Investigation of the work accidents based on the statement of the nurses at the hospital in Turkey

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Abstract

**Aim:** This study aims to investigate the work accidents considering the statements of the nurses working in a university hospital.

**Methods:** This is a descriptive study. All the nurses (527) working in the hospital were tried to be reached. 71.4 percent of them agreed to participate in the study (n=405). By the survey form consisting of nineteen questions; socio-demographic characteristics, work history and the work accidents of the last six months were investigated.

**Results:** The mean age of the nurses was 32.3±0.3. 60 percent of the nurses were married, 29.1 percent had less than four years of professional experience, and 33.1 percent were working in the surgical units. Nurses thought that chemicals like medicines had the worst effects for their health. The ratio of the work accidents experienced by nurses in the last six months was 60 %. Cutting and piercing injuries accounted for the biggest part of the work accidents (42%). Work accident ratio was found higher for the nurses working in the surgical units less than four years. Ratio of verbal violence encountered by nurses was found higher than physical violence. Glide and fall injuries were caused mostly by slippery ground.

**Conclusion:** The results are important for hospital management to take necessary precautions and for other institutions, as well.

**Key Words:** nurse, work accidents, accident type, hospital

Introduction

Healthy and safe work environment is crucial especially for health workers. Considering this fact, International Council of Nurses (ICN) based its 2006 topic on safe environment and safe employment and 2007 topic on positive practice and work environment (1). In a work environment, healthy workers were reported to improve health condition and life quality of the patients (2). 70 percent of the nurses work in hospitals and frequently encounter dangers in the work environment (3).

Nurses spend more time with patients and directly deal with their treatment; therefore, the possibility of health risk is higher for them than other health workers (2). Nursing includes stress related risk factors such as long work periods, excessive workloads, time oppression, difficult or complex tasks, insufficient recreational breaks, monotone and physically bad working conditions (place, temperature, lightning etc.). Nurses work in dangerous and risky environment, which increases the rate of work accidents. Present or possible dangers and risks in the work environment were reported to increase professional diseases, work accidents, health problems related to work, disability and inability situations, and to create similar conditions (4).

Cutting-piercing injuries, being exposed to blood and body fluids, splash of blood and body fluids on mucose membranes, glide-fall and violence are among the work accidents most frequently encountered by health workers (5). Cutting and piercing injuries were reported as the most frequent work accident type, and needle piercing as its most frequent form (6).
Nursing, in which needles are most frequently used, is under serious risk of contagious disease caused by accidental needle piercing. In the study made by National Institute for Occupational Safety and Health (NIOSH) on 2,247 health workers in 2002, 155 individuals were determined to experience cutting-piercing injury (7).

Another important work accident was being exposed to blood and body fluids. In a study conducted among the health workers in seven different rural regions of India, the rate of being exposed to blood and body fluids in the last year was 63%, and the nurses comprised 42.6% of this case (8). These rates show similarity in Turkey, too (9).

Splash of blood and body fluid on mucose membranes is another accident source. In a study conducted in Turkey, splash of blood and body fluid on mucose membrane was seen in 57% of the nurses during cutting tool injuries (10).

Violence at workplace is defined as a problem related to working environment that affects the productivity and safety of the workers negatively (11, 12). In a study conducted in England, 72.8% of the health workers were exposed to non-physical violence and 21.3% to physical violence in 12 months (13). And in the studies conducted in the hospitals of Turkey, violence rate was reported as 60% (14, 15).

Corporal illness was reported to be higher in nurses than other health personnel. And the most important reason of this situation was the glide-fall injuries. In a study investigating the tissue trauma and injuries of 209 medical personnel, 47% of them was caused during the transportation of patients, 45% by collision and 48% by glide-fall (16).

Determining the work accidents encountered during the execution of the work would be beneficial to take necessary precautions. The number of studies on this subject is very limited in Turkey, and there is no national data at present. Findings obtained in this study demonstrate the importance of the subject by determining the work accidents of nurses, and therefore, be guiding principles to take necessary precautions. For this reason, it was aimed to determine the rate of the total accidents of the last six months and effective variables, based on the statements of the nurses working in the hospital.

Methods

Type of Research

Study was planned as a descriptive research.

Sample

In the study, it was aimed to reach 567 nurses working in the Practice and Research Hospital of the Dokuz Eylül University; therefore, no sampling was made. Units of the nurses consisted of emergency, surgery and intensive care units (n=179), internal services (n=132), surgical services (n=167), polyclinics (n=11), special centers (n=67) and administrative units (n=11). 71.4 % of the nurses could be reached (Table 2). The reasons for inability to reach the nurses were 35% of passive denial (postponement to a later date and not showing up or re-postponement), 30% of refusal to participation, 30 % of inability to find in their work plots, and 5% of unclear reasons.

Instrument

Data were collected with questionnaire forms covering socio-demographic characteristics and work history after examining the literature on the subject. Work accidents (within the last six moths) were considered in seven different groups as follows: cutting-piercing injuries, being exposed to blood and body fluids, splash of blood and body fluids on mucose membrane, tissue trauma caused by gliding-falling, being exposed to aggression and having traffic accident on way to work and others.

Data Collection

Data were collected by the researcher. Each interview was completed in 15 minutes.

