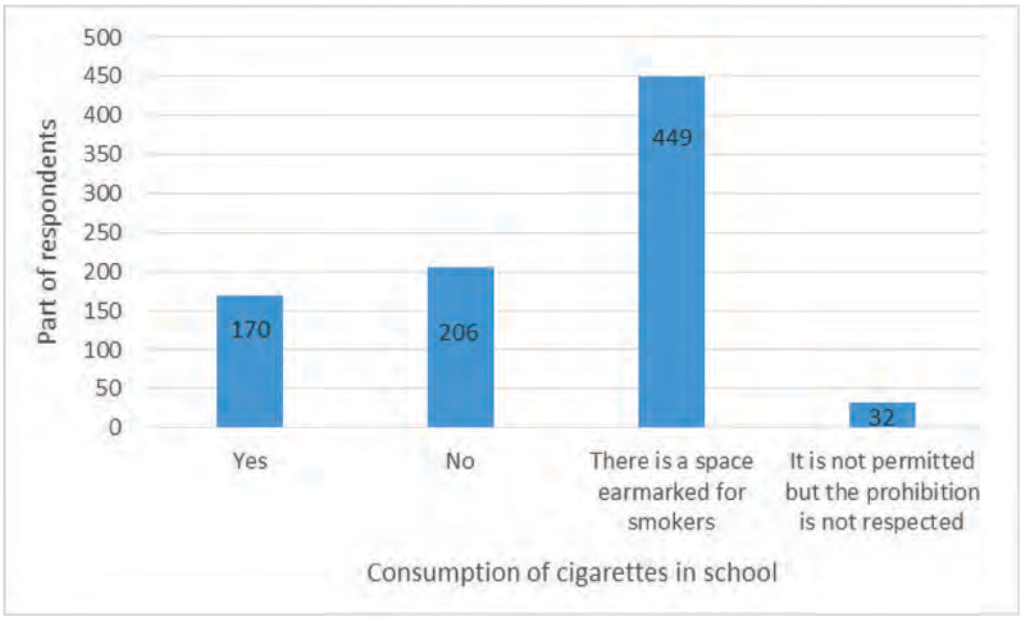

Figure 1. Consumption of Cigarettes by Education Workers During Their Time in School.

To the question "Do you consume cigarettes during working hours?" most of the subjects (136, 64.45%) stated that they consume cigarettes during their working hours, whilst 75 (35.55%) do not consume cigarettes during their working hours. The largest number of subjects, 746 (87%), believed that during their time in school or the school yard, pupils are in a situation where they see educational workers smoking. Most subjects stated that a special place was provided in the school for smokers (449), whilst 170 subjects stated that it was permitted to smoke cigarettes in the work place without any form of restrictions (Figure 1).

To the question "Are you acquainted with the negative consequences of consuming cigarettes?" most of the subjects (759, 88.56%) replied that they were fully aware, 85 (9.92%) were superficially acquainted, and 13 (1.52%) were not acquainted with the negative consequences of consuming cigarettes and they did not care.

Of the total number of subjects who declared themselves to be smokers, the largest share, 82 (38.86%) of them, had not tried to stop smoking and were not thinking about it, 55 (26.07%) of them had tried and reduced their consumption, 53 (25.12%) subjects had tried to stop smoking but had not succeeded, whilst the smallest number of subjects, 21 (9.95%), had tried to stop smoking and had succeeded temporarily. For each subject, a dependence score was calculated, and the total results obtained by analysis of the Fagerstrom test are shown in Table 3.

On the basis of the results obtained, the level of nicotine dependence of education workers in CBC was calculated. In the 211 subjects who declared themselves to be smokers, nicotine dependence was mostly mild, 53.55% (n=113), then moderate, 44.54% (n=94), and severe in 1.89% (n=4) (Figure 2).

Men mostly showed a moderate level of nicotine dependence (57.35%), whilst women most often show mild nicotine dependence (60.83%) (Table 4).

Table 3. Results of the Fagerstrom Test

Nicotine dependence	Male		Female		Total	
	N	%	N	%	N	%
1. How soon after you wake up do you smoke your first cigarette?						
a. Less than 5 minutes	3	4.41	9	6.29	12	5.68
b. 5-30 minutes	43	63.23	57	39.86	100	47.38
c. 30-60 minutes	22	32.35	77	53.84	99	46.91
2. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., at the library, in the theatre, the doctor's)?						
a. Yes	15	22.05	19	13.28	34	16.10
b. No	53	77.94	124	86.71	177	83.87
3. Which cigarette would you hate most to give up?						
a. The first in the morning, after you wake up	31	45.58	61	42.65	92	43.59
b. Any other during the day	37	54.41	82	57.34	119	56.39
4. How many cigarettes per day do you smoke?						
a. < 10 cigarettes	10	14.70	71	49.65	81	38.37
b. 11-20 cigarettes	38	55.88	64	44.75	102	48.33
c. 21-30 cigarettes	19	27.94	6	4.19	25	11.84
d. ≥ 31 cigarettes	1	1.47	2	1.39	3	1.41
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?						
a. Yes	27	39.70	29	20.27	56	26.54
b. No	41	60.30	114	79.72	155	73.45
6. Do you smoke when you are so ill that you are in bed most of the day?						
a. Yes	19	27.94	28	19.58	47	22.27
b. No	49	72.05	115	80.42	164	77.72

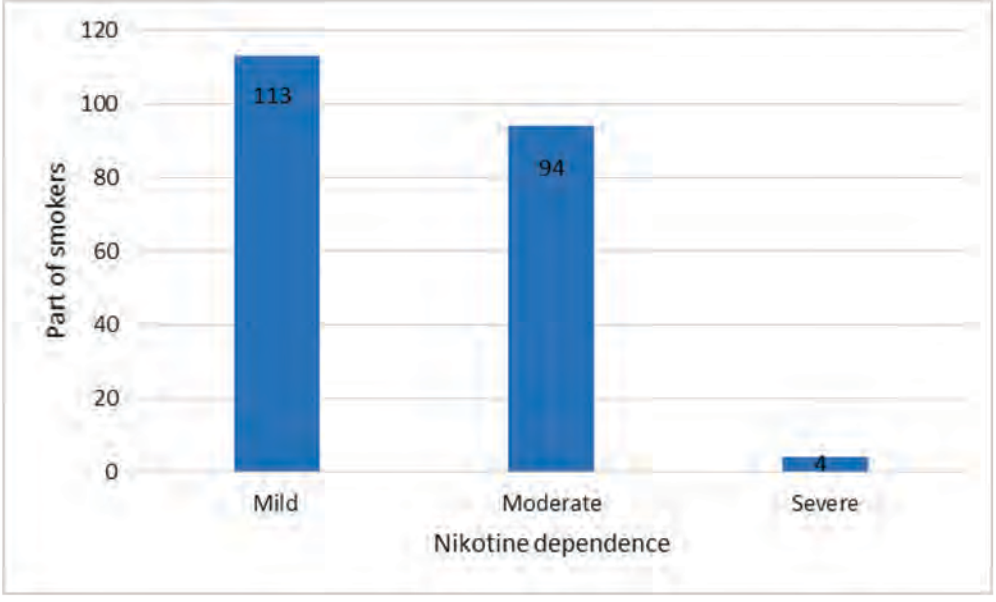

Figure 2. The Level of Nicotine Dependence of Education Workers in the Central Bosnia Canton.

Table 3. Results of the Fagerstrom Test

Level of nicotine dependence	Total	M	F
	N (%)	N (%)	N (%)
Mild nicotine dependence	113 (53.55)	26 (38.23)	87 (60.83)
Moderate nicotine dependence	94 (44.54)	39 (57.35)	55 (38.46)
Severe nicotine dependence	4 (1.89)	3 (4.41)	1 (0.70)

Discussion

Our research showed that every fourth teacher in schools in CBC smokes. These findings also indicate that there is no statistically significant difference in smokers in relation to gender, nor in relation to place of residence (urban/rural). The largest number of smokers had consumed cigarettes for longer than 15 years. In addition, about 30% of teachers who are involved in the teaching process with the task of teaching children about improving their life style habits, also smoke. This shows that there is a significant number of smokers amongst teaching staff, and also that smoking habits are very prominent. Although a statistically significant difference was expected between the subjects who smoke in relation to gender and place of residence, it was not found. This expectation was based on most research which indicates that of the almost 20% of the world's population who smoke, men are represented more in a ratio of 4:1 (about 800 million men and 200 million women) (11). However, mortality from smoking indicates a different relationship. Every year 6 million active smokers die from smoking and also 600,000 passive smokers, 75% of which are women and children (12-14).

On the other hand, our study showed that in the subject population there is a statistically significant difference in their attitude about how far smoking cigarettes by teaching staff encourages pupils to smoke as well. Smokers take the attitude that their consumption of cigarettes does not encourage pupils to smoke as well, but this attitude is significantly different in smokers in comparison to non-smokers. Otherwise, smoking by teachers, parents and friends is described as an important predictor of adolescents' decisions to smoke or not (15, 16). Poulsen et al. discovered that teachers who smoke outside are a significant predictor of smoking by pupils (17).

Some authors did not express any correlation between teaching staff smoking and actual smoking by pupils (18). Others found evidence of a positive link between teachers who smoke in the school yard and smoking by older pupils, but also younger pupils (19). Several mechanisms may explain this connection between smoking by teachers and smoking by pupils. According to the Social Ecological Model and the Social Learning Theory, pupils identify with the behaviour of teachers because teachers are deemed to be adult models and leaders in creating opinions and attitudes (19-21).

Our research showed that the largest number of subjects (87%) were aware of the fact that pupils have the opportunity, during their time in school or in the school yard, to see education workers smoking. In this situation, girls are particularly vulnerable, where a high prevalence of smoking noted amongst the school staff was linked to a greater likelihood of starting to smoke and less likelihood of stopping smoking (22, 23).

Since teachers are models and examples for pupils, the importance is stressed of teachers not consuming tobacco and avoiding smoking in the school yard (24). According to data collected by the GYTS (Global Youth Tobacco Survey) research in

2010, in Italian youth we see that in fact both teachers and pupils often smoke in school and outside of school (44%), whilst almost 56% of the subjects stated that they have seen teachers and pupils smoking inside the school building (25). The GYTS data collected in Croatia in 2011 show that as many as 78.7% pupils are surrounded by people who smoke in closed public spaces (26).

Our research showed that the average age of the subjects who declared themselves to be smokers was 43.61 years, and the average length of time they had been smokers was 14.84 years, which indirectly tells us that education workers start smoking relatively late. Research by various authors shows that most adult smokers lit their first cigarette at an age up to 18 years, or they became dependent before they came of age (25, 27, 28). Analysis on the group level shows a significant increase in smoking from adolescence to young adulthood, and an insignificant fall after the middle of the second decade of life (27, 29).

Due to the risk of developing a state of dependence, preventive programmes should educate young people to take the stance that it is risky to try, and even more to continue to experiment with cigarettes. The results of tests so far suggest that anti-smoking messages sent through schools and the media help to prevent the use of tobacco and the intention to smoke. In contrast, the effects of family warnings of the harmful effects of smoking are, in the best cases, insignificant (30).

Analysis of the results of the Fagestrom test to assess nicotine dependence showed a small number of teachers with severe nicotine dependence. However, a significant number of teachers showed a moderate degree of nicotine dependence, especially when considered in relation to the length of time they had been smoking and the age of the subjects. Women tend to form stronger nicotine dependence than men, although in general they smoke fewer cigarettes a day and have the tendency to use cigarettes with lower nicotine content. Women less often decide to stop smoking and more often relapse after an attempt to stop (31). Our findings indicate that men showed a higher extent of moderate dependence in contrast to women, in whom the predominant dependence was mild.

The effect of teachers who smoke should also be considered in the context of the influence of parents, relatives and friends. The effect increases of the presence of a teacher who smokes in a situation where parents and friends do not smoke (16). The prevalence of smoking in adolescents is significantly lower amongst pupils in countries with moderate to strong national regulations on smoke-free schools in comparison with those in countries with poor implementation or even no school policies in place (32, 33). Prohibition of smoking in the public sector may be seen to be successful in reducing smoking in adolescence. In the twenty-first century many countries have signed the Framework Convention of the World Health Organization on Tobacco Control and in recent years an increasing number of countries and regions are implementing partial or complete prohibition of smoking in order to protect the population from passive exposure to smoke (34). Acts on prevention of smoking are deemed to be self-executing. It seems this concept derives from certain developed countries where the cost of implementation is small. It is characteristics that this is seen in jurisdictions with a lower prevalence of smoking and a relatively long history of tobacco control (35).

A review by the International Agency for Research on Cancer of the World Health Organization, taking into account legislative restrictions of smoking of various strengths, has already pointed out that alignment, although it is often satisfactory in most countries, is inadequate in some (35).

Perkins and Neumayer analyse respect for anti-smoking laws in all countries, re-

gardless of the type of legislation they have, whether comprehensive or limited, complete prohibition of smoking, or only in one or some sectors, or restriction of smoking to certain closed spaces ear-marked for smoking. They found that alignment with the WHO's Framework Convention on Tobacco Control is four times greater in countries with comprehensive national laws, in comparison with countries without strong policies on implementation of legislation on prohibition of smoking (36). So for example, four years after adoption of a comprehensive national law in Greece, the vast majority of its citizens reported smoking in public places. Bosnia and Herzegovina is one of the countries where the rule of law is at a very low level. Further, legislation prohibiting smoking in open spaces could also have other useful effects, such as a reduction in the initiation of young people, the launch of attempts to stop smoking, a reduction in smoking, discussion of and reduction in tobacco consumption in our society, as well as the development of new initiatives dealing with the school environment (37).

The connection between smoking by pupils and smoking by teachers depends on the level of exposure to smoking by teachers. When teachers smoke in schools, this underlines the idea that school anti-tobacco policies are not restrictive and therefore may also reduce the perception of the severity of the penalty linked with violations of policies. It is important to mention that the size of the correlation between teachers smoking and pupils smoking is greater, similar or lower than between parents smoking and children smoking, depending on the level of visibility of the teacher who smokes. Watching a teacher smoke every day, or almost every day, on school grounds, shows a greater correlation with smoking behaviour than in relation to a parent who smokes. The strength of the association is similar when pupils watch a teacher smoke for more than half the day, and lower when the pupils watch the teacher smoke for about half the day (3, 38). Schools provide the ideal environment for preventive initiatives amongst children and adolescents. However, some research has concluded that in the long term there is no consistent effect from prevention programmes in schools (39). Cochrane's review of 2013 considered 49 randomized studies on prevention of smoking amongst school-aged children and found a significant effect in prevention of pupils starting to smoke amongst pupils who had never smoked. The estimated difference in the incidence of smoking between schools with interventions and controls was 12% (40).

However, not all programmes were equally effective. Programmes focussing on social competence for instance were more effective than those merely providing information, or focussed solely on social effects. It seems that the success depends on the programme content, the intensity of the intervention and implementation of the programme (41). Intervention for prevention of and stopping smoking that deals with the social context, including life experience, social relationships, organizational structures and social influences have proved to be significant and relevant for the target audience, as well as effective in changing behaviour in health, which may reduce the risk of development of chronic diseases (42, 43). The Tobacco-Use Prevention Education (TUPE) programme in California has had significant results. In the schools that finance TUPE, teachers believe that prevention of smoking is a priority, that smoking prevention programmes in their school are effective, and they feel better prepared for prevention of the use of tobacco amongst pupils (44).

Limitations of the Study

The limitations of this study stem from the fact that it relies on a self-assessment ques-

tionnaire about something which may be qualified as socially undesirable behaviour. It is important to convince the subjects that their answers are not subject to any form of judgement or disapproval.

In future research it is necessary to test the interest of teachers in participation in programmes to help them stop smoking.

Conclusion

A quarter of the education workers in CBC are smokers, and every second smoker has developed a mild level of dependence on nicotine, whilst only an insignificant number of smokers have developed a severe level of dependence. Every fourth subject stated that they respect the prohibition of smoking in school, whilst nine out of ten subjects are aware of the fact that children in school see education workers smoking. In schools in the CBC there are no preventive programmes to combat smoking by education workers and pupils.

What Is Already Known on this Topic

Smoking is not just a bad habit - it is one of the most significant public health problems today. The quality of a smoker's life is much worse and they die earlier. The worrying fact is that the trend of smoking cigarettes among adolescents is increasing. Scientific evidence undoubtedly confirms the harmful effects of tobacco smoke on human health and the quality of life, as well as the fact that there is no safe level of exposure to tobacco smoke. A special form of exposure to tobacco smoke is so-called passive smoking, to which young people and children are particularly exposed due to the irresponsible behaviour of smokers (teachers, parents...).

What this Study Adds

Our survey showed that one in four teachers in schools in the Central Bosnia Canton smoke. An analysis of the results of Fagestrom's nicotine addiction assessment questionnaire identified a significant number of teachers showing a moderate degree of nicotine dependence, especially viewed in relation to the length of time they have been smokers. A significant number of teachers involved in the teaching process, with task of teaching children how to improve their life style habits, are smokers. Students identify with their teachers' behaviour because teachers are considered to be adult role models and leaders in creating opinions and attitudes. The respondents are aware of the fact that while they are in the school or the school yard, students are able to see their teachers smoking.

Authors' Contributions: Conception and design: SK and MP; Acquisition, analysis and interpretation of data: SK, MP, and KE; Drafting the article: SK; Revising the article critically for intellectual content: SK, MP, and KE; Approved final version of the manuscript: SK, and KE.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report

Case Series
Acta Medica Academica 2019;48(2):205-216
DOI: 10.5644/ama2006-124.259

Subscapularis Myotendinous Junction Tears Presenting with Posterior Shoulder Pain in Overhead Throwing Athletes

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Received: 30 November 2018
Accepted: 13 May 2019

Key Words: Subscapularis ▪ Posterior Shoulder Pain ▪ MRI, Overhead Throwing Athlete ▪ Pitcher.

Objective. Acute inferior subscapularis myotendinous junction injuries are occasionally seen in overhead throwing athletes, and can present with posterior shoulder pain. **Case Reports**. Four professional baseball pitchers presented with acute onset of posterior shoulder pain while pitching. After thorough, routine physical examination of the shoulder by the referring orthopaedic surgeon magnetic resonance imaging (MRI) was performed within 7-10 days of the onset of presenting symptoms and interpreted in consensus by 2 fellowship-trained musculoskeletal radiologists with 9 and 5 years of experience and a musculoskeletal radiology fellow. The patients were then treated conservatively for subscapularis musculotendinous injuries and clinically assessed for symptom resolution before they were allowed to return to play. **Conclusion**. Inferior subscapularis myotendinous junction injuries should be included in the differential diagnosis of baseball pitchers with posterior shoulder pain.

Introduction

Shoulder pain is common in overhead throwing athletes due to the extreme stresses placed on the shoulder during the throwing motion. While shoulder injuries can occur in all types of overhead throwing athletes, including quarterbacks, tennis players, swimmers, volleyball players, and water polo players, professional baseball pitchers are especially prone to shoulder injuries (1). In fact, 57% of professional baseball pitchers will experience shoulder pain during their careers (2). Additionally, shoulder injuries are the most common injuries in amateur and professional baseball players and result in the most total days of pitching missed (3, 4).