Data Analysis

The Statistical Package for Social Sciences (SPSS 15.0) was used to compute frequency and
descriptive statistics related to demographic data. Chi-square analysis was applied between the work accidents of the last six months and the variables of socio-demographic and work history. Statistical significance was set at p<0.05.

Ethics Consideration

Ethical committee approval was granted by Dokuz Eylül University, School of Nursing to conduct the study. Written consent to collect data was obtained from Practice and Research Hospital of Dokuz Eylül University. The purpose of the study was explained to each nurse, and their consent was asked to participate in study.

Results

Demographics

60% of the nurses were married. 66.4% were university graduates, and 28.6% had an associate degree. 72.8% of them had 5-8 hours of daily sleep. The mean age of the nurses was 32.3±0.3. 29.1% of the nurses had 0-4 years of work experience. 38.3% had been working for this institute 0-4 years. 33.1% were working in the surgical services, and 27.4% in the internal services. 58.5% had been working in the same service for 0-5 years. Daily work hours of 79.5% were between 10-14 h. and weekly working days of 95.6% were between 4-6 days. 79.3% were working on shift basis, and 20.6% had 9 or more shifts. 60.7% were pleased with their units. 66.2% thought that there were materials-appliances-machinery in the workplace to affect their health condition negatively. 72.3% didn’t take any training on work accidents and protective methods.

The Accident Types of the Last Six Months

60% of the nurses (243) stated to have work accident within the last six months. Distribution of the work accident types was given in Table 1. 42% of the nurses were exposed to cutting-piercing injuries, 30.1% blood and body fluids, 13.6% splash of blood and body fluids on mucose membrane, 9.6% gliding-falling injuries, 21.5% aggression and 3.7% traffic accident on their way to work (Table 1).

Table 1. Distribution of the work accident types of the nurses within the last six months (n=243)

<table>
<thead>
<tr>
<th>Work Accident Types</th>
<th>Number*</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutting and Piercing Injuries</td>
<td>170</td>
<td>42.0</td>
</tr>
<tr>
<td>Exposing to Blood and Body Fluids</td>
<td>122</td>
<td>30.1</td>
</tr>
<tr>
<td>Splash of Blood and Body Fluids on Mucose Membranes</td>
<td>55</td>
<td>13.6</td>
</tr>
<tr>
<td>Glide-Fall Accidents</td>
<td>39</td>
<td>9.6</td>
</tr>
<tr>
<td>Exposing to Violence</td>
<td>87</td>
<td>21.5</td>
</tr>
<tr>
<td>Traffic Accident on way home or work</td>
<td>15</td>
<td>3.7</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* More than one option selected

Comparison of the Accident Cases according to Work History of the Nurses

Results of the statistical analysis are given in Table 2. Difference between the marital status of the nurses and accident occurrence was found statistically significant (p=0.025). Single nurses had accidents more frequently than married ones.

Comparison of the Accident Types of the Last Six Months Considering the Socio-Demographic Characteristics of the Nurses

Results of the statistical analysis are given in Table 2. Difference between the marital status of the nurses and accident occurrence was found statistically significant (p=0.025). Single nurses had accidents more frequently than married ones.

Difference between the graduated schools and accidents occurrence was found statistically significant (p=0.043). Difference was derived from the nurses graduated from Open University (distance learning). All the Open University graduates had accident. Difference between the daily sleep time and accident occurrence was found statistically significant (p=0.018). Difference was derived from the nurses sleeping over 8 hours a day. These nurses had less accident (Table 2).

Comparison of the Accident Cases according to Work History of the Nurses

Related results of the statistical analysis are given in Table 3. Difference between accident occurrence and occupational period of the nurses was found statistically significant (p=0.002). Nurses with less than four years work experience had more accidents.
Table 2. Comparison of the work accidents within the last six months according to socio-demographic characteristics of the nurses (n=405)

<table>
<thead>
<tr>
<th>Socio-Demographic Characteristics</th>
<th>Having Work Accident</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes Number</td>
<td>%</td>
<td>No Number</td>
<td>%</td>
<td>Total Number</td>
<td>%</td>
</tr>
<tr>
<td>Marital Status</td>
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<tr>
<td>Married</td>
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<td>55.6</td>
<td>108</td>
<td>44.4</td>
<td>243</td>
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</tr>
<tr>
<td>Single</td>
<td>108</td>
<td>66.7</td>
<td>54</td>
<td>33.3</td>
<td>162</td>
<td>100.0</td>
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<tr>
<td>Education Level</td>
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<td>Two Years Degree</td>
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<td>107</td>
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<td>100.0</td>
</tr>
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<td>Open University</td>
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<td>52</td>
<td>44.8</td>
<td>116</td>
<td>100.0</td>
</tr>
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<td>Health Vocational</td>
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<td>0</td>
<td>0.0</td>
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<td>82.4</td>
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<td>Daily Sleep Time</td>
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<td></td>
<td></td>
<td></td>
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<td>60.7</td>
<td>11</td>
<td>39.3</td>
<td>28</td>
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<tr>
<td>5-8 h</td>
<td>188</td>
<td>63.7</td>
<td>107</td>
<td>36.3</td>
<td>295</td>
<td>100.0</td>
</tr>
<tr>
<td>More than 8 h</td>
<td>38</td>
<td>46.3</td>
<td>44</td>
<td>53.7</td>
<td>82</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td>60.0</td>
<td>162</td>
<td>40.0</td>
<td>405</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*p<0.05

Difference between accident occurrence and the period nurses spent in the same institution was found statistically significant (p=0.036) (Table 3). Nurses working in the same institution for less than four years had more accidents.