Diagnosing the cause of shoulder pain in overhead throwing athletes can be challenging for a variety of reasons. Pain is often due to overuse from a complex throwing mechanism and the actual inciting event may be difficult to pinpoint. Moreover, many different types of injuries, or a combination of multiple injuries, can manifest in similar symptoms (1). For these reasons, this topic is important to understand for sports medicine physicians, orthopaedic surgeons, and musculoskeletal radiologists.

During pitching, the thrower must generate high energy levels to reach peak acceleration and velocity. During deceleration and after ball release, the forces must then dissipate through the stabilizing structures of the shoulder (5-8). Because of these complex biomechanical forces, a number of structures, including the glenoid labrum, the glenohumeral joint capsule, humerus, rotator

cuff tendons, muscles, and upper extremity vessels and nerves can potentially be injured, leading to the thrower's pain (1, 5, 9-18).

To our knowledge, the location of where most pitchers experience their pain has not been reported. However, in our experience, acute onset of predominantly posterior shoulder pain occurs in only a small number of these athletes. The most commonly encountered etiologies for posterior shoulder pain in an overhead thrower include posterior muscle strains, internal impingement, which consists of posterior glenoid labral injuries and rotator cuff tendon tears, glenohumeral internal rotation deficit (GIRD), scapular dysfunction, or referred pain from cervical spinal injuries. Occasionally, unusual injuries can be associated with predominantly posterior pain in overhead throwers.

We describe four professional baseball pitchers, each presenting with acute onset posterior shoulder pain while throwing and subscapularis inferior bundle edema along its musculotendinous junction seen on magnetic resonance imaging (MRI) that was felt to represent the main site of recent injury.

Case Report

Methods

Between 2006 and 2015, four professional baseball pitchers, ages 22, 25, 24 and 33, presented to the team's orthopaedic surgeon with acute onset of posterior shoulder pain that developed while pitching. Routine MRI of their symptomatic shoulders was performed within 7-10 days of initial injury. MRI was performed either on 1.5 tesla (T) magnets (Aera, Siemens, Erlangen, Germany) (Symphony, Siemens, Erlangen, Germany) or 3 T magnets (Verio, Siemens, Erlangen, Germany) (Skyra, Siemens, Erlangen, Germany). Routine shoulder MRI pulse sequences were obtained in axial, sagittal and coronal planes with respect to the glenoid fossa utilizing T1-weighted, proton density, and fluid-sensitive sequences. Because our routine shoulder MRI protocol changed during the time period in which these cases were collected there is some variability in the imaging protocols used in the MRIs of these patients. We have provided our current protocol on Siemens 3 T magnets (Table 1). The studies were each interpreted by a fellowship-trained musculoskeletal radiologist and reviewed with the referring orthopaedic surgeon at the time of exam completion. Once a pattern of findings was identified within this set of patients, the clinical presentation and imaging features of each study were again reviewed by 2 fellowship-trained musculoskeletal radiologists (9 and 5 years of experience respectively), a musculoskeletal radiology fellow, and the referring team orthopaedic surgeon (24 years of experience). The case presentations are summarized in Table 2. The study was granted an internal review board waiver by the institutional ethics committee because no experimental diagnostic modalities were used.

Table 1. Routine MRI Shoulder Protocol on Siemens 3T Scanners

Sequence	FOV*	Matrix/NEX†	ST‡	TR§	TE‖	FA¶	ETL**	BW††
Axial T2‡‡	12-16	320x256/2	3	3,000-4,000	50-70	140	12-16	200-300
Sagittal T2‡‡	12-16	320x256/1 (with 100% oversampling)	3	3,000-4,000	50-70	150	12-16	200-300
Coronal T2‡‡	12-16	320x256/1 (with 100% oversampling)	3	3,000-4,000	50-70	150	12-16	200-300
Sagittal T1***	12-16	320x256/2	3	400-800	<20	150	4	200-300

*Field-of-view; †Number of excitations; ‡Slice thickness; §Repetition time (msec); ‖Echo time (msec); ¶Flip angle; **Echo Train Length; ††Bandwidth; ‡‡Fat-suppressed; ***Nonfat-suppressed

Table 2. Summary of Patient Presentations

Patient No.	Age (year)	POT* (years)	Acute injury	Subscapularis appearance on MRI	Additional injuries seen on MRI	Treatment	RBF†
1	22	3	No	Grade 2‡	Posterior glenoid labral tear	Rest and rehabilitation	Yes
2	25	4	Yes	Grade 2§	Moderate supraspinatus tendinosis and small partial-thickness tendon tear	Rest and rehabilitation	Yes
3	24	4	Yes	Grade 2‖	None	Rest and rehabilitation	Yes
4	33	7	Yes	Grade 2¶	Posterior labral detachment	Rest and rehabilitation	Yes

*Professional overhead throwing; †Return to baseline function; ‡Inferior myotendinous junction; §Inferior myotendinous junction strain; ‖Inferior myotendinous junction strain; ¶Inferior myotendinous junction strain with moderate muscle fatty atrophy.

Case Presentation 1

The first patient was a 22-year-old, right hand dominant, professional baseball pitcher with 3 years of experience. The pitcher reported gradual worsening of shoulder achiness and soreness with pitching over his first four games of the season. He also noted loss of speed and accuracy. After an acute exacerbation during his fourth start, the patient reported new onset of shoulder pain that he felt was located posteriorly and was separate from his prior, vaguer symptoms. The pitcher could not recall a specific inciting event. Additionally, the pain was unrelated to arm positioning or phase of throwing. Physical examination by the team orthopaedic surgeon was negative for tenderness to palpation. Strength testing of the affected arm revealed no loss of strength. Reduced internal rotation was noted with asymmetrically decreased internal rotation to the T6 level on

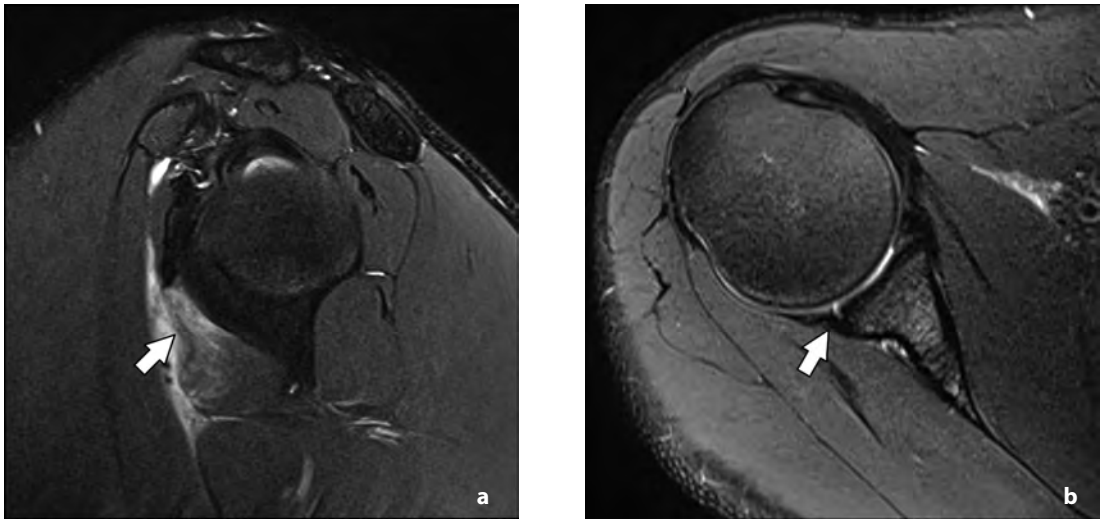

Figure 1. 22-year-old, right hand dominant, professional baseball pitcher with shoulder achiness following throwing: (a) Sagittal oblique T2-weighted turbo spin echo (TSE) fat-suppressed (FS) MR image of the right shoulder shows a focal grade 2 muscle strain of the inferior subscapularis muscle at the musculotendinous junction (arrow) and additional fascial edema extending toward the axilla. Note high signal intensity in the affected region, (b) Axial T2-weighted TSE FS MR image of the same shoulder shows a small focal posterior labral partial detachment (arrow) without significant bone marrow or soft tissue edema around the labral tear, and with preservation of the adjacent articular cartilage.

the symptomatic side compared to internal rotation to the T6 level on the asymptomatic side. A routine shoulder MRI was performed and demonstrated a focal inferior subscapularis muscle partial tear and edema at the musculotendinous junction, 1 cm medial to its humeral attachment with additional fascial edema extending toward the axilla (Figure 1a). Although a small focal posterior glenoid labrum tear with partial detachment was noted from the posterior 8-10 o'clock positions, this was felt to be a finding that was unrelated to his acute discomfort as there was no significant bone marrow or soft tissue edema around the labral tear, and the adjacent articular cartilage was preserved (Figure 1b). The patient was treated with 10 days of rest and nonsteroidal anti-inflammatory medication. The pitcher was also instructed to follow a stretching and rehabilitation throwing program. Following this course of therapy, there was a gradual resolution of pain. Shortly thereafter, the pitcher returned to normal function and regained normal strength/velocity.

Case Presentation 2

The second patient was a 25-year-old, right hand dominant, professional baseball pitcher with 4 years of experience. The pitcher experienced an acute shoulder injury during off-season throwing. He complained of posterior inferior shoulder soreness that radiated to the posterior axillary line following the shoulder injury. The patient could not correlate the injury with a particular arm position or phase of throwing. The physical examination performed by the same team orthopaedic surgeon was negative for tenderness with palpation or loss of strength. Additional orthopaedic tests were negative and the etiology of the pain was unclear. A routine shoulder MRI was performed and demonstrated a grade 2 subscapularis inferior bundle muscle strain, and the site of the tear and surrounding fascial edema were adjacent to the axillary neurovascular bundle (Figure 2a). There was moderate supraspinatus tendinosis and a 2 mm partial-thickness, articular surface tendon tear, both of

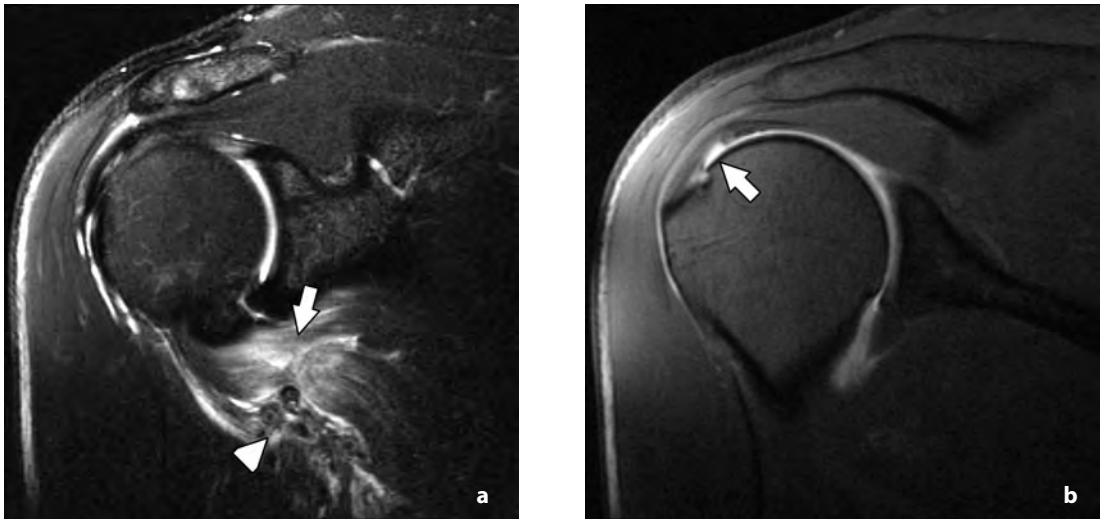

Figure 2. 25-year-old, right hand dominant professional baseball player with an acute shoulder injury during off-season pitching: (a) Coronal T2-weighted TSE FS image of the anterior right shoulder shows a partial-thickness tear at the subscapularis musculotendinous junction (arrow). Note high signal intensity in the affected region. Additionally, the axillary neurovascular bundle is seen in close proximity to the site of this tear (arrowhead), (b) Coronal T1-weighted spin echo (SE) FS MR arthrogram image of the same shoulder at the level of the anterior supraspinatus tendon shows contrast undercutting the articular surface of the tendon (arrow), consistent with partial-thickness, articular surface tendon tear.

which were unchanged dating back at least 2 years (Figure 2b). No additional pertinent findings were seen. The team orthopaedic surgeon treated the patient for a muscle strain with ice and off-season rest. An off-season rehabilitation throwing program was also instituted. Following the course of therapy, the player noted resolution of pain. The pitcher fully recovered his function by the time the start of the next season began.

Case Presentation 3

The third patient was a 24-year-old, right hand dominant, professional pitcher with 4 years of experience. The player experienced an initial onset of burning posterior shoulder pain after throwing a fastball in the 3rd inning of a regular season game. He continued to have posterior pain and achiness with throwing through the 6th inning when he was finally substituted. The patient was unable to pinpoint a time in his throwing cycle when he injured his shoulder. Additionally, the pain was unrelated to arm position during throwing. The team orthopaedic surgeon identified posterior shoulder soreness with abduction and external rotation. There was also reduction in the pitcher's internal rotation to the T6 level on the symptomatic side compared to the T4 level on the asymptomatic side. No tenderness on palpation was present on physical examination, and further shoulder testing for rotator cuff or labral tears was also negative. A routine shoulder MRI was performed and demonstrated a focal partial-thickness tear of the inferior subscapularis musculotendinous junction (Figure 3a). There was additional fluid tracking along the anterior surface of the muscle toward the axilla. No additional rotator cuff or labral abnormality was seen. The patient was treated in a similar fashion to the previous two pitchers who were felt to have symptomatic inferior subscapularis muscle strains, including ice and 10 days of rest. He was given an additional course of oral steroids. The player also gradually returned to

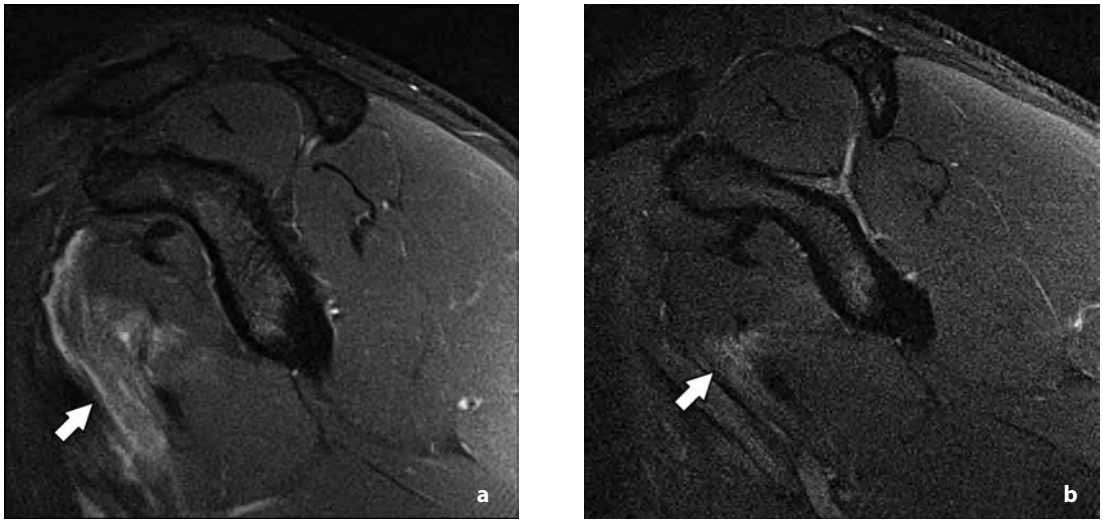

Figure 3. 24-year-old, right hand dominant professional baseball pitcher with posterior shoulder pain after throwing a fastball: (a) Sagittal T2-weighted TSE FS MR image of the right shoulder shows a focal partial tear of the inferior subscapularis musculotendinous junction (arrow) and additional fluid tracking along the anterior surface of the muscle toward the axilla. Note high signal intensity in the affected region, (b) Sagittal T2-weighted TSE FS image of the same shoulder from a follow-up MRI 6 weeks after starting a course of conservative treatment, showed improvement in the inferior musculotendinous subscapularis muscle edema (arrow), consistent with resolving injury. Note decreased high signal intensity in the affected region.

activity with a rehabilitation throwing program. The patient noted mild improvement of symptoms for a brief period, but had a relapse injury 6 weeks later while warming up before a game. A follow-up MRI was then performed and showed mildly progressive inferior subscapularis musculotendinous partial-thickness tear (not shown). No other acute abnormality was seen. Once again, the patient was treated for an exacerbation of a muscle strain with conservative therapy. A second follow-up MRI examination 6 weeks later was performed and showed significant decrease in the inferior musculotendinous subscapularis muscle edema, consistent with resolving injury (Figure 3b). Over the remainder of the season, the pitcher experienced gradual resolution of pain and returned to normal function.

Case Presentation 4

The final, most recent patient was a 33-year-old, right-hand dominant professional baseball pitcher with 7 years of experience. The pitcher experienced acute onset of progressive posterior shoulder pain while warming-up prior to entering a game. Similar to the prior cases the patient could not identify a phase of the pitching cycle in which the in-

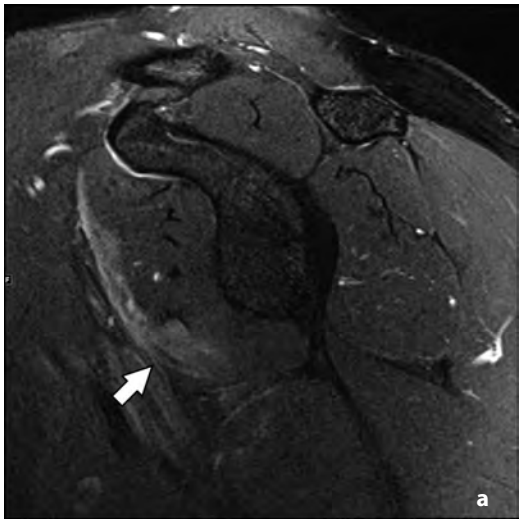

Figure 4. 33-year-old, right hand dominant professional baseball pitcher with posterior shoulder pain experienced during warmups: (a) Sagittal T2-weighted TSE FS MR image of the right shoulder shows a focal partial tear in the region of the subscapularis musculotendinous junction (arrow) and additional fluid tracking along the anterior surface of the muscle toward the axilla. Note high signal intensity in the affected region, (b) Sagittal T1-weighted nonfat-suppressed MR image of the same shoulder demonstrates moderate inferior subscapularis muscle fatty atrophy (arrow). (c) Axial T2-weighted TSE FS MR image of the same shoulder on a preseason MRI performed 8 months prior depicts a posterior labral tear (arrow). The patient was asymptomatic at the time of initial MRI.

jury occurred, and the symptoms did not worsen in particular positions or with certain movements. The patient had suffered a shoulder strain 5 years prior that was treated with rest followed by a program of physical therapy, and was able to recover full function. Physical examination revealed weakness with shoulder abduction and a deficit in internal rotation, but was negative for tenderness to palpation. Additional testing for rotator cuff or labral pathology also was deemed negative. A routine shoulder MRI was performed and demonstrated a focal partial-thickness tear of the inferior subscapularis musculotendinous junction (Figure 4a). There was additional fluid tracking along the anterior surface of the muscle toward the axilla. There was moderate inferior subscapularis muscle fatty atrophy (Figure 4b). Of note, the patient had a preseason MRI examination 8 months prior demonstrating a posterior labral detachment at the posterior 9 o'clock position (Figure 4c), but was asymptomatic at that time. On the repeat MRI examination, the detachment was inconspicuous, suggesting healing (not shown). No additional rotator cuff or labral abnormality was seen. The patient was again treated conservatively for muscle strain, including off-season rest and gradual return to overhead throwing with a rehabilitation program. He has noted significant reduction in his discomfort.