Difference between the units where the nurses worked and the accident occurrence was found statistically significant (p=0.001) (Table 3). Nurses working in emergency, surgery and intensive care units had more accidents. And the difference between the shift number per month and accident occurrence was found statistically significant (p=0.000) (Table 3). Nurses having nine or more shifts had more work accidents. On the other hand, the difference between the accident occurrence and working with appliances and materials having harmful effects on human health was found statistically significant (p=0.000) (Table 3). Nurses working with this kind of elements had more work accidents.

Discussion

In this study, more than half of the nurses were found to have work accident within the last six months. In similar studies, accident rate changed between 25-60% (13,16). In a study carried out in Turkey, it was reported that 55% of the nurses working in the hospital had work accident (17). In this study, rate of having training on protective methods against work accidents was determined highly low. From this respect, high accident rate of nurses could be caused by the lacking knowledge on protective methods, and the lacking precautions due to the fact that many accidents weren’t reported. Every accident is important and suggestive for the later possible accidents. Therefore, accidents should be investigated in detail, and necessary precautions should be taken.

Characteristics of the Work Accidents of the Last Six Months

In the study, the most frequent accident was found cutting-piercing injuries. Majority of the nurses had this kind of accident 1-2 time(s), which was a highly significant result. In a study carried out in Egypt, the most frequent work accident experienced by the health workers was found to be cutting-piercing injuries (35.6%), and the reasons were the inadequate use of protective methods (18). In a study carried out in Turkey, it was reported that 51.9% of the nurses had cutting-piercing injuries during closing the cover of the syringe (17). In this study, the reason for the most frequen-
Table 3. Comparison of the Work Accidents Considering the Work Histories of the Nurses (n=405)

<table>
<thead>
<tr>
<th>Work History Characteristics</th>
<th>Having Work Accident</th>
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<th></th>
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</thead>
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<td>Yes</td>
<td>No</td>
<td>Total</td>
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<td>No</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>%</td>
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<td>Professional Experience</td>
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<tr>
<td>0-4 year</td>
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<td>74.6</td>
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<td>25.4</td>
<td>118</td>
<td>100.0</td>
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</tr>
<tr>
<td>5-9 year</td>
<td>49</td>
<td>51.6</td>
<td>46</td>
<td>48.4</td>
<td>95</td>
<td>100.0</td>
<td></td>
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<tr>
<td>10-14 year</td>
<td>53</td>
<td>55.8</td>
<td>42</td>
<td>44.2</td>
<td>95</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>More than 15 year</td>
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<td>54.6</td>
<td>44</td>
<td>45.4</td>
<td>97</td>
<td>100.0</td>
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</tr>
<tr>
<td>Work Period</td>
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</tr>
<tr>
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<td>69.0</td>
<td>48</td>
<td>31.0</td>
<td>155</td>
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<tr>
<td>10-14 year</td>
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<td>54.4</td>
<td>36</td>
<td>45.6</td>
<td>79</td>
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<tr>
<td>More than 15 year</td>
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<td>53.7</td>
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<td>Working Units</td>
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<td>Emergency, Operating Room and Intensive Care</td>
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<td>59</td>
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<td>Internal Units</td>
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<td>polyclinics</td>
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<td>6-11 year</td>
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<td>More than 12 year</td>
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<td>10-14 hours</td>
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<td>Hiç</td>
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<td>50.0</td>
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<td>1-2 times</td>
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<td>10</td>
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<td>3-5 times</td>
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<td>Use of equipments that might be harmful for health</td>
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<td>56.9</td>
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<tr>
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<td>57.7</td>
<td>124</td>
<td>42.3</td>
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<tr>
<td>Total</td>
<td>243</td>
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<td>162</td>
<td>40.0</td>
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</tbody>
</table>

*p<0.05
ent accident, cutting-piercing injuries, could be caused by the effort to close the cover of the needle and insufficient use of the protectors. In addition, the nurses were forced to carry out many tasks in a short time, protective materials (like gloves, treatment tray etc.) were inadequate and nurses didn’t follow the necessary protection procedures. Rate of the nurses reporting an accident was rather low, which indicates that they should be more informed on the subject.

Nurses were exposed to blood and body fluids 1-2 time(s) during the treatment of the patients (aspiration, mouth care etc.) and the intravenous injection. In a study carried out in France, this kind of accident was reported to occur most frequently during injection or taking blood (19). In this study, majority of the nurses exposed to blood and body fluids were employed in the services requiring urgent intervention. Most important reasons for this were found as the inadequate numbers of protective materials, lacking knowledge on the subject and intensive working conditions. Nurses should be trained on the possible health risks and the protection methods.

In the study, it was determined that nurses were exposed to splash of blood and body fluids on mucose membranes maximum 1-2 time(s). These accidents occurred during the treatment of the patients and in the mid and last four hours of the shift. Participant nurses stated that they couldn’t be protected due to the lack of protective materials, and most of them didn’t report the accident. Trape et al. reported that 55% of the nurses had accident during intravenous injection, and 92% didn’t report accidents because they disregarded the accidents due to the high occurrence frequency, and also they didn’t have sufficient time (20). In this study, the reasons why the nurses didn’t use protective materials were the high work density and the inexistence of these materials in the work units.