Discussion

Although shoulder pain is common in overhead throwing athletes, acute onset of predominantly posterior shoulder pain seems uncommon. Posterior shoulder pain in these patients is typically the result of high stresses placed on the internal structures of the shoulder with alteration of biomechanics due to repetitive, forceful throwing (13, 19). The commonly encountered etiologies of posterior shoulder pain include internal impingement with posterior rotator cuff strains or tendon tears, and posterior labrocapsular injuries, or GIRD (11, 13, 20, 21).

Posterior rotator cuff pathology is typically due to degeneration of the cuff from the extreme repetitive forces generated during pitching (22). The most common locations for injury include the undersurface of the posterior half of the supraspinatus and anterior half of the infraspinatus tendon (23). Imaging findings range from tendinosis, to partial-thickness and full-thickness rotator cuff tendon tears (24, 25).

Internal, or posterior, impingement is a mechanism that has been proposed for the cause of a spectrum of injuries in the overhead throwers that includes posterolateral humeral head subcortical edema and pseudocyst formation, posterior labral fraying/tearing, and partial-thickness articular surface rotator cuff tendon tears (24, 26, 27). Impingement occurs during the late cocking phase of throwing when the arm is in abduction, external rotation, and extension. This positions the posterior superior labrum between the greater tuberosity of the humeral head and the posterior rotator cuff, which places these structures at risk for injury (13, 19).

The propensity for posterior glenoid labral tears in throwers is believed to be the result of tightening of the posterior band of the inferior glenohumeral ligament (IGHL), as a result of repetitive tensile forces during the follow-through phase of throwing (13). A contracted posterior band of the IGHL shifts the humeral head contact point on the glenoid and results in GIRD. This ultimately allows hyper-external rotation, which leads to increased torsional forces transmitted through the biceps labral complex (BLC), resulting in SLAP type II posterior superior labral lesions (13, 28). The changes seen with GIRD are initially adaptive in overhead throwers and allow for the generation of greater throwing forces. In fact, many professional overhead throwing athletes exhibit

physical examination findings of GIRD even if they do not experience findings often seen in symptomatic GIRD, which includes progressive/chronic shoulder pain and achiness that is frequently posteriorly located, along with decreasing throwing velocities (13). It is possible the alterations in biomechanics experienced with GIRD, such as the posterosuperior shift of the humeral head, could increase strain on anteroinferior supporting structures, such as the inferior bundle of the subscapularis muscle, and predispose these athletes to subscapularis injuries.

To our knowledge, subscapularis muscle injury as the cause of posterior shoulder pain in adult overhead throwing athletes has not been previously reported. A literature search revealed two solitary case reports and one case series of subscapularis injuries causing shoulder pain in baseball players. One report described a case of subscapularis muscle strain that an outfielder endured from direct impact against a wall (15). A second case reported spontaneous subscapularis tendon rupture in a 50-year-old patient playing recreational baseball who experienced sudden pain after forcefully throwing a ball to a catcher from second base (29). This particular patient reported anterior shoulder pain. A third, more recent case series by Polster et al. described 11 baseball players with muscle strains involving the inferior half of subscapularis at musculotendinous junction (30). However, the case series included both pitchers and non-pitchers, and none of the cases reported a clinical history of posterior shoulder pain. Additionally, the series did not discuss whether the injuries were isolated.

We have found four professional baseball pitchers presenting with acute posterior shoulder pain while throwing, with unexpected subscapularis inferior bundle musculotendinous junction strains diagnosed on MRI. Similar to the work by Polster et al. the common finding in each of the four athletes was muscle edema within the inferior bundle of the subscapularis muscle at the musculotendinous junction, suggesting a low-grade muscle strain. The soft tissue edema also extended into the axilla along the inferior glenohumeral ligament.

In one of the four patients, no additional findings to explain the etiology of the patient's pain were seen. Specifically, there was no injury to the supraspinatus or infraspinatus muscles or tendons, MRI findings of internal impingement, or posterior labral pathology. The imaging of another of our patients did show moderate supraspinatus tendinosis with a small partial-thickness articular surface tendon tear. However, these findings were unchanged dating back 2 years, and therefore felt unlikely to be the source of acute pain. In the third patient, there was a small posterior labral detachment. However, the labral injury was not believed to be the cause of the patient's acute shoulder pain as there was no associated bone marrow edema to suggest an acute event, nor was there underlying glenoid articular cartilage loss that could explain posterior shoulder pain with throwing.

In the most recent case the patient had a posterior labral detachment seen on a preseason MRI examination 8 months prior. However, the patient was asymptomatic at that time, and on the subsequent study demonstrating a subscapularis musculotendinous strain, the labral tear was no longer visible suggesting healing. In addition to the acute subscapularis strain, there was inferior subscapularis muscle atrophy, likely related to a remote muscle strain sustained while pitching 5 years earlier, suggesting a recurrent or acute on chronic injury. Finally, although at least three of our patients demonstrated clinical findings of GIRD, which often does present with posterior shoulder pain, the patients presented with abrupt onset of pain that was either new or felt to be different in quality to existing symptoms. The sudden onset of symptoms is atypical of

GIRD but more commonly seen with musculotendinous injuries.

The findings of each study were reported to the team orthopaedic physician at the time of the exam, who agreed with the diagnosis of subscapularis inferior bundle muscle strain at the musculotendinous junction as the cause of the patients' posterior shoulder pain. The patients were treated conservatively with rest followed by a throwing rehabilitation program, and their symptoms improved within several weeks. No further potential etiologies for the pitchers' pain were detected in the subsequent weeks following presentation. Although three of the patients performed at a pre-injury baseline level for a substantial length of time following treatment, one of the patients did demonstrate gradual decline in function the following season. However, this was favored to be due primarily to the development of throwing shoulder microinstability and rotator cuff fatigue, with the subsequent development of rotator cuff tears.

While not frequently injured in overhead throwers, the subscapularis muscle, like the other rotator cuff muscles, has a dynamic role in pitching and experiences strong forces during the violent throwing motion (15, 31). The subscapularis muscle serves multiple functions in shoulder stabilization and motion. Its major roles include internal rotation, shoulder abduction, humeral head depression, and anterior stabilization (17, 18). During pitching, the subscapularis muscle begins eccentric contraction during the late cocking stage to halt external rotation while applying an anterior stabilization force to the glenohumeral joint (31, 32). The muscle reaches its maximal activation during the acceleration phase of throwing, contributing to violent internal rotation (16). Internal rotation forces reach as high as 185% of its maximum muscle strength, while internal rotation angular velocities can be as high as 7000 to 9000 degrees per second (16, 28, 31).

The mechanism of inferior subscapularis musculotendinous junction muscle injury in this group of throwing athletes is not entirely clear. Subscapularis injuries in general have mostly been described in the setting of rotator cuff tendon tears, as these would be more surgically relevant. Rotator cuff tears involving the subscapularis muscle are far less common than those involving the supraspinatus and infraspinatus muscles (33). When the subscapularis tendon is torn, it usually occurs in conjunction with supraspinatus tendon tears (34). Subscapularis tendon tears are typically degenerative (35). Although when acute traumatic tears do occur, they predominantly occur in younger patients (36).

The most common mechanism of injury of the subscapularis tendon is in the setting of external rotation (36). The greatest forces exerted on the musculotendinous complex occur in the setting of hyper-external rotation and abduction, when the subscapularis is maximally stretched during eccentric contraction (35). This occurs during the late cocking phase of throwing. However, it has recently been shown that throwers with a shorter arc of motion are more likely to experience subscapularis strains, suggesting that the greater angular acceleration and power generated by the subscapularis in the early acceleration phase may be the major contributing factor to injury (30). Either way, it is likely that these massive forces exerted on the subscapularis during extreme external rotation and by the subscapularis during powerful acceleration are major factors in subscapularis injury in the setting of pitching.

The subscapularis muscle is composed of two functionally independent bundles with separate nerve innervations, the upper and lower subscapular nerves which originate from the posterior cord of the brachial plexus (33, 34). This configuration allows each bundle to achieve different activity lev-

els during muscle activation (34, 37). As a result, the two bundles have different roles during shoulder motion. The lower subscapularis, which is more transversely oriented to the humerus during cocking motion, activates earlier and has greater activity during abduction and flexion (30, 37). Thus, it has a greater role in humeral head depression and resistance to anterior translation (37). The differences in the roles of the two bundles may help explain why the inferior bundle is more prone to injury that could occur during the late cocking hyper-external rotation/abduction phase.

The clinical presentation of subscapularis injuries varies depending on the mechanism of injury. The pain experienced in subscapularis musculotendinous injuries is typically more anterior than the pain experienced in other rotator cuff tears (35, 38). Additionally, patients with subscapularis injuries may present with night pain and shoulder weakness (37). Physical exam findings may demonstrate anterior shoulder tenderness, increased passive external rotation, and weakness with internal rotation, which can be tested by the subscapularis lift off test, subscapularis lag sign, and subscapularis belly press test or strength test (11, 35, 38).

The cause for posterior pain in our cohort is uncertain. Since the muscle and its musculotendinous junction are anterior structures, the sensation of posterior pain is unlikely to originate from the subscapularis itself. For this reason, it is postulated that the posterior pain from this injury is a referred pain caused by local irritation or stretching of nerves, which course along the site of injury. In each of our cases, soft tissue edema tracked along the musculotendinous junction toward the axilla, and is in close proximity to neurovascular bundles.

In particular, there are two nerves that are possible candidates for causing referred pain in the setting of subscapularis inferior bundle muscle strain. One candidate, the axillary nerve, courses near the inferior margin of the inferior glenohumeral ligament. The posterior branch of the axillary nerve gives rise to the superior lateral cutaneous nerve, which is a sensory nerve that supplies the skin over the lower two/thirds of the posterior deltoid. The second candidate is the radial nerve, which courses along the posterior wall of the axilla on the subscapularis muscle. The nerve directly gives off a posterior sensory branch, the posterior cutaneous branch of the radial nerve, which supplies the skin over the posterior upper arm and forearm. Of the two potential causes of referred pain, the posterior sensory distribution of the superior lateral cutaneous nerve branch more closely corresponds to the reported site of pain in this cohort. Therefore, referred pain from irritation of the axillary nerve as it courses alongside edematous soft tissues tracking toward the inferior glenohumeral ligament could result in the sensation of posterior shoulder pain in the setting of subscapularis inferior bundle strain.

Conclusion

While uncommon, inferior subscapularis musculotendinous junction strains do appear related to the symptom of acute onset posterior shoulder pain in our cohort of professional baseball pitchers. This injury is likely related to the violent mechanism of throwing, with extreme stretching of tendon fibers during hyper-external rotation and abduction, and extreme force generation during rapid acceleration of the arm through its arc of motion. While the exact etiology of the posterior pain is uncertain, it could be the result of local irritation of the axillary nerve and referred pain from its superior lateral cutaneous branch. Knowledge of this association in overhead throwing athletes, along with the signs and symptoms during presentation, may be helpful to clini-

cians to make the diagnosis and implement appropriate treatment more quickly.

What Is Already Known on this Topic

Very few published reports of subscapularis injuries in baseball players exist. 2 are case reports, one in player with direct trauma and the other in an older, amateur player. There has been one case series by Polster et al. in 2016 on subscapularis injuries in baseball players. However, this work does not directly address the location of presenting symptoms.

What this Study Adds

Although pitchers with posterior shoulder pain may most commonly present with posterior labrocapsular or posterior rotator cuff injuries, our work tries to highlight an injury to an anterior/inferior structure, the subscapularis muscle and tendon, that may seem somewhat remote from the site of presenting symptoms. As a result, this finding was initially surprising both to the referring orthopaedic surgeon and interpreting radiologist. To our knowledge this is the first description of this injury pattern presenting with posterior shoulder pain.

Authors' Contributions: Conception and design: IMO, EMT and SMG; Acquisition, analysis and interpretation of data: IMO, EMT, KJB and SMG; Drafting the article: EMT and IMO; Revising it critically for important intellectual content: IMO, EMT, KJB and SMG; Approved final version of the manuscript: IMO, EMT and SMG.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report

Case Report and Mini Review
Acta Medica Academica 2019;48(2):217-224
DOI: 10.5644/ama2006-124.260

A Case of Early FAP Diagnosis with Extraintestinal Manifestations on the Face

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Received: 10 December 2018
Accepted: 26 March 2019

Key Words: Gardner's Syndrome ▪ Osteoma ▪ Polyposis of the Colon.

Objective. Gardner's syndrome is a variant of familial adenomatous polyposis, characterized by gastrointestinal polyps, multiple osteomas, and skin and soft tissue lesions. Diagnosis by means of an understanding of its various symptoms is of great importance. **Case report.** This report presents the case of a 32-year-old man with obvious asymmetry and disfigurement on his face, due to multiple osteomas and soft tissue lesions. These clinical signs prompted us to further investigation, leading to the diagnosis of a case of Gardner's syndrome, with asymptomatic polyposis of the colon. Polyps have virtually an almost 100% risk of undergoing malignant transformation. **Conclusion.** Early diagnosis and treatment of this pathological entity are crucial, while the presence of osteomas should alert us to this diagnosis.

Introduction

Gardner's syndrome (GS), a phenotypic variant of familial adenomatous polyposis (FAP), is a rare autosomal dominant disease, characterized by a classical triad of signs: a) gastrointestinal polyps, b) multiple osteomas and c) skin and soft tissue lesions. Polyps have a very high risk, of almost 100%, of undergoing malignant transformation, in untreated cases (1, 2). Although Menzel was the first to describe the adenomatosis of the colon in 1721 (3), it was Gardner in 1951 who noted the significant correlation between extra-intestinal osseous and cystic tumours and FAP, a relationship which presented a very high probability for future malignancy (4). In the following years, alongside his colleagues, he proved that the syndrome had a dominant hereditary pattern of inheritance (5, 6). Multiple osteomas and soft tissue lesions with obvious asymmetry on the face, as the first manifestation of the disease, have been reported rather rarely as an early diagnostic sign of GS. We therefore present this case to alert clinicians to the possible existence of gastrointestinal pathology associated with signs in the maxillofacial region.

Case Report

A 32-year-old Caucasian man of Hellenic origin presented with an asymptomatic mass causing disfigurement in the left preauricular area. The mass had appeared 8 years previously and was gradually growing in size. During the physical examination a hard, fixed mass was palpable, while similar masses, smaller in size, were revealed at the angle of the mandible, bilaterally. The lat-

Figure 1. A: Photograph of the patient showing disfigurement of the left preauricular region and the right forehead. B: Photograph of the patient showing a swelling at the left angle of the mandible.

ter had been present since adolescence, and had been forgotten and somehow neglected by the patient. There were also three small sclero-elastic swellings in the soft tissues of the right forehead, with an appearance similar to lipomas or epidermoid cysts (Figure 1 A and B). The patient mentioned no other symptoms of acute or chronic character.

Orthopantomography revealed multiple well-defined opacities of the mandible and maxilla (Figure 2). During a computed tomography (CT) examination, an osteoma,

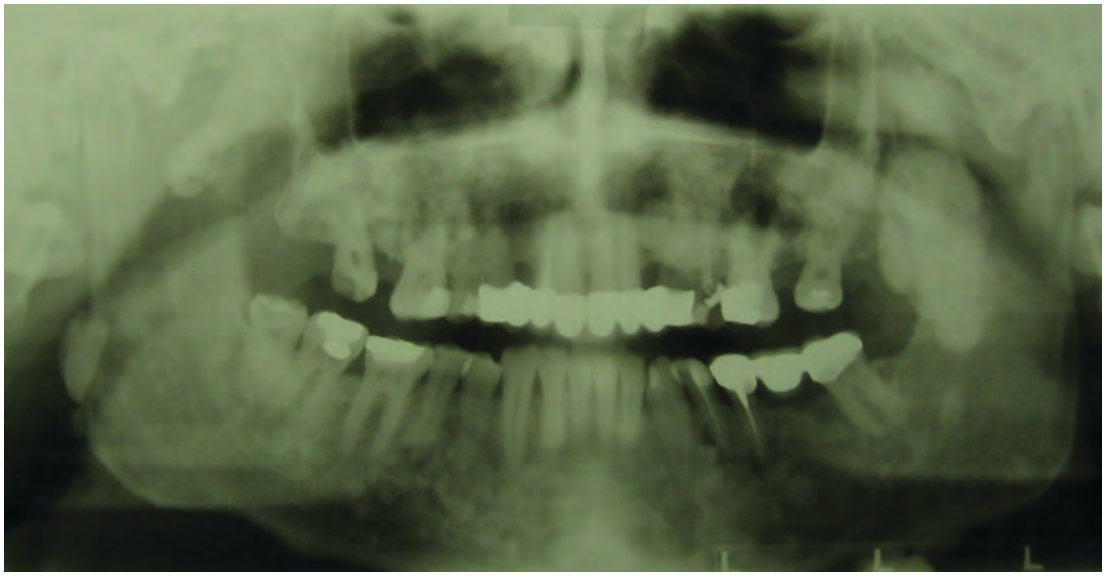

Figure 2. Panoramic radiograph showing multiple radiopaque lesions of the mandible and maxilla.

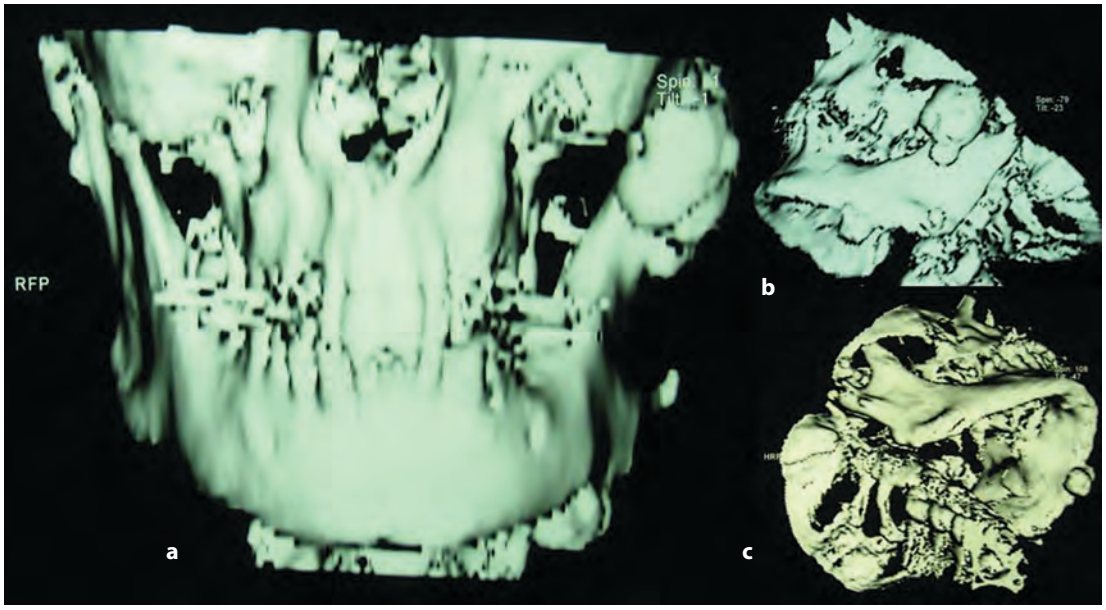

Figure 3. A, B and C: Computed tomography scans showing multiple osteomas of the mandible, maxilla, left ramus, maxillary antrum and pterygoid processes.

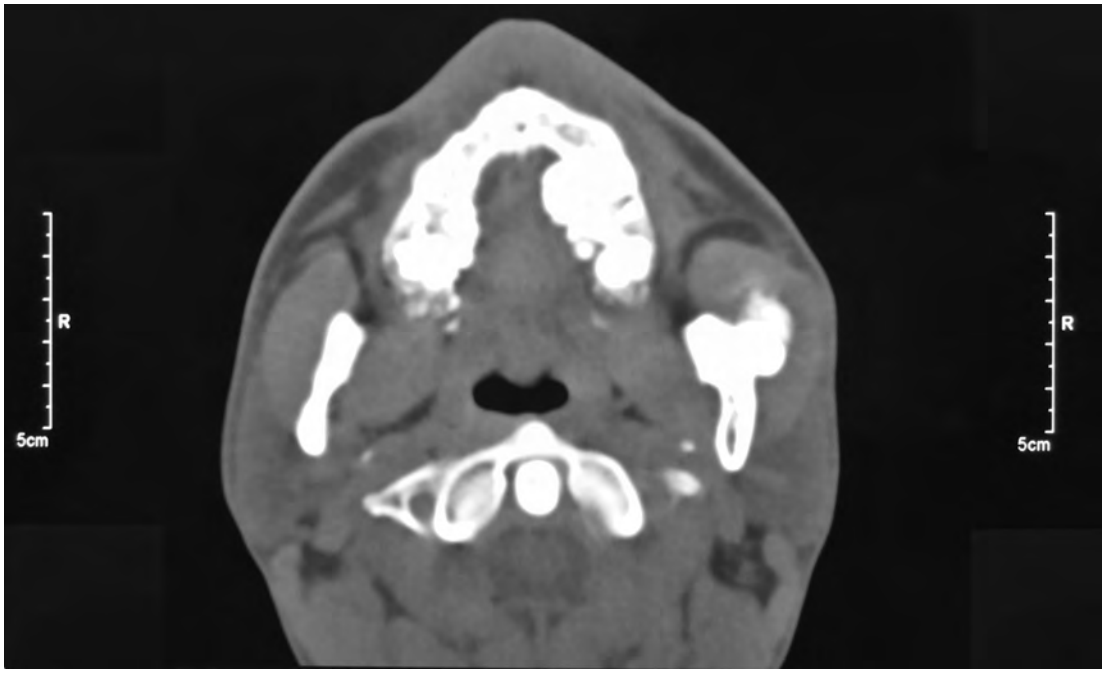

Figure 4. Three-dimensional CT-scan demonstrating the multiple osteomas.

with a maximum diameter of 3cm, was detected on the superior part of the left ramus. Similarly, multiple smaller osteomas of the mandibular angles, right ramus, the temporomandibular joint, maxilla, maxillary antrum and pterygoid processes were also detected (Figure 3 A, B and C; Figure 4).

The suspicion of possible GS guided our team towards a more detailed clinical examination and further diagnostic investiga-

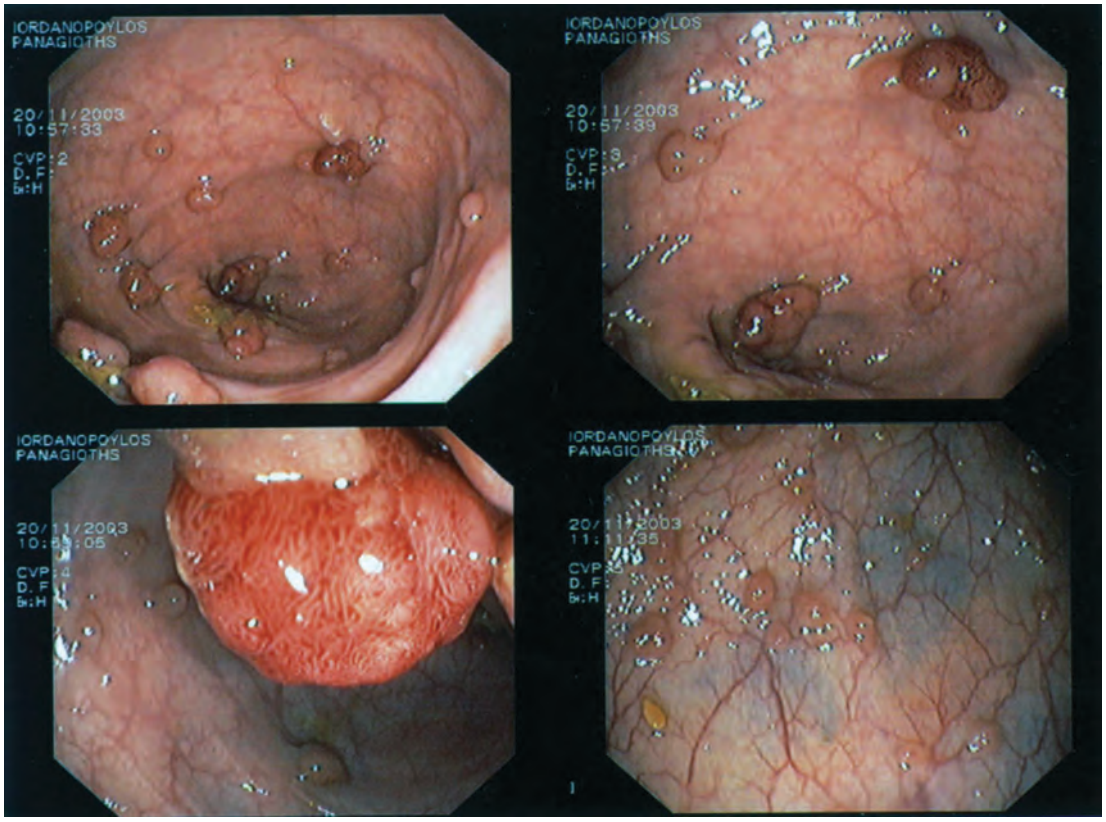

Figure 5. Multiple polyps of the intestine revealed by colonoscopy.

tion. The patient mentioned no complaints regarding his bowel function, while a routine physical examination showed no other positive relevant findings. According to his family history, there were no recorded cases or suspicion of familial polyposis syndrome. Routine laboratory examination showed all results within the normal limits. Although it might have been a case of lipomas, or multiple osteomas, a possible diagnosis of a GS was considered. Thus, the patient was referred for an urgent colorectal examination. Barium passage of the intestine and colonoscopy up to 120 cm were requested. Myriads of small, sessile polyps carpeting the colon were eventually detected (Figure 5). Histological analysis of the biopsied polyps showed adenomas of the colon. Moreover, a CT scan of the abdomen showed no evidence of soft tissue tumours intraperitoneally or retroperitoneally, while the ophthalmological examination was negative for congenital hypertrophy of the retinal pigmented epithelium (CHRPE). An ultrasound examination of the patient's neck showed the normal size and echogenicity of the thyroid gland. Long bone x-rays revealed no signs of other bone lesions.

The extraintestinal manifestations of the face combined with the plethora of polyps resulted in the final diagnosis of GS. A proctocolectomy with ileal pouch-anal anastomosis was performed a few days later. The postoperative course was uneventful and histological examination of the specimen confirmed a case of a plethora of adenomatous polyps, none of which had transformed malignantly. A thorough screening examination of all first-degree relatives of the patient was negative for FAP. During the follow-up the patient presented no signs of malignancy over a two year period, while

after that he neglected his preventive examinations.

Discussion

GS is inherited, with an autosomal dominant pattern with complete penetrance and variable expressivity, while one third of cases are spontaneous mutations, with no family history reported (1, 2). It is linked genetically to chromosome band 5q21, and the adenomatous polyposis coli (APC) locus. More than 200 mutations have been reported in the literature (7-10). The exact position at which the APC gene is mutated determines the manifestations and severity of the GS (11).

GS occurs in 1 person per million, while the incidence of FAP occurs in between 1 case out of 8,300 people and 1 out of 14,025 (12, 13). GS is characterized by extensive adenomatous polyps in the colon, which may also develop in any anatomical position of the gastro-intestinal tract (14). Although they begin to form in puberty, the average age of diagnosis is 22 years. Nevertheless, a number of paediatric cases have also been reported with a mean age of GS appearance of 4.1±3.6 years for males and 5.0±3.7 years for females (15). The patient may present with intermittent mucus discharge with defecation, bleeding per rectum, diarrhoea and abdominal pain. On the other hand, many patients seek medical care for cosmetic reasons (16), being unaware of the significance of the GS. Polyps tend to progress to malignancy in nearly 100% of untreated cases. However, in studies rates vary from 58% to 100% (17-20), while there are some reports of patients who received no treatment and remained free of malignancy (21). Malignancy is usually diagnosed in patients between 30 to 50 years of age, with an average age of diagnosis of 39.2 years (2, 22).

Extracolon manifestations, such as osteomas and soft tissue tumours, in many cases may precede the polyposis and therefore may contribute to an early diagnosis of a case of GS. Studies showed that 62-80% of patients with GS have osteomatous lesions, which are commonly detected during their second decade of life, as opposed to osteomas non-related to GS, which are revealed during the sixth decade (23, 24). Osteomas can be detected either during routine examinations or when they reach a considerable size, causing disfigurement. The mandible, especially its angle, is the most common location. However, osteomas may grow in the maxilla, the skull and the long bones (25-28). The case reported here presented with obvious asymmetry on the face, particularly in the left preauricular area and mandibular angle, due to multiple osteomas. Although a solitary osteoma of the jaw is a common incidental radiographic finding, if more than three are discovered, there is a high possibility of GS (29, 30). These manifestations, along with the soft tissue lesions on the patient's forehead, alerted us, resulting in an early diagnosis of GS with asymptomatic, benign polyposis of the colon, and therefore a postoperative course with an excellent prognosis.

Soft tissue lesions in GS include epidermoid cysts, in 50%-65% of patients, as well as lipomas, leiomyomas, neurofibromas and pigmented skin lesions (17). In our case, we also diagnosed three epidermoid cysts on the forehead. Cysts may also be detected in the scalp and extremities. However, the clinical examination of our case was negative. Osteomas and epidermoid cysts constitute early manifestations of GS, as they usually develop during puberty and may precede the formation of FAP. The late onset of symptoms, with the first suspicion of a facial deformity at the age of 24, and their diagnostic identification at the age of 32, with no manifestation of a FAP and its symptoms, is a rare case, in terms of a diagnostic approach to GS. The chronic asymptomatic presence of multiple colon polyps may limit the clini-

cal signs in the anterior skull area, rendering the task of correct diagnosis particularly difficult. Other clinical signs, such as CHRPE, which is frequently present in up to 80% of cases and may appear shortly after birth, or various dental abnormalities (e.g. unerupted, impacted, or supernumerary teeth) were not detected (31-34). Moreover, our clinical examination for fibromatous lesions was negative, while no other neoplasms, which may also appear simultaneously (e.g. thyroid carcinoma, periampullary carcinoma, osteosarcoma, liposarcoma, hepatoblastoma, adrenal adenoma and carcinoma) were identified (35-41). All those facts further diminished an obvious diagnosis of GS.

As colon polyps are an extremely high-risk lesion for malignant transformation, a surgical proctocolectomy with ileal pouch-anal anastomosis was quickly performed. An alternative surgical approach suggested was colectomy with ileo-rectal anastomosis, which presents a 25% to 59% chance of rectal carcinoma and requires annual endoscopic surveillance of the rectum, so it was therefore excluded (2). The postoperative follow-up necessitates a multidisciplinary approach. Duodenal cancer occurs in about 5% of FAP patients following colectomy, and therefore upper gastrointestinal endoscopy is also required, intermittently, until the patient reaches the age of 50 years (41). The increased prevalence of thyroid carcinoma (100-fold of the general population), also necessitates periodical diagnostic screening (36, 42). Finally, a periodic radiographic examination of the jaws should be performed regularly, as osteomas continue to develop in adulthood.

Genetic tests may reveal a mutation in the APC gene in about 85% of individuals with FAP. This involves the simple and accurate screening of the family (43, 44). It is particularly useful for children who, if they have not inherited the damaged gene, will never have to undergo a bowel screening procedure. In families where a mutation cannot be detected, the only possible way to identify affected individuals is by bowel screening. All first-degree family members have to be screened from the age of 12. However, if no polyps develop by the age of 40, the screening may be discontinued (45).

Knowledge about maxillofacial findings which are a hallmark for diagnosis of GS is of fundamental importance (18, 46). Our case of GS was an isolated familial incident, maybe the first in this family. Several studies suggest that osteomas are important manifestations of GS, and regardless of the absence of a family history of FAP, their occurrence should prompt a diagnostic evaluation related to this disease (47).

Conclusion

GS is characterized by gastrointestinal polyps, multiple osteomas and cutaneous and soft tissue tumours. Although it is rarely diagnosed, clinical physicians and dentists should be aware of the common and early manifestations which may appear before any FAP symptomatology, as there is an almost 100% risk that the polyps will undergo malignant transformation at an early age. Therefore, early stage detection and treatment of GS is crucial.

What Is Already Known on this Topic

GS is a rare syndrome consisting of hereditary intestinal polyposis, accompanied by osteomas, and multiple cutaneous and subcutaneous lesions. In some cases, osteomas emerge before the diagnosis of GS could be established.

What this Study Adds

Maxillofacial demonstrations of GS may be the first to be noticed. Our case demonstrates how a patient, referred for mainly cosmetic reasons, could suffer from GS, a syndrome which should be diagnosed early by practitioners due to the very high risk of malignant transformation of the FAP.

Authors' Contributions: Conception and design: VT; Acquisition, analysis and interpretation of data: VT, AV, GME and KS; Drafting the article: GT and KR; Revising it critically for important intellectual content:

VT; Approved final version of the manuscript: VT and AF.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
Acta Medica Academica 2019;48(2):225-229
DOI: 10.5644/ama2006-124.261

MR Imaging of the Hypermobile Lateral Meniscus of the Knee: A Case Report

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Received: 17 December 2018
Accepted: 6 June 2019

Key Words: Hypermobile Lateral Meniscus ▪ Knee ▪ Locking ▪ Popliteomeniscal Fascicle.

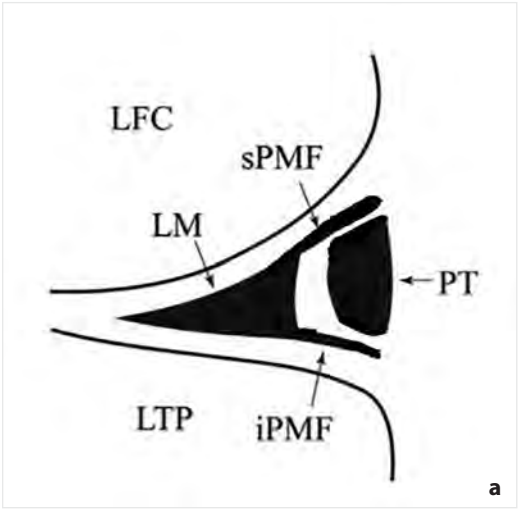
Objective. The hypermobile lateral meniscus of the knee is a rarely described entity. In this case report we aim to draw attention to the clinical presentation and MR imaging findings of this pathology. **Case Report.** We review the clinical and imaging findings that led to the diagnosis of hypermobile lateral meniscus with transient subluxation causing intermittent locking, and which subsequently led to successful surgical treatment. **Conclusion.** Hypermobile lateral meniscus is rarely diagnosed prospectively on MR imaging. A better understanding of this uncommon condition will lead to prompt diagnosis and effective treatment, with a better outcome for the patient.

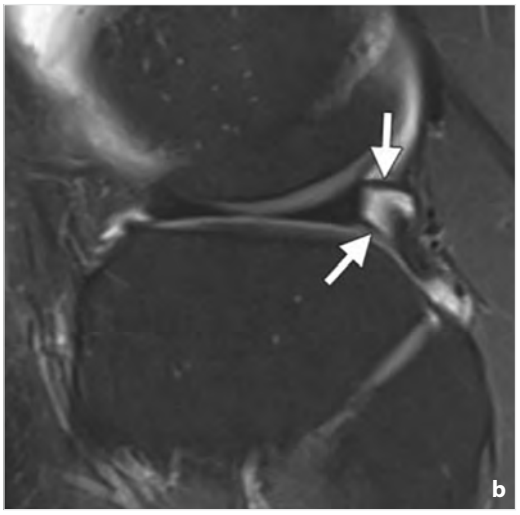
Introduction

The menisci are two C-shaped fibrocartilage structures in the knee joint (medial and lateral), which act as stabilizers and 'shock-absorbers'. Both menisci are attached firmly to the tibial plateau by anterior and posterior roots. There are also peripheral attachments to these menisci, which are less extensive and laxer on the lateral meniscus (LM), making it more flexible and less prone to injuries compared to the medial meniscus (1).

In a seminal paper, correlated with an excellent anatomical demonstration, Resnick et al. (2) demonstrated the presence of ligamentous bands that anchor the posterior horn of the LM in its normal position so that it does not translate anteriorly upon knee flexion. They demonstrated the presence of popliteomeniscal fascicles (PMFs) that hold the posterior horn of the LM in place. The fascicles, namely the anteroinferior and the posterosuperior fascicles (Figure 1a-b), were shown to be fairly constant in most people, while an inconstant fascicle, the posteroinferior fascicle (Figure 1c) is seen rising more medially on the level of the attachment of the meniscofemoral Ligament of Wrisberg to the posterior horn. Injury to these fascicles or a congenital absence has been shown to be a major factor in excessive mobility of the posterior horn of the LM, a condition aptly named the 'hypermobile lateral meniscus' (3).

Hypermobile LM of the knee has not been widely described and is rarely diagnosed on prospective imaging. To our knowledge, based on a literature search, there are two other case reports of hypermobile lateral meniscus diagnosed on MRI and proven by arthroscopy (4, 5). In a case series of 13 patients with proven hypermobile lateral meniscus on arthroscopy (6), only one out of the 13 cases demonstrated an abnormality of the meniscus on MR imaging, where the knee was imaged in the locked position.