Nurses had glide-fall injuries 1-2 time(s). These accidents occurred in the services, and most of the nurses didn’t report the accidents, nor did they take any action afterwards. In another study, it was reported that 48% suffered from muscle-skeleton injuries due to the gliding, and 47% due to the impact (21). In a study carried out in Turkey, wet ground and that nurses had to lift the patients during the transportation were showed as the reasons for glide-fall accidents (22). To prevent glide-fall accidents, environment should be kept in order, and ground shouldn’t be left wet after cleaning. In this study, high rate of glide-fall accidents could be caused by these facts.

27.6% of the nurses were exposed to violence five or more times, and the most common of them was verbal violence. In a study carried out in Kuwait, it was determined that 48% of the nurses were exposed to verbal violence, and 7% to physical ones (23). In a similar study carried out in İzmir-Turkey, it was reported that the nurses were exposed to verbal violence more frequently (24). They stated to encounter violence generally at the beginning of the dayshift. In another study it was reported that the 38 nurses were exposed to mobbing. And 55.2% of them were graduates of bachelor degree and 52.7% of them were single. Moreover 86.9% of them are service nurses and 63.2% of them are working in clinics of interior branches (12).

In this study, the reason why the nurses were exposed to violence in the first hours could be the fact that their work stress was increased, for they had to carry out daily routines in a little time. Violence generally occurred in patient rooms. Most of the nurses didn’t report the violence, and 47.1% didn’t take any action. This problem should be reported to management to take the necessary precautions. The dimensions of the communication between patients and nurses should be evaluated. The reason why nurses didn’t take any action after being exposed to violence could be either their disregard or the insufficient safety precautions. Most of the nurses stated to have traffic accident 1-2 time(s) on the way to home or work. The most important reasons of the accidents were overstrain, weakness and the lack of attention caused by the work.

Intoxication and burns caused by chemicals came first among the other accidents nurses had. The rate of the mercury intoxication caused by the breaking degree was 15.4%. Nurses had their accidents in the first and last four hours of the shifts. Most of the accidents occurred in the services, and nurses generally didn’t report them. Kır et al. determined the rate of mercury intoxication as 12% in his study (25). Intoxications caused by chemicals could lead serious health problems. Therefore, nurses should be more careful for the protective methods. Common reason why nurses
didn’t take any action after work accidents could be the fact that they didn’t have enough time and didn’t believe the existence of a responsible department to apply.

**Comparison of the Work Accidents Considering the Socio-Demographic Characteristics of Nurses**

In the study, single nurses were found to have more accidents than married ones. Tabak et al. reported similar findings in their study, as well (26). In this study, most of the nurses began their professional lives in the same hospital where they still worked. With the increasing work experience, they were employed in the units with lower work intensity. Most of the single nurses were the new recruits, and had less work experience than married ones; however, they were employed in the services with high risks. Theses facts could be linked to the high accident rate of single nurses.

In the study, the difference between the graduated schools and accidents occurrence was found statistically significant. All the Open University graduates had work accidents. In a study, nurses with higher education level were found to have less needle accident (26). In a study carried out in Izmir-Turkey, accident rate was found lower for the nurses with higher education level (27). Open University graduates were older; therefore, they didn’t use protective equipments, which could have caused the accidents. In addition, Open University was a distant education system for the nurses graduated from health vocational high schools, and it is not being implemented in Turkey at present because it was not found beneficial. Therefore, high accident rate of these nurses could be attributed to the lacking education and skills.

In the study, nurses sleeping more than eight hours a day had fewer accidents. In a study carried out in Norway, it was reported that work accidents were caused mostly due to the loss of balance after night shift (28). In another study carried out in Turkey, it was determined that nurses having sleep disorder were exposed to violence more often than others because they became more aggressive and agitated when they were sleepless (29). In this study, it was possible that the fear for not finishing jobs in time, loss of attention and nervousness of the nurses with sleep disorder could lead the accidents.

**Comparison of the Work Accidents Considering the Work History of the Nurses**

In the study, work accident rate was found higher for the nurses working four or less years in the institute. There have been studies reporting that work accident rate was higher in less experienced nurses (8,19). However, there have also been studies stating no significant difference (29). Results obtained in this study could be attributed to the inability and inexperience of the newly recruited nurses.

In the study, it was found that nurses working in the surgical units were exposed to work accident more frequently. Operating rooms, emergency and psychiatry services were the placed where work accidents occurred more frequently (10,26,29). These results could be caused by the fact that newly recruited and inexperienced nurses were employed in these services.

In the study, nurses having nine or more shifts per month were found to have more work accidents. Many studies have reported that physiological, psychological and neurological problems like insomnia, loss of attention, fatigue, etc. were observed in the person working on shift, and these increased the accident possibility (18,30). In this study, shift number of the nurses was found to decrease with growing experience. Therefore, newly recruited and inexperienced nurses had more shifts in a month, and that increased their accident rate.

In the study, nurses with the opinion that work equipments were harmful for their health were found to have more work accidents. In another study, the most frequent accident rate was determined for the nurses using cutting-piercing equipments like needles (31). Similar results were reported in a study implemented in Turkey (32). Working with harmful equipments increased the accident rate. Despite the high number of materials that increase the accident possibility, the number of protective materials is rather inadequate; therefore, a healthy and safe work environment should be taken into consideration once again.
Conclusions

Nurses constitute a great part of the health workers. They encounter many risks and accidents during their professional lives. Lacking regulations for work environment, inadequate protective materials, low conscious and training level of nurses regarding protective methods for work risks are the factors that increase the accident rate.