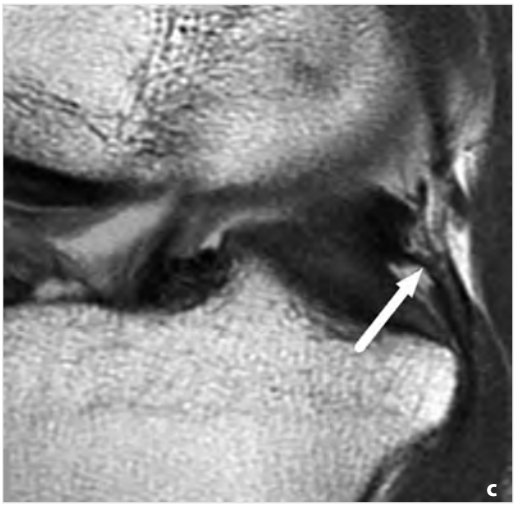


Figure 1.
(a) A drawing showing PMFs of knee joint. iPMF= Anteroinferior popliteomeniscal fascicle; LFC=Lateral femoral condyle; LM=Lateral meniscus; LTP=Lateral tibial plateau; PT=Popliteus tendon; sPMF=Posterosuperior popliteomeniscal fascicle.
(b) Sagittal PD FS image of a normal left knee. The sPMF and iPMF are clearly visualized (arrows).
(c) The posteroinferior popliteomeniscal fascicle (arrow) is not consistently visualized. It is seen near the root of the posterior horn and courses from the inferior margin of the meniscus posteroinferiorly to attach to a capsular extension of the popliteus tendon.

This abnormality was misdiagnosed as a bucket handle tear.

Case Report

An 18-year old girl presented with recurrent, intermittent episodes of painful locking of the left knee from 2010 up to the time of the last presentation in October 2015. In the interim, she had been examined in the orthopedic clinic with MR imaging of the affected knee in October 2013, which was reported as normal.

In the last presentation at the orthopedic clinic in October 2015, physical examination of the left knee revealed no swelling, redness or muscle wasting. There was limited range of motion of the knee joint. There was no patella apprehension but tenderness was felt over the medial patella region. Due to her unresolved symptoms, the patient was subsequently referred for a second MR imaging of the left knee.

In October 2015, MRI examination of the left knee of this patient was planned to be performed with the department's routine protocol. However, when the patient presented at our department on the day of the appointment, her knee was in a locked position, flexed at approximately 60o. The mus-

culoskeletal radiologist on duty that day decided to perform the MRI in this 'provoked' position. However, the patient could not fit into the GE Optima MR 430S-Extremity (GE Healthcare, Chicago, Illinois, United States) scanner or into the knee coil. As a result, the scan was performed on the 1.5T MAGNETOM Avanto (Siemens Healthcare, Erlangen, Germany) scanner instead, with a body array coil used around the left knee. The sequences obtained were proton density (PD) weighted fat saturated (FS) images in axial, coronal and sagittal planes, coronal T1 weighted and 3D gradient echo in the sagittal plane. After the scan, the patient was able to extend her knee again. The MRI exam was then repeated in the neutral position utilizing our routine knee MRI protocol on the GE Optima MR 430S-Extremity (GE Healthcare, Chicago, Illinois, United States) scanner with a 145 mm knee coil. The sequences obtained were the PD weighted FS, in the axial, coronal and sagittal planes.

In the neutral position, the LM was essentially normal in appearance and position on all planes (Figure 2 a-c). In the 'provoked' position, the posterior horn of the LM was anteriorly subluxated and interposed in the lateral tibio-femoral joint space (Figure 2d-f). The PMFs were absent (Figure 2f), which could be a result of a previous injury or a congenital absence. No meniscal tear was identified, but a LM flounce was noted. The final diagnosis was hypermobile posterior

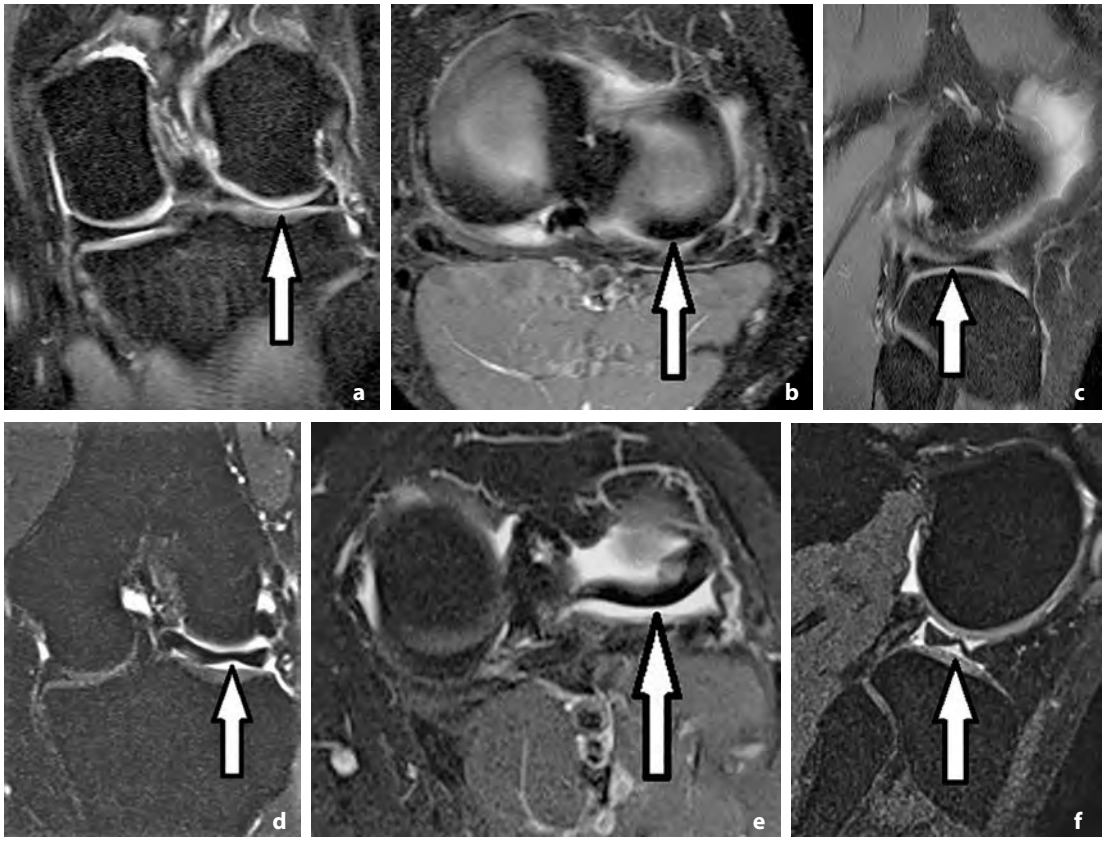


Figure 2. (a) Coronal (b) axial and (c) sagittal PD weighted FS MR images in the neutral position demonstrate a normal LM (arrows). (d) Coronal weighted PD FS MR image in the provoked position demonstrates a 'flounce' in the LM (arrow). (e) Axial PD FS MR image in provoked position demonstrates anterior subluxation of the posterior horn of the LM (arrow). (f) Sagittal gradient echo MR image demonstrates anterior subluxation of the posterior horn of the LM with a 'flounce' (arrow). This image also demonstrates the absence of the PMFs

horn of the LM with transient subluxation causing intermittent locking.

After the diagnosis was made, the patient went on to have arthroscopic stabilization surgery. On examination under anesthesia, there was a positive pivot and Lachman test. Arthroscopy revealed an intact anterior cruciate ligament (ACL) and posterior cruciate ligament (PCL). The medial meniscus was intact. There was hypermobility and subluxability of the posterior horn of the LM. No tear was detected. Stabilization of the posterior horn was performed with fixation of the posterior horn of the LM to the posterior capsule using Fast-Fix 360 meniscal repair device (Smith & Nephew, London United Kingdom), which is a device normally used for a minimally invasive repair of posterior and middle third tears of the meniscus using a vertical mattress suture. The patient was discharged in good health the following day. Her symptoms have resolved since then.

Discussion

The hypermobile LM refers to the condition where the LM loses its posterior anchoring i.e. the PMFs becomes very lax and the LM subluxates, mainly with flexion. The patient presents with a range of symptoms in terms of severity, most commonly pain and locking of the knee joint, in the absence of a meniscal tear or discoid meniscus (7). PMFs are structures that are not routinely assessed by most radiologists, hence the low incidence of the diagnosis of the hypermobile lateral meniscus of the knee on MR imaging. The diagnosis is usually made by arthroscopy (8).

In our case, the initial MR imaging performed in October 2013 with the routine protocol i.e. with the knee in the neutral position, was reported as normal. In retrospect, the anteroinferior and the posterosuperior PMFs were not visualized while the posteroinferior PMF was partly seen. On the subsequent MR imaging performed in October 2015, imaging the left knee in the provoked, symptomatic flexed position, it was possible to demonstrate the anterior subluxation of the posterior horn of the LM and the condition of the absent PMFs became more apparent. In the same setting, with the knee in the neutral position, none of the three PMFs are seen. A helpful pointer in this regard is the presence of an LM flounce, which is often considered normal in the medial meniscus, but its presence in the LM should prompt a search for the condition of the absent PMFs (9), in the absence of a meniscal tear. In our patient, the flounce of the LM was detected in the provoked position.

Conclusion

Hypermobile LM is a poorly recognized entity which is rarely diagnosed prospectively on MR imaging. Increased awareness of the clinical presentation, and the anatomy and pathology of the PMFs, will lead to accurate and prompt diagnosis with effective treatment and better outcome for the patient. Pointers that could help radiologists in diagnosis are looking for the presence of a lateral meniscal 'flounce' and performing MR imaging of the knee in the provoked position, whenever feasible, in a patient with typical symptoms.

What is Already Known on this Topic

The posterior ligamentous bands, called the popliteomeniscal fascicles, anchor the lateral meniscus to its normal position, preventing it from anterior subluxation during flexion. The absence of these bands, either due to trauma or congenitally, could lead to an entity called 'hypermobile lateral meniscus'. The patient commonly presents with knee pain and locking. This entity is not widely described and commonly missed on prospective imaging, if not scrutinized carefully.

What this Case Report Adds

We present a case of the hypermobile lateral meniscus which was not diagnosed on prospective imaging in 2013. On the repeat MR imaging of the affected knee in October 2015, the pathology was demonstrated when the knee was in the flexed and locked position. A lateral meniscal 'flounce' was also demonstrated. These could be pointers to help radiologists in seeking out this pathology when presented with a similar case.

Authors' Contributions: Conception and design: ND; Acquisition, analysis and interpretation of data: ND and RN; Drafting the article: RN; Revising it critically for important intellectual content: ND; Approved final version of the manuscript: ND and RN.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Case Report

Images in Clinical Medicine
Acta Medica Academica 2019;48(2):230-231
DOI: 10.5644/ama2006-124.262

A Case of with an Incidental Penetrating Foreign Body in Lumbar 2 Vertebra

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Figure 1a

Figure 1b

12-year-old male was admitted to the pediatric emergency department with stomach ache. On physical examination the abdomen was normal, the rebound sign was negative, but mild defense was observed. According to standing AP X-ray of the abdomen, a penetrating foreign body was seen on the left and at the level of the stomach (Figure 1A). The patient was questioned again about swallowing any foreign body or penetrating body access holes on the patient's skin but there was no history of ingestion of foreign bodies or any access hole of a penetrating body observed on the patient's skin. Therefore the patient was hospitalized in the pediatric surgical service. On abdominal computed tomography, the foreign body was seen at the level of the L2 vertebra left pedicle (Figure 1B) and as a result the patient was referred to our clinic (neurosurgery) for surgery. The patient was laid down in the prone position. After L2 level had been determined

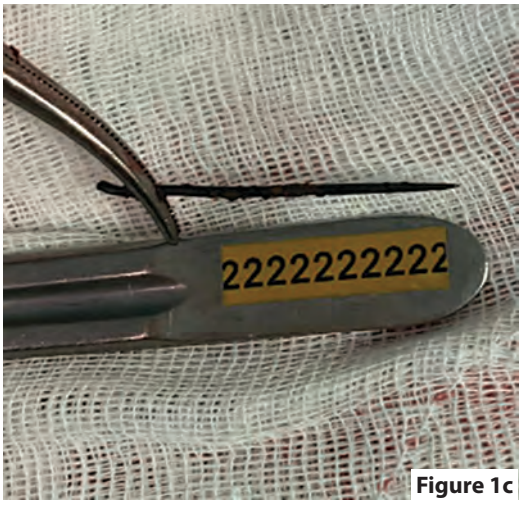

Figure 1c

by fluoroscopy, the operation began with a vertical left paramedian incision. After the fascia was cut, the foreign body was sought by fluoroscopy between the paravertebral muscles. When the foreign body was found, it was removed by pulling with a rongeur. It was a rusty nail (Figure 1C). Subsequently, no cerebrospinal fluid was observed and the operation was completed.

Key Words: Foreign Body ▪ Vertebra ▪ Incidental.

Authors' Contributions: Conception and design: ÖFT; Acquisition, analysis and interpretation of data: VMÜ; Drafting the article: ÖFT; Revising it critically for important intellectual content: VMÜ; Approved final version of the manuscript: ÖFT.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Received: 20 December 2018; Accepted: 29 March 2019

Historical Article

Narrative Review
Acta Medica Academica 2019;48(2):232-249
DOI: 10.5644/ama2006-124.263

Bogusławą Keckova: An Official Female Doctor in Bosnia and Herzegovina, 1893–1911

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Received: 25 July 2019
Accepted: 30 August 2019

Key Words: Official Female Doctors ▪ Bogusławą Keckovą ▪ Mostar ▪ Osvit ▪ Bosnia and Herzegovina 1878–1918.

The purpose of this paper is to bring to light the biographical details, the professional work and the publishing activities of Bogusławą Keckovą (Bohuslava Kecková in Czech and Keck in German), who functioned as an Austro-Hungarian health officer in Mostar from 1893 to 1911 during the period of the Austro-Hungarian occupation of Bosnia and Herzegovina (BH). Keckovą, who came from Prague, was the second of nine female physicians to be employed by the Austro-Hungarian authorities between 1892 and 1918. Keckovą contributed significantly to the improvement of public health and hygiene in BH, especially by organising the medical treatment of Muslim women. She published a series of popular medical articles, both in Czech and in Bosnian. Her medical articles in the Mostar newspaper, 'Osvit', were among the first in BH to promote public health education and aimed at improving the health of the population. In the Czech Republic, 'Bohuslava Kecková' is renowned for being the first Czech female physician to graduate, who, due to Austria's conservativism and anti-feminism, had been forced to study and practise abroad. After Keckovą's efforts to have her Swiss MD degree (1880) recognised in Austria failed in 1882, she acquired an Austrian midwife's diploma and established a maternity home in Prague. In 1892, she accepted the invitation to serve as an Austro-Hungarian female health officer in Mostar, where she initialised and popularised the utilisation of public health among (Muslim) women. Conclusion. Bogusławą Keckovą's work as a physician, medical writer and health educator, which she continued tirelessly until her death in 1911, was based on gender-specific social-medical concepts, which were at the core of the contemporary Czech feminist movement.

Introduction

Throughout the Austro-Hungarian occupation (1878–1918) of Bosnia and Herzegovina (BH), nine official female doctors were employed in the territory from 1892 onwards (1, 2). Bogusławą Keckovą was the second official female physician from the Austro-Hungarian Empire (AHE) to come to BH and worked in Mostar from 1893 to 1911. At that time, the standard of living was very low throughout BH; public health and hygiene had not yet been introduced and the hygienic standards of the population were poor, particularly in the rural areas. Infectious diseases often had the character of epidemics, and the mortality of the population was significantly high, especially among infants and children. In many aspects, so-

ciety was based on 'customs', which were deemed to be the best and only way to live, and folk medicine was deeply rooted and often included quackery. The common people believed that 'God had given disease' and that 'God would make the disease disappear'. A special challenge in providing health care to the population involved Muslim women and girls. According to the traditional customs that prevailed at the time, Muslim women should not be treated by male doctors, nor should they receive medical help outside of their own home (3).

Bogusławą Kecková finished her medical studies in Zürich in 1880 but was denied permission to practice as a physician in the *AHE*. After having conducted a midwife's practice in Prague, she accepted the offer to enter service as a female health officer (*Amtsärztin*) in BH in 1892. As indicated by her official title as a '*Landesärztin (für Herzegowina)*' (provincial health officer [of Herzegowina]), her responsibilities involved the treatment of female patients from Mostar and the surrounding area as well as other Herzegovinian districts. From the beginning of her appointment, she worked tirelessly at the medical clarification of her patients. In her earlier years, she frequently visited patients in their homes, as they refused to come to the outpatient clinic; visiting possibly unwelcoming patients at home was even a requirement in the guidelines for female health officers (4).

Kecková pursued a social and educational concept of modern hygiene which, in the AHE, had been first promoted by the Czech feminist movement. Czech feminism advocated women and children's need for female physicians in order to remove the general ignorance of the facts of life that prevailed at the time and to popularise modern medicine, hygienic standards, public health utilisation and a healthy lifestyle among the female population. Based on her experiences as a midwife in Prague and a female health officer in Mostar, Kecková published popular medical articles that aimed to provide health education in the Czech feminist press from the late 1880s onwards. In BH, she published four comprehensive medical educational articles that appeared in 1904 and 1905 in 'Osviť, a newspaper founded and edited by Ivan Aziz Milićević in Mostar from 1898 to 1907.

In this paper, Bogusławą Kecková's life, her professional work from 1893 to 1911 and her publishing activities as a medical educator are outlined more specifically.

Kecková's Short Biography

Bogusławą Kecková finished her medical studies at the University of Zürich in 1880 and became known as the first Czech (Austrian) female physician to graduate. In the Czech Republic, Kecková's publicity lags behind Anna Bayerová's prominence as the 'first Czech female physician', which is owed to Bayerová's historical role as the figurehead of Czech feminism. Although Kecková has been neither forgotten nor omitted from the Czech medical and feminist historiography (5-9), no detailed biography has been dedicated to her memory. Her life and activities have predominantly been the subject of numerous collective biographies of the pioneers of female academic and medical education in the AHE (10).

The Czech historian Ctibor Nečas provided a monograph on the lives and activities of the Austro-Hungarian female health officers who were deployed in BH as an extraordinary administrative measure to popularise public health utilisation, particularly among the female Muslim population, from 1891 to 1918 (11). He based his short biographical note on Kecková (12) on an autobiographical account that she had shared with Eliška Krásnohorská, the editor of the Czech femi-

nist journal, *Ženské Listy* (13). This principal biographical source for Keckovą's life and activities was revised and complemented by Krásnohorská, thus constituting a tribute to Keckovą's absolute commitment to the cause of social medicine designed from and for 'women and children' as the core concern of 19th century popular Czech feminism.