Therefore, in terms of ensuring the safety of personnel, institutional culture plays a very important role in dealing with the risks (33). Especially hospital managements should take necessary precautions and make surveillance on site to prevent work accidents, and the trainings should be constant. Moreover, a workplace health unit should be established to observe and investigate work accidents in every hospital, and accident reports should be kept in order.

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A Novel model for inference of gene regulatory networks

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Abstract

Gene regulatory networks (GRNs) are complex networks consisted of nodes representing genes, transcription factors, microRNAs and edges that represent the interactions between nodes. GRNs can expose and depict underlying cells' gene regulatory mechanisms. In this paper, we propose a new model for inference of GRNs. This model includes prior knowledge into network inference. Our model was applied on generated gene expression time series with different number of genes and time points and one subset of experimental data received from colorectal cancer microarray experiment. To validate the inference capabilities of the proposed model, we compare the ROC curves and AUC values for proposed model and the common used models: dynamic Bayesian networks, Boolean networks and graphical Gaussian models. The proposed model has shown competitive inference capabilities in comparison with other models.

Key words: gene regulatory networks, inference models, validation, ROC curve, AUC value

1. Introduction

Gene regulatory networks (GRNs) are consisted of genes, proteins, metabolites and other network components, which interact mutually. For clearer presentation and understanding of these complex biological processes, inferring of gene regulatory interactions between genes, RNAs, proteins and metabolites is required. The inference of GRNs based on available gene expression data is a very complex and difficult task considering that the raw data used for inferring contain biological and technical noise. Additionally, microarray gene expression data refer to a smaller number of experimental conditions or time points, compared to the number of genes whose expressions are measured. These shortcomings of microarray data lessen the precision and accuracy of the networks’ inference. The accuracy and precision of an inferred network can be increased using other types of biological data and prior knowledge such as knowledge from scientific papers, protein-DNA interactions data and other available databases for gene regulatory pathways [1].

In this paper, we propose a new model for inference of GRNs. The network inference is performed in two phases. In the first phase, the model derives prior knowledge for gene regulations, whereas in the second phase, this knowledge is integrated into inference of the GRNs.

The remainder of this paper is organized as follows. In the second section, we briefly describe the commonly used inference models: Boolean networks, Bayesian and dynamic Bayesian networks and graphical Gaussian models. In the following section, we describe the methodology of the proposed model for GRNs inference. The data simulations, experimental data and data transformation are described in the fourth section. Finally, the discussion and concluding remarks, as well some further directions towards improvement of GRNs inference are presented in the last section.

2. Related works

One of the simplest models for reconstruction of GRNs is that based on Boolean networks. These networks are consisted of a set of nodes and
3. Methodology of the proposed model

Inferring networks using GGMs compared to Boolean networks and DBNs has shown better inference capabilities. GGMs can deal with large datasets comprised of thousands of genes, and the network inference is very fast, compared to DBNs, which can infer only small networks having tens of genes at the most [14]. Hence, we have chosen the GGMs in the first phase of the proposed model, because they are a good base for uncovering the “hub” genes, which are included in many regulatory pathways.

3.1 First phase of proposed model

The GRNs structure $G$ represents a directed acyclic graph which can be represented by an adjacency matrix. The elements of the adjacency matrix $G_{ij}$ can be either 1 or 0, which refers to the presence or absence of a directed edge between the $i$-th and $j$-th node of the network $G$, respectively.

Bayesian networks are extended to model time features by the introduction of dynamic Bayesian networks (DBNs). The main disadvantages of dynamic Bayesian networks are their inference accuracy that depends on number of genes and the excessive computational time needed for networks learning and inference.

Graphical Gaussian models (GGMs) employ partial correlation coefficients to determine the conditional independencies between genes. GGMs can distinguish direct or indirect interactions between genes, unlike the correlation networks where the edges present correlation between genes [15].

Beside of above-mentioned models, other models are used too, such as models based on Petri networks, neural networks, linear and nonlinear differential and difference equations systems. The methods which include prior biological knowledge into GRNs inference are described in [2] and [5].

To reduce insufficient accuracy of the inferred networks by above-mentioned models, we propose a new model that includes a priori knowledge.
For the elements of the matrix of prior knowledge $G_{priorij}$, the following is valid:

$$0 \leq G_{priorij} \leq 1 \quad \text{...(4)}$$

The value $G_{priorij}=0$ denotes that there is no directed edge between $i$-th and $j$-th node, and the value $G_{priorij}=1$ signifies the greatest clue of the presence of a directed edge between node $i$ and node $j$. The closer $G_{priorij}$ to 0 is, the greater clue for absence of edge is and the closer to 1 $G_{priorij}$ is, the greater clue for presence of a directed edge between $i$-th and $j$-th gene is.

The obtained matrix of prior knowledge $G_{prior}$ presents a basis for the second phase of the proposed model.