'Bohuslava Josefa Kecková' was born in Bukol, a small hamlet in the Czech district of Mělník to the northwest of Prague. She was the second of three daughters of the wealthy local landowner and businessman Adolf Keck and his wife Jana Kecková, née Kubičková. In 1862, the family moved to Prague-Karlín, after Bogusławą's father had sold his estate and acquired a limestone quarry to the south of Prague, in Braník, which would earn him a fortune (13, 12). Therefore, Bogusławą and her family spent their lives under materially favourable conditions, which made her efforts to 'support women and children' (13, p. 68) a genuine mission that she at no point had to pursue to earn a living. As a girl from the milieu of the emerging Czech nationalist and pro-feminist middle class, Bogusławą was sent to a higher girl's school in Prague and considered to be extraordinarily gifted. According to her own account, she urged her father to allow her to 'continue learning' after she had finished school at the age of 16. Her father arranged for her additional private lessons in all subjects taught at Austrian higher boys' schools. Finally, he also succeeded in persuading the principal of the famous Czech Malostranské Gymnasium in Prague to permit Bogusławą to take the exam for general qualification for university entrance (*Matura*) (13). Keckovą took her *Matura* exam privately at the sports hall of the same school in July 1874 (13), thus becoming the first girl in the AHE to qualify for academic studies.

Although Austrian medical faculties did not accept female students prior to 1900, some Swiss universities did, particularly the 1833-founded University of Zürich, where women were admitted to all courses from the 1840s onwards. In October 1874, Keckovą travelled to Zürich accompanied by her father and enrolled at the university to study medicine (13). Keckovą claimed that she learned 'easily', and she was awarded her MD degree on August 4, 1880. Her thesis on the statistics of struma operations (14) was dedicated to Edmund Rose (1836–1914), university professor of surgery in Zürich, who had chosen her as his assistant physician (13). At an earlier point, she had also assisted at the clinic of gynaecology (13). Keckovą returned to Prague immediately after graduation. According to Nečas (12), she seemed to be convinced that she would succeed in publishing her thesis and establishing her own doctor's office in Prague. Both goals, however, proved to be unachievable. Her expertise was neither acknowledged by the association of Czech physicians nor was her Swiss degree recognised in Austria (12). After two years of intervening with the academic and administrative authorities and filing lawsuits at the different courts, in 1882, she received the negative decision of the Supreme Court in Vienna purporting that her femininity excluded her from the right to practice as a physician in Austria (12; 13). Forced to change her plans, Keckovą initially considered settling in Hungary, where female physicians were admitted practicing when male physicians were unavailable (12). However, she ultimately chose the second option available to her, which meant to abandon her MD qualification. Still in 1882, she travelled to Vienna to attend a midwifery course at Vienna University's medical faculty, which entitled her to work as an Austrian midwife. Based on the wealth that she and her sisters had inherited from their father, who died in 1880, Keckovą established a maternity home in Prague in 1883, where 'women of all classes' were welcome (8). Krásnohorská (8) later highly praised

Keckovą's activities as a midwife, emphasising her philanthropic practice of also taking care of destitute women, whom she treated free of charge, even providing for their medication at her own expense. Krásnohorská remarked that Keckovą's maternity home was 'unusually popular', the 'Czech ladies' rushing to her like 'the faithful to a wonder-working spring' (8). According to Krásnohorská, Keckovą, as a 'genuine democrat', had abandoned all professional pride in order to 'support her Czech sisters as a midwife'. Krásnohorská considered Keckovą's final appointment as a female health officer in BH to be a 'gratification and triumph' of the Czech women's movement (8).

Keckovą reported that she had been unexpectedly invited by the Austro-Hungarian administration in BH in November 1892 to assume the newly-created position of a 'female health officer (*Landesärztin*) of Herzegovina'. As documented by Nečas (12), two positions for female health officers in BH had been created and advertised regularly in the same year. Since none of the five applicants with Swiss MD degrees met the official requirement to be a native speaker of a Slavic language, the administration of BH took the initiative to enquire about the vocational aptitude of the second Czech female physician, Keckovą, with the association of Czech physicians. After the latter had consulted the Czech women's association, Keckovą was recommended for the office (8). Keckovą accepted 'with pleasure' (13), giving up her life and work in Prague immediately and without hesitation. On January 1, 1893, she headed for Mostar, interrupting her journey in Vienna for an audience with BH's de-facto governor, Bénjamin de Kállay, and in Sarajevo, to be introduced to the Austro-Hungarian supreme medical officer, Joseph Unterlugauer. On January 11, she arrived in Mostar, where she was introduced to the local religious and secular dignitaries. Two days later, she was sworn into her still provisional office, which obliged her to wear an Austro-Hungarian 'military blouse with three rosettes' (12). Her appointment for life followed in 1896. As a health officer for Herzegovina, she was officially responsible for the women and children of a population of roughly 220,000 people (13). Keckovą, as we later elaborate, performed her medical duties and educational activities in Herzegovina tirelessly for 18 years. Suffering from diabetes, she spent her annual six-week leave, besides family visits, on health cures in Karlovy Vary. In the autumn of 1911, her health condition suddenly deteriorated to a degree that she travelled again to the Czech lands. She died on October 17, 1911, in her elder sister's house in Kostomlaty nad Labem, where she was buried in her parents' tomb (7, 12, 15).

Keckovás Professional Activities in BH

From 1898 onwards, Keckovą shared the official annual reports that the female health officers in BH were required to submit (16, 17, 18) in a processed form with the Czech women's journals, *Ženské Listy* (13, 16, 19) and *Lada* (20). These unofficial reports reveal her 'bottom-up' approach to a job that, in modern terms, might be described as the task of a development worker. Based on her experience as a midwife to 'women of all classes' in Prague, Keckovą seems to have been well-prepared for the eventuality that Herzegovinian women would neither promptly seek her medical help nor closely observe her professional advice. She reported having had the inspiration to 'talk' to the Muslim officials of Mostar and was soon asked by the local Mufti to treat his wife, who had been in need of medical attention for a long time. When her treatment of this 'Muftinica' proved to be a success, she began to receive invitations to consultations in the houses of the wealthy Muslim ladies of the region, and subsequently, her clinic was increasingly frequented by the local

common women (13). According to the official statistics, Keckovą treated 710 patients in 1893, 763 in 1894, 829 in 1895, 925 in 1896 and 987 in 1897 (13), all of whom were 'women, female adolescents and children'. In 1904, she reported having treated 9,102 patients over the 10-year period of her stay, roughly half of whom were Muslims (16). These statistics include women and children from different Herzegovinian areas where Keckovą was detailed to practice during official trips. At a later point, she spoke of the 'annual six-week trips' that she undertook after 1895, accompanied by two gendarmes and a local Muslim guide, who organised the announcement of the 'doctorica's' arrival in the villages (19). These trips also served to allow Keckovą to carry out her official duties of vaccinating children and combating regionally widespread infectious diseases, particularly endemic syphilis.[1] Despite representing the unpopular Austro-Hungarian authorities, Keckovą enjoyed her increasing respect and popularity in Mostar and throughout the country. She reported having been fetched by mostly remotely-living rural people. Her latest official report gave the number of 1,213 patients who had sought her advice and treatment in 1909 (19).

Her popularity is also demonstrated by the numerous gifts that she received from her patients and students. She gathered the folk jewellery, handkerchiefs and scarves embroidered with silver and gold thread, clothes made from fine linen and lightweight cloth woven on traditional looms into a collection, which she donated to the National Museum in Prague (8) (Picture 1).[2] Her collection included a considerable number of photographs, which demonstrate her serious interest in the local handicraft related to her apparent personal taste for needlework.

In 1900, the authorities expanded Keckovą's scope of duties to include the office of a school physician at the higher girls' school in Mostar.[3] She was simultaneously charged with the task of preparing courses for prospective midwives at the same school, Keckovą herself teaching anatomy, anthropology and nursing care in the Bosnian language (16, 19). Her personal educational commitment is underlined by her private initiative to promote literacy among local Muslim girls from 1893 onwards. According to Keckovą, the daughters of Muslim families, if at all, attended only girls' religious schools, which did not teach them how to read and write.[4] Since the local Muslims were opposed to her suggestion to allow girls to learn how to write, she organised private lessons in 'reading Croatian'[5], arithmetic and needlework, which occasionally met Muslim fathers' approval. In 1904, she

[1]It was the locally widespread occurrence of not sexually transmitted syphilis ('Frenjak') in BH that prompted the Austro-Hungarian authorities to create the office of female health officers, who at first were required to perform genital examinations of Muslim women, who, according to the standards of Bosnian Muslim leaders, were not allowed to be examined by male doctors (21, 22).

[2]Krásnohorská (8), however, complained as early as 1924 about the 'deplorable condition' of the photograph collection.

[3]The employment of school physicians who should monitor the pupils' health and teach them hygiene in Austrian schools had been urged by the Austro-Hungarian pioneers of social medicine, among whom Czech patriots and feminists were prominent. Keckovą's appointment as a school physician was an Austro-Hungarian school pilot project in BH, the office of school physicians being introduced to Austria only in 1909/1910.

[4]Keckova states that local Muslim (elite) men expressed the attitude that women and girls were not allowed to "study the Quran" and, therefore, did not learn how to read and write Arab letters (13). The correctness of this statement is questionable, however, since the sense of attending a "religious school" must have been the study of Quran and, therefore, how to read (and write) Arab.

[5]Today, "Croatian" is considered the language of Croats living in Bosnia and Herzegovina. Muslims (Bosniaks) call the same language Bosnian. Kecková called the local language "Croatian" regardless whether the speakers were Serbs, Croatians or Muslims (Bosniaks).

Picture 1. A: Scarf, decorative, of starched white muslin, embroidered in four corners with gold thread on both sides fully, the edge of the corner is trimmed. Motif: bird (Inventory no.: H4-NS3505, Collection of the National Museum, Prague, Czech Republic); B: "Children's waistcoat called 'barley', a miniature display of light blue silk, decorated around the edges with a gold portico, lined with printed chintz (Inventory no.: H4-NS3552, Collection of the National Museum, Prague, Czech Republic); C: A silk blanket called 'bošča', sewn a square of purple silk in the middle with a yellow woven pattern – purple and yellow flowers. There is yellow silk around the purple stripe. The blanket is lined with white cloth. Gifts are wrapped in the blanket during baptism (Inventory no.: H4-NS3523, Collection of the National Museum, Prague, Czech Republic); D: Ceremonial wedding shawl, made of red sparse cotton fabric in the manner of soft organza, decorated with gold coins, the region is lined with yellow machine lace. So-called 'telija' bridal veil bride (Inventory no.: H4-NS3516, Collection of the National Museum, Prague, Czech Republic).

reported home that she had thus far been able to recruit 37 young Muslim women and girls, whom she taught obviously the virtues of a modern 'Bosniak' identity (16). Keckovą's commitment was rewarded with the affection that she enjoyed in Mostar (15), and, on the part of the administration, with her repeated official commendation by the de-facto governor, de Kálley (8).

Keckovą's Publishing Activities

As the first female medical doctor in the AHE, Keckovą did not have the option of publishing scientifically.[6] She had published articles of literary and medical content from the 1880s onwards in the Czech feminist press, predominantly in the Czech women's magazine, *Lada*, which was introduced in 1889. Keckovą's preference for *Lada* seems to be due to the fact that *Ženské Listy* called for the opening of higher education for women in the AHE, while Keckovą preferred the es-

[6]Contemporary male supporters of the idea of 'women's need of female physicians', in fact, tended to conceptualise female doctors as academically trained midwives, because midwives served throughout the 19th and early 20th century as the main health care providers for the common people.

tablishment of separate universities for girls and women, 'as in Russia and the United States' (13). When she depicted rural women's work in agriculture as an obstacle to a healthy lifestyle, which she conceptualised as requiring the full-time work of a well-informed and qualified housewife and mother, she proved to be a follower of 19th century moderate feminism. This movement assumed a detrimental effect of women's double and triple burden as workers, mothers of numerous children and caretakers on female health and aimed at transforming working women into domestic, voting 'agents of modern health and hygiene' rather than at gender equality. Keckovą's mission to popularise medical and nursing knowledge among women, girls and pupils corresponds with the contemporary middle-class feminists' social-medical reform movement, which drew on sources such as Florence Nightingale's hygienic standards in nursing (23) and popular medicine (24). Keckovą was convinced that the introduction and dissemination of hygienic standards were the most important means to prevent and contain diseases. In BH, Keckovą reported on her continuous attempts to persuade her (former) patients to prepare healthier meals and take care of their personal hygiene as well as the hygienic condition of their living spaces and garments (16). *Lada* printed Keckovą's remarks on female health education, nursing care, achieving a balanced diet, and healthy living and ways to dress (13).

In the Bosnian language, Bogusławą Keckovą published four medical articles of similar content in the 'Society and Science' section of the Mostar newspaper, 'Osvit'.[7] These articles are entitled 'Raising Healthy Infants' (1904), 'Man and His Health' (1905), 'Caring for Patients at Home' (1905) and 'About Tuberculosis, and How It Can Be Contained' (1906) and convey an impression of the state of public health and hygiene in the Mostar district and the health consciousness of the local population at that time. Keckovą's familiarity with the local population's social conditions, health culture and local customs allowed her to address the specific challenges to public health and hygiene that she had observed and to formulate practical suggestions for improvement. Keckovą, therefore, is the author of the first popular medical articles ever published in BH. (Please see the supplementary material entitled: Medical Articles in the Mostar Newspaper *'Osvit'*.)

Concluding Remarks

Bogusławą Keckovą/Bohuslava Kecková is known in the Czech Republic, her native country, as the first female physician to graduate but who was not permitted to practice at home. As a female member of the prosperous Czech 'patriot' middle class, Keckovą was strongly influenced by the Czech feminists' claims for a social/social-medical reform triggered by female physicians designed to treat women and children 'of all classes'. Keckovą evidently internalised this aspiration and considered bringing medical help to whomever and educating 'women and children' in modern hygiene and public health utilisation to be her personal mission. When she was denied the right to practice as a physician in Austria in 1882, she decided to work as a midwife in Prague (1883–1892). When the Austro-Hungarian authorities of occupied BH invited her in 1892 to assume the office of a female health officer for Herzegovina in Mostar, she accepted without hesitation. Arriving in Mostar in January 1893, she continued her mission in BH, where she worked as a physician, instructor of midwives and health educator until her

[7]Ivan Aziz Milićević (1868–1950), Bosnian journalist and writer. http://bosnae.info/index.php/knjizevni-dvojac-osman-nuri-hadzic-ivan-milicevic-jedinstven-kulturoloski-fenomen-u-historiji-bosanskohercegovacke-knjizevnosti

death in 1911. She published educational articles in popular magazines and newspapers, both in Czech and in Bosnian, which she based on her extensive professional and health education experience in BH. Her familiarity with the local population's social conditions, health culture and local customs allowed her to address the specific challenges to public health and hygiene that she had observed and to formulate practical suggestions for improvement.

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Authors' Contributions: Conception and design: HT and BF; Acquisition, analysis and interpretation of data: BF and HT; Drafting the article: HT and BF; Revising the article critically for intellectual content: HT and BF; Approved final version of the manuscript: BF and HT.

Conflict of Interest: The authors declare that they have no conflict of interest.

Supplementary material

Medical Articles in the Mostar Newspaper 'Osvit'

In the Bosnian language Bogusławą Keckovą published four medical articles in the 'Society and Science' section of the Mostar newspaper 'Osvit'[8] The first article was entitled 'Raising Healthy Infants', the second was 'Man and His Health', the third, 'Caring for Patients at Home' and the fourth, 'About Tuberculosis, and How It Can Be Contained '. These were the first articles in BH with health education content published in a newspaper with the aim of raising the level of health culture and improving the health of the wider population.

Raising Healthy Infants was the first article published in the 'Osvit'[9] newspaper in installments. In the introductory part of this article, Dr. Bogusława Keckovą writes about the importance of nutrition of infants from the perspective of the wider community, about the happiness of parents when their infants are healthy and advanced, and about their misfortune when they are ill, about their illnesses and mortality in the world, pointing out that the cause of mortality in the first year of their life is 'inappropriate and insufficient food'.

She pays particular attention to breastfeeding and the negative effects of artificial nutrition, and points out that hindrances and exceptions to breastfeeding are not quite as common as many women think, and that barely a tenth part of these 'imaginary' exceptions are justified. Furthermore she states, in an advisory manner, that when there are justified hindrances to feeding infants a strong breastfeeding woman should be engaged ('wet nurse') and when those opportunities have been exhausted the only thing that remains is to accept artificial nutrition.

This is followed by a range of tips on how to prepare cow's milk, a meal schedule, the delusions of mothers about the causes of crying in infants who are fed with cow's milk, on the unnecessary use of various teas instead of water to dilute milk, and the possible consequences of giving milk prepared in this way to infants. The chapter ends with a text about the serious consequences of long-term feeding with rice flour and similar surrogates.

Hereinafter, Dr. Keckovą talks about nurturing a newborn, listing the most common

[8]Ivan Aziz Milićević (1868 – 1950). Bosnian Journalist and Writer. http://bosnae.info/index.php/knjizevni-dvojac-osman-nuri-hadzic-ivan-milicevic-jedinstven-kulturoloski-fenomen-u-historiji-bosanskohercegovacke-knjizevnosti

[9]Keckovą B. Raising Healthy Infants. Osvit.1904; 7(88):3-4.; Osvit.1904;7(90):4.; Osvit.1904; 7(91): 4.; Osvit.1904;7(93):3.; Osvit.1904; 7(94):4.; Osvit. 1904;7(98):3.; Osvit.1904; 7(10):3.

mistakes she has learned about in dealing with patients who caused the newborn to become ill, sometimes with a fatal outcome, and she provides a range of tips on how and in what way to achieve and maintain a healthy environment for a newborn which will allow their normal growth and development.

This part of the text also discusses teething with a note that the teething follows the period of illnesses of the child followed by various signs and symptoms until all milk teeth erupt, but that a small number of these illnesses originate from tooth eruption, while other illnesses have other causes. Late tooth eruption is discussed in particular and the text indicates that it is commonly caused by malformation of the bony system, or the existence of rickets. Thereafter she describes her understanding of unnecessary and inappropriate methods which parents still use to support tooth eruption, noting that reasonable parents do not ask for jaw incision any more as was practised before.

The article ends with a text on 'child care in the family' which mainly discusses the nutrition of a child in the second year of life. The following section states that sour, spicy and irritating dishes, as well as spirits are not given to a child at that age. It is interesting that the author immediately makes an exception for spirits, stating: 'in the early childhood years, we can give them spirits, but only a small amount, diluted with water and very rarely.' However, Dr. Kecková immediately points out that in this respect, many mistakes are made in areas where wine is made and where the adults mistakenly think that the children's teeth will come out more easily after giving them wine, or that children will learn to walk more easily, etc. if they take a little bit of wine with each meal. In the remaining text of this subchapter Dr. Bogusława Kecková points out that it takes a long time to prepare liquid, nourishing and tasty food and, if it is not well-prepared, even the best dishes become less good and tasty, stating that this often happens in the homes of farmers, where the woman's cooking skills are still low-leveled because of their involvement in cultivating the fields and grooming livestock.

Man and His Health is the second article by Dr. Bogusława Kecková published in the newspaper 'Osvit'[10] in installments. The first chapter is entitled *Housing*. The author describes housing as the place where people live, the place which protects them from external influences and disasters, a place that should be airy, spacious, bright and arranged according to each person's wishes.