### 3.2 Second phase

To integrate the prior knowledge obtained from first phase, the second phase defines a function $G_{prior'}$ as a measure of matching between the given network $G$ and the obtained prior knowledge $G_{prior}$. This function is named as a network energy $E$ [2]. The function $G_{prior'}$ of network $G$ with $N$ nodes and prior knowledge $G_{prior}$ obtained from the first phase is calculated according to the following equation:

$$G_{prior'}(G) = \sum_{i,j=1}^{N} \left| G_{ij} - G_{priorij} \right| \quad \text{...(5)}$$

If prior knowledge matches with the network structure $G$ completely, then $G_{prior'}=0$. The $G_{prior'}$ value increases if there is a significant deviation between prior knowledge and the true structure of the network $G$.

The integration of prior knowledge $G_{prior}$ is according to prior distribution of the network structure $G$, which follows Gibbs distribution [1], given by the following equation:

$$P(G|\beta) = \frac{e^{-\beta G_{prior'}(G)}}{Z(\beta)} \quad \text{......... (6)}$$

where the denominator is normalization constant calculated from all possible network structures $\Gamma$ by the equation:

$$Z(\beta) = \sum_{G \in \Gamma} e^{-\beta G_{prior'}(G)} \quad \text{......... (7)}$$

The parameter $\beta$ determines the influence of prior knowledge employed in network inference. When $\beta$ tends to 0, prior distribution does not provide information about network structure. When $\beta \to \infty$, prior distribution defined by Eq. 7 has a maximum for structures with smallest energy [1].

In the second phase of the proposed model, a structure Bayesian learning is carried out using Markov chain - Monte Carlo simulations [8] [16].

### 4. Data and results

To validate the proposed model for network inference, we have employed simulated and experimental gene expression time series data. We performed two kinds of simulations to gain different size data-sets: Gaussian and SynTReN gene expression data.

Apart from networks inference from simulated data, we used experimental data to perform GRNs inference. We used gene expression time series data obtained from colorectal cancer microarray experiment. To validate inferred networks we employed the commonly used validation criteria such as receiver operating characteristic (ROC) curve and AUC value.

ROC curve is a chart where on the $x$-axis the false positive rate $(fpr=FP/(FP+TN))$ and on the $y$-axis the true positive rate $(tpr=TP/(TP+FN))$ are applied. $TP$, $FN$, $TN$ and $FP$ denote true positive, false negative, true negative and false positive, respectively [19] [21]. The values of the ROC cu-
The ROC curve belongs to the unit square in the first quadrant. If the ROC curve follows the equation \( y = x \) then it means a random guess of the class of absent or present edges. When the ROC curve is above the line \( y = x \), the inference performance is better. The AUC value is the area covered by the ROC curve with the x-axis. The AUC values belong to the interval \([0, 1]\), where the closer to 1 the value is, the better inference performance is [19] [21].

The inference with Boolean networks is by using the R package BoolNet [6], the dynamic Bayesian networks are inferred using the R package G1DBN [18]. The inference of GGMs networks, as well as obtaining of prior knowledge for proposed model is by employing the R package GeneNet [7]. The MCMC simulations for the second phase of the proposed model and the representation of the ROC curves is performed using BNSL MATLAB toolbox [8].

4.1 Inferring GRNs from simulated Gaussian data

The simulation of Gaussian data is performed by simulation of partial correlation matrix \( P \) with entries uniform distributed over interval \((-1, 1)\). The matrix \( P \) should be positive-definite matrix, so its diagonal elements are calculated by the following equation:

\[
p_{ii} = \sum_{j: j \neq i} p_{ij} + \varepsilon \quad \cdots \cdots \cdots \cdots \cdots (8)
\]

where \( \varepsilon \) is a small constant. The covariance matrix \( \Sigma \) is obtained by inverting of the matrix \( P \). Time series of gene expression data are generated according to multivariate normal distribution \( N(0, \Sigma) \) [20]. We have simulated 9 time series gene expression datasets denoted as DataGi_j, where \( i \) refers to the number of genes (5, 10 and 15 genes) and \( j \) refers to the number of time points (5, 10 and 50 time points). Datasets are simulated by the R package GeneNet [7], so the percent of present edges in the networks is 15%.

To compare the inference capabilities of the proposed model with GGMs, Boolean networks and DBNs, we have employed these models on simulated Gaussian datasets. From the ROC curves illustrated in Fig. 1, it can be concluded that the proposed model has better inference performances than GGMs, especially than Boolean networks and DBNs. For small number of genes, DBNs shows the worst inference performance compared to other models.

Table I shows the corresponding AUC values for Boolean networks, DBNs and GGMs. The charts presented in Fig. 2 illustrate AUC values depending on data dimensions: number of genes and number of time points. Only for DataG15_10, GGMs surpass the proposed model. Fig. 2 and Table I show that the proposed model shows the best inference capabilities for the most datasets.

4.2 Inferring GRNs from synthetic gene expression data generated by SynTReN

To validate the proposed model, additionally, we generated 9 time series gene expression data by

<table>
<thead>
<tr>
<th>Number of genes</th>
<th>Model</th>
<th>5 time points</th>
<th>10 time points</th>
<th>50 time points</th>
</tr>
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<td>5</td>
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<td>0.857</td>
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<td>0.632</td>
<td>0.766</td>
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</table>
the generator of synthetic gene expression data – SynTReN [17]. The SynTReN generator generates data, which are very plausible to the real biologic gene expression data [20]. Generated data by SynTReN generator refer to the referent gene regulatory network of E. coli. Time series gene expression datasets are denoted as DataSi_j, where i refers to the number of genes (5, 10 and 15 genes) and j refers to the size of time series (5, 10 and 50 time points).

The obtained ROC curves are shown in Fig.3. These ROC curves show that the proposed model has better inference performances than GGMs, especially than Boolean networks for most datasets. The proposed model is followed by DBNs, which show better inference capabilities for 3 datasets.
Figure 2. AUC values of inferred GRNs from simulated Gaussian data referred to a) 5 genes, b) 10 genes and c) 15 genes depending on time series size.

Figure 3. ROC curves obtained by GRNs inference from simulated datasets by SynTReN generator a) DataS5_5, b) DataS5_10, c) DataS5_50, d) DataS10_5, e) DataS10_10, f) DataS10_50, g) DataS15_5, h) DataS15_10 and i) DataS15_50.
To survey the inference capabilities of these models, Table II shows the corresponding AUC values. The charts presented in Fig. 4 illustrate AUC values depending on data dimensions: number of genes and number of time points. Only for DataS5_5, GGMs surpass the proposed model. Fig. 4 and Table II show that the AUC values, which correspond to the proposed model, are the highest for most datasets, and for several cases are in the second place. Unlike GGMs that can deal with smaller time series data, inference with DBNs show better capabilities when the number of time points increased.

4.3 Inferring GRNs from experimental colorectal gene expression data

Apart from networks inference from simulated data, we used experimental data to perform GRNs inference. We used microarray gene expression time series data that refer to genes in colorectal cancer measured in 4 time points. The data from experiment M-EXP-390 are downloaded from ArrayExpress [9]. After preprocessing and logarithm transformation of the gene expression data, 13 genes were selected according to available scientific databases and publications that described regulatory mechanisms between genes, transcription factors and other regulatory networks’ components such as: KEGG [12], TRANSFAC [3], JASPAR [4], DAVID [11] and [10]. The official names of the selected genes are: MLH1, MSH6, APC, DCC, SMAD4, SMAD2, TP53, KRAS, MSH3, MSH2, TGFB2, PTEN, and LKB1 (STK11).

The obtained ROC curves are depicted in Fig. 5 and the corresponding AUC values are shown in Table III. These ROC curves and the AUC values have shown that the proposed model, DBNs and GGMs have similar inference capabilities surpassing the inference with Boolean networks.

<table>
<thead>
<tr>
<th>Number of genes</th>
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<th>5 time points</th>
<th>10 time points</th>
<th>50 time points</th>
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<td>0.589</td>
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</table>
Figure 4. AUC values of inferred GRNs from synthetic datasets generated by SynTReN referred to a) 5 genes, b) 10 genes and c) 15 genes depending on time series size.

Figure 5. ROC curves obtained by GRNs inference from experimental colorectal gene expression subdataset consisted of 13 genes.

In the case of GRNs inference from experimental colorectal gene expression, a validation problem occurred because there are no “gold standard” networks with true interactions between genes. In other words, there is no “true” referent network in terms of which the inferred networks would be compared.

Table III. AUC values obtained by inference from selected 13 genes’ expression time series data received from experiment E-MEXP-390.

<table>
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<td>0.690</td>
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</table>

5. Discussion and concluding remarks

We propose a novel model for GRNs inference. The inference of this model is achieved in two phases. In the first phase, prior knowledge was extracted using partial correlations from GGMs and in the second phase, this knowledge was integrated performing structure Bayesian learning and Markov chain – Monte Carlo simulations.

The proposed model for GRNs inference has shown a very good inference performance when it was applied to simulated Gaussian data. Except in one case, the model has shown better inference performance compared to Boolean and dynamic Bayesian networks, as well as GGMs. For the most simulated time series data by SynTReN generator, the inference of the proposed model was better than Boolean networks and GGMs. For SynTReN generated data, DBNs follow the proposed model. In the third case, the inference by DBNs has a better inference performance than the proposed model, which was followed by GGMs and Boolean networks. The AUC values of the proposed model are ranged either in the first or second place.

In the case of GRNs inference from real gene expression data obtained by experiment E-MEXP-390, a validation problem occurs because there are no “gold standard” networks with true interactions between genes. When the proposed model, DBNs and GGMs are applied on gene expression data, similar AUC values are obtained. Otherwise, the inference with Boolean networks has shown significantly lower capabilities in comparison with other models.

Expectedly, the inference with GGMs was better when the models were employed on simulated Gaussian data in comparison with GGMs inference of data generated by SynTReN. Unlike GGMs that can deal with smaller time series data, inference with DBNs was better when the number of time points increased.

The validation of the employed models has shown that the proposed model in the most cases is able to infer GRNs more accurately compared to other models. The comparison of inference model point to that beside microarray gene expressi-
on data, prior knowledge and other data such as ChIP-chip, ChIP-Seq, microRNA data and should be used for GRNs inference.

Another problem for validation of inferred networks is that there are no “gold standard” networks, which present the true regulatory interactions between genes. Thus, as a further work, additional efforts towards upgrading the existing databases for regulatory mechanisms between genes, proteins, metabolites and other components should be made.

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Abstract

Dental erosion is defined as the irreversible loss of hard dental tissues caused by the long-term and often repetitive acid activity that dissolves the surface layer of hydroxyapatite and fluoroapatite crystal structure, where the aggressive factor is not of bacterial origin.