The text about air begins with the sentence: 'We always need clean air; it is a major requirement of a healthy life, therefore even though a person cannot live from the air itself, one can do without it even less.' In addition, the author describes the procedures that people used to refresh the air in the apartment, such as burning spruce or other spices and here she gives her critical opinion: 'If the window remains open for the same length of time that these fragrances were burning, then the stench would be removed and the room would be filled with clean and fresh air. ' To be more convincing to readers of the newspaper 'Osvit' she states the opinion of the English writer Miss Nightingale[11] from her famous work about

[10]Kecková B. Man and His Health. Osvit.1905;8(2):3.; Osvit.1905;8(4):3.; Osvit.1905;8(5):3.; Osvit.1905;8(6):3.; Osvit.1905;8(12):3-4.; Osvit.1905;8(12):4.; Osvit.1905;8(13):3.; Osvit.1905;8(15):3.; Osvit.1905;8(17):3.; Osvit.1905;8(20):3.; Osvit.1905;8(21):3.; Osvit.1905;8(22):3.; Osvit.1905;8(25):3.; Osvit.1905;8(26):3.; Osvit.1905;8(27):3.; Osvit.1905;8(30):3.; Osvit.1905;8(31):3.; Osvit.1905;8(32):3.; Osvit.1905;8(34):3.

[11]Florence Nightingale (1820–1910). During the Crimean War, she and a team of nurses improved the unsanitary conditions at a British base hospital, reducing the death count by two-thirds. Her writings sparked worldwide health care reform. In 1860 she established St. Thomas' Hospital and the Nightingale Training School for Nurses. She died August 13, 1910, in London. https://www.history.com/topics/womens-history/florence-nightingale-1#section_2

health care: 'Where there is a stench there is a waste of health and money, therefore, never dwell in such a house.'

Thereafter, she describes places and position where a house should be built, the arrangement of the rooms in apartments, the places where the windows should be placed etc. Dr. Bogusława Keckovą devotes special attention to the ventilation of the apartment, its dryness and humidity, the formation of unclean air and its harmful effects on the human body. At the same time she highlights the danger of unclean air in rooms such as halls for school children and inns for adults, where the air is not only polluted by carbon dioxide but also other pollutants such as vapors, dust and tobacco smoke. As one of the preventive measures for polluted air in inns, the author asks for a resolution from the authorities, so that besides just issuing a license to work, they require a test of integrity and the good management of inns so they are provided with the necessary ventilation. This section ends with an overview of the health problems that most often arise from local pubs where the air conditions are limited and unfavourable. Further in the text the origin of moisture in the apartment is described and its adverse effects on human health, i.e. how frequently various diseases are generated in the wet apartments in comparison to dry apartments. This is followed by short texts about light in the apartment which the author begins with the old proverb: 'Where the sun can't go, that's where the doctor comes in' by which the author wishes to accentuate the importance of sunlight for human health; and heating of apartments which begins with a warning about prevention of the generation of the 'fiercest' poisons of carbon monoxide and carbonic acid in heat-generating devices due to their improper use, stating that despite the fact that this is often spoken about as fatal, it still occurs; and about the temperature of the patient's room and heating devices and their place in the apartment, as well as the ventilation of the heated rooms. The following text is about the colors with which the apartment is painted and the author writes about the colors that make the apartment more comfortable and those that make it uncomfortable, the poisons found in the paints or wallpapers covering the walls, which cause poisoning or diseases which are frequently fatal, citing as an example arsenic toxic substances contained in paint and wallpapers.

The next chapter is entitled *Clothing*, with an introduction in which author talks about creating heat in the body, the range of normal human body temperatures, the body's reaction to the ambient temperature, i. e. about the role of clothing in regulating body temperature. Dr. Bogusława Keckovą further writes about woven materials and the types from which clothing is sewn, and their effect on body temperature and human health. The influence of colour on the regulation of body temperature is especially emphasized, as well as the health hazards of toxic substances found in the colours with which the fabric is dyed, and the clothing of patients which may transmit 'germs of disease' to the healthy population, and the necessity for disinfection.

The following text, entitled *Kinds of the Clothing* is dedicated to clothing used for certain body parts, the consequences of its improper use and the negative effects on human health, all of which is supported by the customs that people kept and nurtured. The final text of this chapter is entitled *Beds*. The author calls them 'night-suits' and writes about the importance of maintaining hygiene of all its parts. As in other parts of the text, she critically comments on irregularities in the use of beds which she had observed during home visits to the sick, and further explains how certain 'irregularities' affect health, explaining the common mistake of warming beds, as well as the use of heavy feather beds and duvets, both in win-

ter and summer, which suck up the vapors of bodies which are not easily discharged from them.

The chapter entitled *About Movement* discusses in the introduction the importance of movement in the newborn for its further psychomotor development, pointing out that 'the child must be given the opportunity for his limbs to stretch and stretch without obstruction' and points to the example of the English: '... who leave their children for hours lying on their rugs and they can roll at will, which contributes more to the tremendous development of their children than the most cautious caregiver and or nanny, who wrap their children tightly, so that they cannot move for hours, and carry them around the room.'

The following text, *Children's Mental Development,* considers the importance of movement for children's mental development. Then, with a great deal of criticism the author looks back on the need for early school attendance, in respect of whether the child's mental and physical development has reached the level to enable them to meet school requirements. In doing so, she does not hesitate to mention the fact that '... the beginning of school should not be determined by a certain age, but rather by the child's mental and physical development', pointing out that , '... the sixth year is too early and the child would profit more if they had another year to devote to their development.'

In continuation of the text about school she states that there is a general complaint about school regarding 'overloading children with educational activities' as a real issue. She states: '... it would undoubtedly be more appropriate and healthier for the physical development of children, if they went to school for only two hours instead of 6 or even 8 hours a day, where they are squeezed behind their school desks'. However, Dr. Bogusława Kecková quickly relinquishes this interesting reasoning with the explanation: 'The present time places great demands on the individual, social life has evolved so much that the simplest occupation requires much more knowledge than before' and she goes on to explain why it is difficult to change school laws, stating that: '…it is still not possible to stand against the current of time and carry out a thorough revision of school institutions' but she notes that it is necessary to fight for clean air and enough light in school classrooms. She also states that sitting for long periods of time in classrooms has harmful effects on children's bodies, and points out the necessity of exercising with special focus on posture. This section ends with advice about how children should be educated, emphasizing in particular 'the development of independent thinking which is the foundation of later life'. The following text is about *School Diseases* in which the author talks about spinal distortion, nearsightedness and the occurrence of goiter, a common disorder in children at school. Attributing these illnesses only to '... inconvenient school seating' is questionable for Dr. Bogusława Kecková, because she sees the causes 'in the lack of care and supervision of children by their parents and teachers.' The author further points out that spinal distortions as well as nearsightedness cannot be easily repaired later and that the onset of these disorders: '... must be limited in development ...' with particular emphasis on exercise and adequate nutrition. It is interesting that goiter, which at that time was a common illness in school children in Bosnia and Herzegovina, is not mentioned further in this text.

There follows a description of the importance of systematic exercise for the health of school children and the views of parents on its practice in school. According to her knowledge, parents, especially those from villages, find school exercise unnecessary because they believe that rural children have

enough physical activity in the fields and vineyards. However, Dr. Bogusława Keckovą believes that this is a consequence of parents' ignorance about the importance of systematic exercise for their children's health. She believes when when this is resolved, with good supervision the resistance to exercise will disappear.

The chapter entitled *Occupation* talks about the impact of work on the health of a person. It starts with a text about household tasks and work, which children often do at the request of their parents, and their harmful effect on the child's development. It specifically describes the adverse effects of work on children's health if they leave school early and go to work immediately in factories. Dr. Bogusława Keckovą touches on this issue later in life in the text *Choice of Occupation* linking the occupation learned and the person's physical condition where using practical examples she shows that their disharmony has an unfavorable effect on health and life expectancy. In this regard, she also refers to a lifestyle which brings together the individual's way of life, health and life expectancy, whereby she quotes and comments on the statement by the French physiologist, Jean Pierre Flourens,[12] 'A man does not die, he kills himself.'

The chapter entitled *About Skin Health Care*, followed by the phrase 'Cleanliness is half of health' is the fifth chapter of the article *Man and His Health*. Although the author begins this chapter with the medical characteristics of the skin, she presents it in a picturesque, clear and understandable way, taking care to make it easily understandable to the readers of the newspaper 'Osvit'. To present readers with the compelling importance of skin for health she states that as a student she attended an experiment, where her physiology professor Ludimar Herman[13] covered the skin of various animals with an airtight blanket, thus preventing the physiological activities of the skin which, after a short or long time, ended with the death of the animals. In this chapter she further writes about skin care products, noting that maintenance is most important for its purity. She goes on to write about how we can best maintain the cleanliness of our skin in the easiest way and in doing so, she notes that we care the least about the cleanliness of our skin and that in general in life we do not treat any organ so mechanically in terms of reasonable and the most ordinary care as our skin. There follows a text entitled Bathing in which the author writes about the use of bathrooms in the world through the long history of human civilization, and then discusses the situation regarding baths in our cities, pointing out that they are rare and usually too expensive for poor people to use, especially in winter, and that as a result: '... thousands of people for many months, even years, do not see any baths and their skin pores are clogged.'

Dr. Bogusława Keckovą then mentions bathing children whereby she points out '... that laziness in bathing is passed on from parents to children, that children are not taught to bathe themselves because they lack public baths and bath utensils in their homes, so children stop bathing as well'. To illustrate the situation better regarding bathing children, Dr. Bogusława Keckovą states that she saw the baby of a woman who looked after a fruit orchard, who was several weeks old and had never been bathed. She concludes this observation with the sentence: 'The poor thing looked a little like a flower, its skin was wrinkled, pale, and there was barely a trace of life in it.' The next thing

[12]Marie Jean Pierre Flourens (1794–1867). Yildirim FB, Sarikcioglu L. Marie Jean Pierre Flourens (1794–1867): an extraordinary scientist of his time. J Neurol Neurosurg Psychiatry. 2007;78(8):852. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2117745/pdf/852.pdf

[13]Ludimar Hermann (1838-1914) German Physiologist. Professor of Physiology, University of Zürich, 1878–1880. https://de.wikipedia.org/wiki/Ludimar_Hermann.

she describes is the importance of bathing children for their health, with specific advice. After that, we come to a text that tells us what needs to be done to improve the population's attitude to bathing, which is summarized in the sentence: 'If our people, who have been weaned from bathing, embrace bathing again, the public baths need to be restored to life, and they will be again the most important and the most blessing institutes for nurturing public health!'. To be more persuasive to the readers of the newspaper 'Osvit' Dr. Bogusława Keckovą ends the chapter with a statement from 150 English doctors: '... if the cities have a sufficient number of public cold and hot baths, such as wash-hand basins, and if they work properly, the incidence of disease as well as mortality should be reduced!'

The last chapter of this article is entitled *Soothing Agents in Early Childhood*. It is likely that the inspiration for Dr. Bogusława Keckovą to write this text was the sudden death of an infant after his mother gave him water to drink in which poppy seeds had been boiled in order to calm him down at night. The text below lists the agents that have been used to soothe infants since ancient times. In doing so, she distinguishes teas from plants that contain opium, which she considers harmful for infant health because they have a 'devastating' effect on the brain. She further explains why infants cannot sleep and why they cry, pointing out that crying is often a 'protest of nature,' listing its frequent causes. She concludes that the mother may be greatly mistaken if she does not take these causes into consideration and that in such situations she should seek medical advice. The chapter concludes with a series of educational responses to the question: 'How and in what way can this widespread and deeply rooted evil in the children's world be eliminated.'

Caring for Patients at Home is the third article by Dr. Bogusława Keckovą published in 'Osvit'[14] magazine in installments. At the very beginning, in the introduction, the author states in a few sentences that it is the custom for a female person to care for a patient at home, considering that this is natural and predetermined, and that in every respect the woman is capable of '.. doing this great work of loving one's neighbor.' This is followed by the first chapter, entitled *The Patient's Room*, which describes the importance and necessity of thinking about the patient's room in a timely manner, and not just in situations where a member of the household falls ill. In her description of what the patient's room should be like, Dr. Keckova begins with a text about *Clean Air*, explaining why and how the air in the room is polluted, how 'foul' air affects the patient, and how to prevent it from being polluted. *Cleanliness* is the following text that describes the necessity of constant maintenance of cleanliness in the patient's room. She writes the following: 'Not only the floor, but all the furniture in the room, including the ceiling and walls, should be kept at the highest cleanliness.' She also states that ceilings and walls, cracked tiles, carpets and wallpaper with many 'adornments' with rough surfaces create places for the collection of evaporated substances and dust, which gradually return into the air and are inhaled by the patient. In this process, the possibility exists of the spread of the agents of disease. This is followed by a series of practical tips on how to maintain the cleanliness of the patient's room on a daily basis, and when and how '... general cleanliness

[14]Keckovą B. Caring for Patients at Home. Osvit. 1905;8(38):3.; Osvit.1905;8(9):3.; Osvit.1905;8(40):3.; Osvit.1905;8(6):3.; Osvit.1905;8(12):3-4.; Osvit.1905;8(12):4.; Osvit.1905;8(13):3.; Osvit.1905;8(45):3.; Osvit.1905;8(45):4.; Osvit.1905;8(46):3.; Osvit.1905;8(48-49):4.; Osvit.1905;8(22):3.; Osvit.1905;8(25):3.; Osvit.1905;8(26):3.; Osvit.1905;8(27):3.; Osvit.1905;8(56):3.; Osvit.1905;8(58):3.; Osvit.1905;8(59):3.; Osvit.1905;8(60):3.; Osvit.1905;8(62):3.; Osvit.1905;8(63):3.; Osvit.1905;8(64-65):3.

should be established.' The following text is about *Light,* in which the author talks about the importance of light in the context of patient care, citing the words 'More light,' the last words spoken by Goethe[15] on his death bed. The next thing she describes is the effect of the 'marvelous power' of sunlight on all living creatures, quoting the famous folk proverb, 'Where the sun can't go, the doctor comes in'. The conditions are specifically described when doctors advise staying in a dark room because of eye ailments, when they must be hidden from daylight for the purpose of treatment. *The Temperature of the Patient's Room* is the following text that presents the reader with the doctor's opinion about the temperature in the room, i.e. its adjustment to the temperature of the patient and the opinion of most of the people: '... that the patient should always be kept warm, despite the differences, as far as the disease is concerned ...'.The author goes on to explain with practical examples that the careful care of patients with raised bodily temperature, if they are kept warm, has an adverse effect on the patient and that people's opinion that patients should always be kept warm is caused by the great fear of catching a cold. The text ends with a tip on how to maintain favorable humidity and temperature in the patient's room. The following text is about *Peace in the Patient's Room*, in which the author gives a range of tips for keeping peace in the patient's room, skillfully describing the situations in Bosnia-Herzegovina that create 'restlessness' and excitement in patients. Whispers or semi-silent conversations in the patient's room with those who came to visit the patient, or with the doctor, visits to the patient, cleaning the room and various noises nearby or further away are mentioned as situations that adversely affect the health of the patient.

In the second chapter *The Patient's Bed* in the opening text, entitled *The Form and Construction of Beds* the author points out the differences between the bed of a healthy and that of a sick person, stating that the patient's bed's shape, quality and bedding are of great importance. She describes what the patient's bed should look like in terms of practicality and the specificity of the disease. The text about *Quality of Beds* deals with the issue of the warmth and firmness of the bed in relation to the nature and course of the disease, that is, the patient's bodily temperature and the softness of the bed. At the end of this chapter there is a text entitled *Characteristics of a Good Bed*, in which it is emphasized that a good bed should be airy, light, long enough and not too cold, and the properties of the materials used to make individual parts of the bed are mentioned in relation to the disease of the patient.

The third chapter *Patient Care in Bed* includes four subtitles. *Bedding and Bed Bases* is the first text that looks at the various aspects of the physical and psychological effects of bedding and bed bases on the patient, with the aim of identifying, eliminating and preventing any negative effects on the patient's health, that is, that 'bedding and bed bases' should be in a condition that will help the physical and mental healing of the patient. What bedding, mattresses, pillows and blankets should look like, how everything should be washed and maintained is described in the text in a simple and understandable way. As in the previous chapters, in this text also, in the context of the topic, the harmful customs of the people of our region, which can have a detrimental effect on the patient, are emphasized. *Bed Care* is a follow-up text that begins with criticism by Dr. Keckova, referring to the placement of beds in the usual places, i.e. in the corners of the room near cold and wet walls, and in

[15]Johann Wolfgang von Goethe (1749–1832). German poet, playwright, novelist, scientist. https://www.britannica.com/biography/Johann-Wolfgang-von-Goethe.

the context of this, in view of the assistance provided to the patients in bed, advises that in patient rooms the bed should be in the middle of the room, emphasizing the advantages of that position. This is followed by practical advice on the importance of moving the bed and adjusting it from the point of view of the patient's medical condition or mobility. The following text *Patients Lying in Bed* talks about the position of the body in bed, which will ease problems and contribute to the faster healing of the patient. The last text in this chapter *Bed Warmers* describes the importance of warming the patient‘s bedding and the various items that serve this purposes.

The Food and Nourishment of Patients is the fourth chapter that begins with a text entitled *Food for Patients*, in which the author writes about the nutrition of patients from different points of view, while pointing out the differences with respect to the diet of a healthy person. *Drinks for Patients* is the next text that describes the preparation and use of Chinese tea, lemonade, suma root powder and almond milk. It should be noted that nothing is mentioned in the text about these drinks in the context of various diseases, which leads the reader to the conclusion that these drinks are taken solely for the purpose of introducing fluid into the body, that is, that their curative value is not important. This chapter also contains a text entitled *Measurement of Body Temperature, Pulse Checking and Measurement and Measurement of Breathing*, which is presented under three headings. In the first part, entitled *Measurement of Body Temperature*, the author describes ‘fever’ as a side effect of many diseases, the reasons for its occurrence, and the way it is measured. The following text entitled *Pulse Checking and Measurement* talks about the importance of measuring a pulse in diagnosing a disease, how it is measured, and about its normal rate at different ages. At the end of this text, the author states that any irritability, the presence of a doctor or medical intervention in many people will greatly increase the pulse rate by 20 to 30 beats. The third text entitled *Measurement of Breathing*, provides instructions on the technique of counting breaths, how many there are in one minute in healthy persons in relation to their age, and situations where the number of breaths increases and when signs of activation of the accessory respiratory muscles are present.

The following chapter is the fifth chapter *Bathing Patients* which begins with their division into the parts of the body to be washed (full baths, half baths, sitting baths, foot baths, forearm baths and hand baths), and the temperature of the water to be used (ice baths, cold baths, lukewarm baths, warm baths, hot baths, sea baths and steam baths). For each bath mentioned Dr. Kecková describes its purpose in several sentences, how it is prepared and used, the temperature of the water to be used and its effect on the organism. The end of the text refers to ‘Salt or Mineral Water Baths’, ‘Mud Baths’, ‘Iron Baths’, ‘Alum Baths’, ‘Sulfur Baths’ and ‘Pleasantly Fragrant Herbal Baths’, that is, ‘Baths with Admixtures (medicines)’. For each of these baths, the preparation and the amount of admixture used are described.

The sixth chapter *Compresses* also begins by dividing them into three types: cold, warm (warm dry and warm moist compresses), and leg compresses. For each type of compress, brief professional and informative texts describe the way they are prepared and used, and the purpose of using them. At the end of this chapter there is a text entitled *Disinfection*, which begins with the sentence: ‘I return to cleanliness one more time and repeat that the most important aspect of patient care is cleanliness to the fullest possible extent.’ To make herself even more convincing, she emphasizes to the readers in the next sentence the importance of cleanliness, stating: ‘… wound cleanliness is the most important

thing in the great successes of surgery.' Then, in a separate paragraph, she writes about infectious diseases and the main cause of their occurrence, that is, their source is uncleanliness transmitted in different ways from person to person, stating that it is not uncleanliness that can be 'caught by hands' or 'swept up' but it is uncleanliness in the air and fluids that can only be detected by 'a microscope'. Thinking of germs, she calls them a dangerous invisible enemy. The following text describes ways infectious disease agents are transmitted to a person and what, how, when and with what they should be disinfected.

About Tuberculosis, and How It Can Be Contained is the fourth and last article by Dr. Bogusława Keckova published in the 'Osvit'[16] newspaper in installments. It contains an introductory text and two chapters: Prophylaxis and School. The introductory wording begins with the proverb 'Good health is the greatest wealth!' and continues with the sentence 'Since it is the hellish and greatest enemy of our health, I consider, therefore, that the fight against tuberculosis is highly important'. With these words, Dr. Keckovą already at the beginning of this article, turns her attention to tuberculosis, a serious illness at the beginning of the nineteenth century, which we simply need to combat.

She goes on to describe the history of tuberculosis, its prevalence and mortality in the world as well as its formation, duration and spread, signs that occur in the sick, pathways for tuberculosis to enter the body, the most common parts of the body that are affected by the disease, and the measures to be taken against its formation and spread.

Prophylaxis is the title of the first chapter that begins with the question: 'The question is certainly justified, can the body defend itself against tuberculosis?' and the answer: 'If a healthy or sick man keeps the rules mentioned here, and if by doing so keeps the most basic laws of cleanliness, the risk of infection is halved'. There follows a detailed and illustrative description of various prophylactic measures to be used by the population, with the support of the state, to keep the disease from occurring, and to reduce the number of tuberculosis patients, emphasizing that all these measures and instructions are in vain if they remain merely on paper.

Chapter two of this article is entitled 'School' and describes school as a convenient place where the application of preventive measures will begin to be used. She says '... modern health science is turning to youth, because to strengthen youth means to cultivate a better generation of human beings in the future'. In the next sentence, she states: '... that there is no 'Institution' so convenient for tuberculosis to be decisively and with a such great success destroyed as a school.'

Dr. Keckovą supports these claims with statistical data indicating that the incidence of tuberculosis among school children was steadily increasing and states that this could not be attributed to uncleanliness and other circumstances in the home, but that one must believe 'that the school itself is where tuberculosis is being spread' and that it is the duty of the state to take care of the fact, '... that the situation where children spend their day is such that they do not suffer any harm to their health.' One part of this text is dedicated to the general health education of school children as well as their training regarding tuberculosis stating that children should be told that '... blowing their nose on the floor or spitting are nasty and dangerous habits and it must be explained why it is written on the walls: 'It is forbidden to spit on the floor!'

[16]Keckedą B. About Tuberculosis, and How It Can Be Contained. Osvit.1906;9(4):3.; Osvit.1906;9(5):3.; Osvit.1906;9(6):3.; Osvit.1906;9(8):3.; Osvit.1906;9(9):3.; Osvit.1906;9(4):3.; Osvit.1906;9(4):3.; Osvit.1906;9(4):3.; Osvit.1906;9(4):3.; Osvit.1906;9(4):3.; Osvit.1906;9(12):3.; Osvit.1906;9(13):3.

She goes on to explain in detail why tuberculosis is a common disease in children, how it comes about and what can be done to prevent it in schools, explaining how this procedure should be applied and what benefit the procedure brings, and what happens if it is not applied. In addition, there are positive examples of schools from abroad (France) and their role in the suppression and prevention of tuberculosis.

In the last two shorter installments of this article Dr. Keckovą refers critically from a medical point of view to the burden on children in terms of the subject matter they need to learn in school, the time burden of school activities, and the working conditions in their schools, stating examples of diseases and conditions in children which result from the burden on children and the inconsistencies in our schools.

This article by Dr. Keckovą ends with a request based on the previous text '… that basic training must be changed immediately and fundamentally.' Her thinking, about changes in schools is expressed in the final sentence of this article: 'Maybe these desires in this country and elsewhere would be quickly met, if the highly necessary and beneficial institution of school doctors was introduced and if there was sincere will and support in municipalities', and is worth attention because she sees the solution to all the difficulties which contribute to the disruption of the school children health in the establishment of the institution of school doctor.

Historical Article

Original Research Article
Acta Medica Academica 2019;48(2):250-254
DOI: 10.5644/ama2006-124.264

Adrianus Spigelius' (1578 – 1625) Ocular Anatomy

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Received: 2 December 2018
Accepted: 18 March 2019

Key Words: Adriaan van den Spiegel ▪ Ocular Anatomy ▪ History of Anatomy ▪ Padua.

The aim was to study Adriaan van den Spiegel's ideas on ocular anatomy. He is better known by his Latinized name as Adrianus Spigelius (1578 – 1625). He was a Flemish physician and anatomist who lived and worked in Padua, where in 1605 he was elected to be Professor of Anatomy and Surgery. Chapter IX of book ten of Spigelius' work on human anatomy, entitled *De humani corporis fabrica libri X tabulis aere icisis exornati* (1627) was devoted to an anatomical description of the eye. Corresponding to contemporary ideas of the production of knowledge Spigelius endeavoured to enhance Andreas Vesalius' (1514–1564) anatomy, he did not repeat his predecessor's theories of ocular anatomy. He conceptualised that the eye has six muscles, five tunics and three humors, while he gave a brief description of ocular physiology combining anatomy and the functional role of the anatomic ocular parts. **Conclusion.** He managed to correct Vesalius' errors and to present ocular anatomy with original notes, which so far, have been ignored and are highlighted now.

Introduction

Adriaan van den Spiegel, or as he is better known by his Latinized name, Adrianus Spigelius (1578 – 1625), was a Flemish physician and anatomist who lived and worked in Padua. He was born in Brussels and studied medicine at the Universities of Leuven and Padua. In Padua he was a student of Hieronymus Fabricius, or Girolamo Fabrizio, known also by his Latinized name as Fabricus ab Aquapendente (1537–1619). After his studies he returned for a while to his own country but from 1605, when he was appointed Professor of Anatomy and Surgery at the University of Padua, he settled in that city until his death (1). He was considered one of the best physicians of the time. Two years after his death, in 1627, his most important work on anatomy was published, entitled: *De humani corporis fabrica libri X tabulis aere icisis exornati* (2). The title of this book was influenced by the work (*De humani corporis fabrica*, 1543) by his fellow-townsman Andreas Vesalius (1514–1564) who had also studied at Padua (3). The aponeurosis of the transversus abdominis (Spigelian fascia), the linea semilunaris (Spigelian line) and the caudate lobe of the liver (Spigel's lobe) bear his name. A hernia of the Spigelian fascia is also called "Spigelian hernia". In his work, *De semitertiana libri quatuor* (1624) we find the first accurate description of malaria. In his anatomical works we can also find detailed descriptions of blood vessels and of the nervous system. Adrianus Spigelius also studied botany, giving his name to the genus Spigelia, while the rhizome and roots of *Spigelia*

marilandica were used as a remedy against intestinal parasites. Apart from his other pioneering work in medicine, his contribution to ocular anatomy has a distinct place in the history of medicine and anatomy (4).

The aim of our paper is to highlight Adrianus Spigelius' contribution to the history of ocular anatomy, because it is very important and mainly unknown.

Adrianus Spigelius as an Ocular Anatomist

Chapter IX of Book Ten of Spigelius' work on human anatomy, entitled *De humani corporis fabrica libri X tabulis aere icisis exornati,* is devoted to an anatomical description of the eye. Spigelius described the ocular muscles, ocular tunics, ocular humors and ocular nerve (2).

According to him the eye has six muscles. Four of them arise from the bottom of the orbit, leading to its middle, and accompany the ocular nerve. They are placed above, below and to the right and left of the orbit, to move the eyeball in these directions. The fifth muscle, which is considered to be the longest and slenderest, arises in the same place as the right muscle mentioned earlier, but when it reaches the Glandula Lachrymalis in the inner corner of the orbit, it ends in a slender tendon which is suspended in the insertions of the muscle that moves the eyeball upwards, and the other which moves it to the inner corner. The sixth one arises from a small hole in the lower part of the orbit, from which the nerve of the third conjunction also stems and ascends transversely to the outward corner in order to turn the eyeball in that direction. Spigelius underlined that the tendon of this muscle, which helps it to pass through the small hole, and the tendon of the outward muscle of the eyeball are often considered mistakenly as one due to their slenderness (2).

Regarding the tunics of the eyeball, Spigelius recognized five of them. According to him the first one found first during a dissection derives from the epicranium and extends over the white of the eye up to the iris. He believed that its role was to bind and give more strength to the orbit of the eyeball. He reported its three known names: Conjunctiva, Adnata and Epipephycos.

As the second tunic Spigelius listed the Cornea, pointing out that it had been given its name due to its resemblance to a horn. He noticed that it extended from the end of the conjunctiva, covering the iris, but also that it is clear and perspicuous in its forepart as far as the iris, but obscure in the hind part due to diverse polishing. On the forepart it is dense because it may preserve the crystalline humor, but transparent in order not to block the crystalline humor. He thought that it derived from the Dura Mater (Crassa Meninx).

The third tunic is the Uvea or Grapy coat, because it is similar in shape to a grape. Its origin was thought to be the Pia mater and that it encompassed all the eye except the pupil. It is nourished by the veins and arteries of the Cornea. At the level of the crystalline humor, it descended deep into the eye and then curved in order to protect it from the Albugineous humor. He also had the idea that the different colors of the uvea, such as black, brown, green and blue, had a special role in allowing people to see all the different colors of light.

The forth tunic, according to the physician, is the Amphiblistroides or Retiformis because it derives from the optic nerve, extends into the back pole of the eye and has a net-like shape, due to the net-like complex of the veins and arteries. It was described as a soft tunic in contrast to the crystalline humor and the cornea, because it has a special role, to form the image of the objects seen after the visual spirit has passed through the other anatomical structures. Therefore,

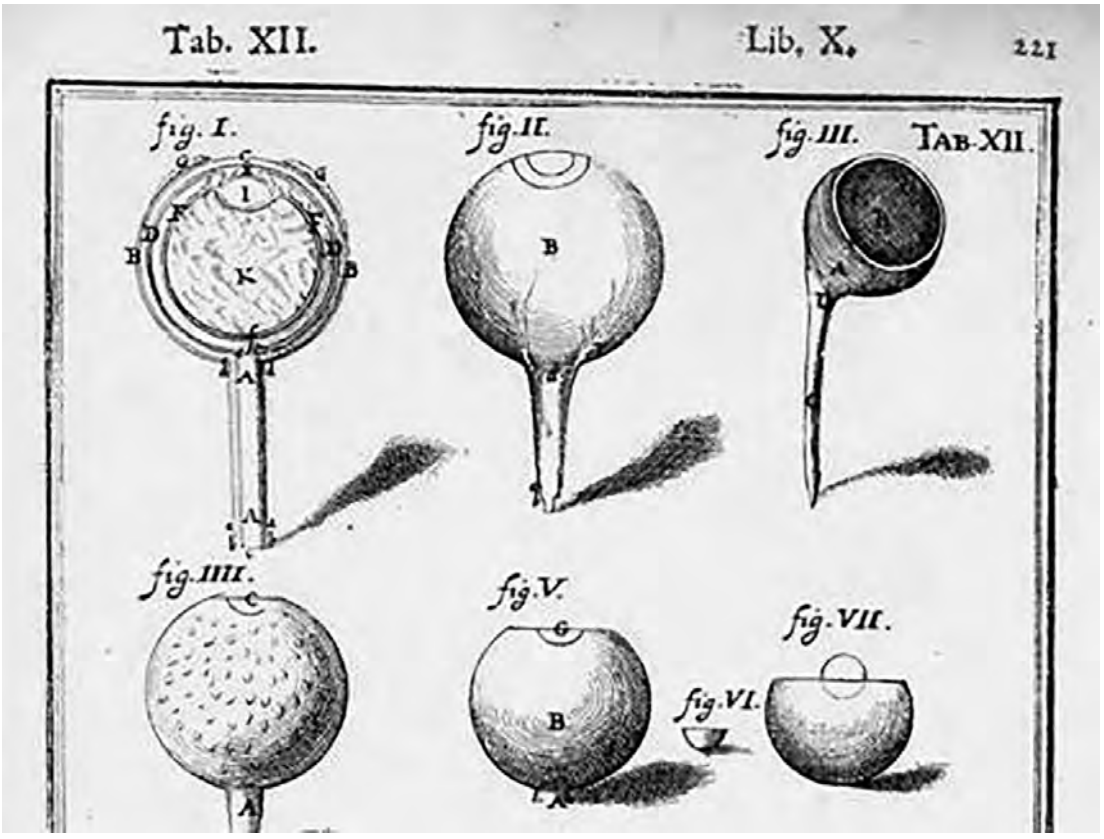

Figure 1. Giulio Cesare Casseri's design on ocular anatomy. Spiegel A. van den, Valasius F. De humani corporis fabrica libri decem. Venetiis, Deuchinus, Evangelista, 1627.

according to Spigelius, it had the most crucial role in vision and was considered to be a delicate structure.

The physician considered the fifth and last tunic to be the Arachnoides coat. Its name derives from its consistency, like a spider net. It is white, clear and the thinnest coat. It encompasses the crystalline humor on the fore side in order to protect it and to act as the vision structure in case the other humors are injured. It was believed that it derived from the excrementitiously humidity of the crystalline humor, which was hardened in order to form a coat by the coldness of the adjacent parts (2).

Spigelius did not forget to describe the three humors in the eyeball. The first was called the Aqueus or water humor, due to its resemblance to water. It took its place between the transparent part of the cornea and the portion of the crystalline humor lying towards the apple of the eye. According to the physician, this humor had a dual role. On one hand, it could be used as a barrier in order to distinguish the cornea, and on the other to protect the crystalline humor to prevent it from losing its moisture due to the light.

The second humor is the crystalline one already mentioned. It received its name due to its crystalline characteristics in brightness and color. Its shape, although round, is flattened on the foreside but not so much as from behind. The construction of this humor was believed, by the physician, to serve a practical purpose. The form of this anatomical part allows the image to be seen in its real shape, like in a mirror, and not to be transformed as it would be seen through a crystalline sphere. The back side of this humor, which is also considered to be the middle humor, was

believed to swim in the third humor from which it is nourished by the transposition of matter, but mainly of the net of the fifth coat which encompasses it.

The third humor was the so-called Vitreus, glassy or Hyaloides of Albugineous humor, which received its name due to its resemblance to molten glass or to egg albumen. It is located behind the crystalline humor, filling the empty space up to the fifth coat, and is therefore the largest humor of the eye in terms of quantity and is nourished by the vessel net of the fifth coat. Its use, according to Spigelius, was to protect the brain from the violence of the light and colors (2).

Discussion

Adrianus Spigelius wrote his treatise on the anatomy of the human body without any illustrations. This work was published two years after his death by Daniel Bucretius (?-1631) as was his wish expressed in his will. In order to fill this book with illustrations, Bucretius used 77 of 86 anatomical sketches designed by Giulio Cesare Casseri (1552-1616), the servant of Fabricus ab Aquapendente and which were presented for the first time in Casseri's treatise *Tabulae anatomicae* (1627) (5). These sketches were drawn by the painter Odoardo Fialetti (1573-1638?) and engraved by the painter Francesco Valesio (1560? - 1643?) (6).

Although the concept of Spigelius' anatomical treatise was influenced by Vesalius' anatomical treatise, mentioned above, as we may infer from the similar title, the analogous division of the chapters and the similar method of using the Greek and Latin terms of the anatomical parts (7), this was nevertheless an original work in anatomy which had a pioneering character, not only in the different format of the anatomical sketches, which were more detailed and mainly focused on anatomy and medicine, but also in the context of the anatomical descriptions.

This originality is also found in the anatomical description of the eyeball.

Namely:

- Spigelius went further than Vesalius, not mentioning the extra retractorius musclulus, while he located the crystal lens in the foreside and not in the middle of the eye ball as Vesalius did (8).
- He located the lens even more to the fore, as Giovanni Battista Della Porta (1535?-1615) did (9).
- He avoided describing the eye ball as a sphere, pointing to its more, but not completely, oval shape, which was an original note. He did not avoid considering the eyeball as a projection of the cerebrum and to locate the ocular nerve almost in the middle of the back pole of the eyeball (10).
- The descriptions of the veins and the arteries were very detailed, which allows us to remember similar detail, especially in the drawings found in the work of Georg Bartisch (1535–1607) (11).
- He tried to correlate ocular anatomy with the physiology of vision.

The significance of the progress in ocular anatomy as it was presented by Spigelius should be considered to be the fact that he rethought ocular anatomy, giving an opportunity to later anatomists and ocular surgeons to be more careful in their surgical or anatomical approach to the eyeball.

Conclusion

In conclusion we may say that Spigelius' ocular anatomy is characterized by its originality. Although Spigelius tried to imitate Vesalius in the form of his anatomical treatise, he did not repeat the theories of ocular anatomy presented by his predecessor. He managed to correct Vesalius' errors and to present ocular anatomy with original notes. However, we should bear in mind that even Spigelius did not succeed in giving an accu-

rate anatomical description of the eye ball, which was achieved much later by Johann Gottfried Zinn (1727-1759) (12). It is impressive that Spigelius tried to explain the characteristics of the humors and tunics of the eye, connecting them with the theories of vision and the physiology of the eyeball, albeit in a primitive way, very far from modern medicine and physiology (13). Nevertheless, this effort demonstrates the originality of his anatomical work about the eyeball.

What Is Already Known on this Topic

The history of ocular anatomy is very interesting because anatomists have many difficulties in understanding ocular anatomy without making mistakes. Andrianus Spigelius had a special role in the understanding of ocular anatomy but very little is known about it.

What this Study Adds

Our manuscript presents for the first time a detailed analysis of how Adrianus Spigelius conceived ocular anatomy and the differences to earlier treaties on ocular anatomy. Adrianus Spigelius' contribution to the theme is also highlighted.

Author's Contributions: Conception and design: KL; Acquisition, analysis and interpretation of data: KL; Drafting the article: KL, EM, KM, EL, PLS and MP; Revising it critically for important intellectual content: KL; Approved final version of the manuscript: KL and MP.

Conflict of Interest: The authors declare that they have no conflict of interest.

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ISSN 1840-1848