The aim of this paper is to present general dental erosions as a consequence of exogenous factors and the potential cumulative effect of gastroesophageal reflux.

This case report describes a 42-year-old man, who visited the Clinic for Dentistry of Vojvodina looking for the treatment of the wears on his teeth. Certain defects have been perceived in gingival thirds of vestibular surfaces of the upper and lower front teeth, in particular, yellow-brownish alterations on the bottom. Furthermore, the horizontal grooves have also been found in dentin. On the incisal edges of the upper incisors, one could observe worn sinusoidal form, whereas the incisal edges of lower anterior teeth turned into a recessed striate surface. These teeth wears react to coldness and mechanical irritation caused by a toothbrush. Composite restorations in teeth 23 and 24 appeared to be prominent.

The first phase of the therapeutic process comprised the identification of the etiologic factor and introduction of the patient with the latter, preventive measures and the need to eliminate the cause of the disease. The patient was recommended to use the straw while drinking, rather than shaking the content in the mouth. Furthermore, it is also advisable to increase the intake of cheese, milk and almond. Chewing gums lead to the increased salivary secretion, and thus, they are highly recommendable. The patient was also told to reduce the intake of tropical and berry fruit and consume plain water instead of mineral water, Fanta and Cola. The Protocol on Oral Hygiene emphasizes the importance of brushing the teeth in the morning and after meals (at least three times a day), with a soft toothbrush, avoiding the use of abrasive paste and in accordance with the Bass brushing technique.

The teeth should be brushed 30 minutes after waking up in the morning and after the consumption of acidic food and drinks. The resistance of the enamel to the acid agents can be increased by the use of fluoride in the form of a solution, lozenge or varnish.

The second phase of the treatment was reflected in the reconstruction of anatomical and morphological characteristics of teeth with composite and elimination of dental hypersensitivity.

The third phase will be done after obtaining the results of gastroenterologist and control of the implementation of precautionary measures. If the reflux disease is diagnosed, the patient will be advised to wear occlusal night guards. The problem of the partial edentulism will be solved with upper and lower removable partial denture.

Key words: dental erosion, dental defects.

Introduction

Dental erosion is defined as the irreversible loss of dental hard tissues caused by the long and often repetitive acid activity which dissolves the surface layer of the crystal structure of hydroxyapatite and fluoroapatite, where cause is not of an aggressive bacterial origin [1]. The formation of defects in dental tissues is caused by the dissolution of calcium and phosphate from the enamel, which leads to the collapse of surface enamel structure. Depending on the origin of the acid, erosions can be divided into: endogenous, exogenous and idiopathic. Endogenous erosion develops as a result of the maturity of gastric hydrochloric acid into the oral cavity; this is the case of anatomical abnormalities such as reflux disease, hiatus hernia and

Erosive nature of dental defect - case report

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diverticulosis of the esophagus, mental disorders-bulimia, chronic alcoholism, rumination, general medical conditions and diseases such as uremic state, peptic ulcers, morning vomiting in pregnancy. Exogenous erosion is caused by the acids that are inserted into the oral cavity through the diet, by the consumption of fruit, various juices, sodas and special drinks, medicines such as vitamin C, aspirin etc. Some occupations can also cause the appearance of exogenous erosion. These include wine tasters, professional swimmers and workers in the chemical industry. Erosive changes, whose existence cannot be explained by any currently known cause, are called idiopathic erosions.

In the earliest stages, dental erosions are manifested in the form of smooth, glossy, seemingly polished tooth surface, whereas the advanced changes have a specific morphology. Changes that occur on the vestibular and oral tooth surfaces can be manifested in different round, oval, irregular shapes; however, one thing is common for all of them— their width is larger than their depth. Sometimes they may appear in the form of flattened surface. Defects in the enamel-cement border appear in the wedged shape. The changes that occur on the biting surfaces of the teeth of the lateral region are manifested as cupped recesses on the locations of nodules, whereas the incisal edge turns into a grooved surface, with the bottom in dentin.

Case Report

A 42-year old man came to the Dental Clinic of Vojvodina, Faculty of Medicine, University of Novi Sad, Department of Diseases of the teeth with erosive changes in the vestibular surfaces of upper and lower teeth and worn incisal surfaces of incisors.

Primarily, he complained about the yellowish changes on the upper and lower front teeth and wear of incisal edges of lower front teeth. In addition to these symptoms, the patient also complained about bad breath, a burning sensation over the heart, belching, grinding and tension in the muscles of mastication. Three years ago, the patient was diagnosed with gastritis. As far as the medicines are concerned, the patient consumes Ranisan and Andol.

The dietetic history lists made on a daily basis (for the past year) note the consumption of citrus fruit and berries as well as the daily intake of at least 1 liter of soda in the form of mineral water, Fanta and Coca Cola. The patient used to eat pickled food three times a week. History data about the oral hygiene reveals that patient brushes his teeth twice a day, using the horizontal technique.

Objective examinations showed good oral hygiene. The patient has the erosive changes that occur on the vestibular surfaces of upper and lower teeth, the vestibular and occlusal surfaces of upper and lower molars and the incisal edges of upper and lower teeth. Voluminous defects are observed in the gingival third of the vestibular area of the upper and lower front teeth, in particular yellow-brownish changes in the form of the tertiary dentin, that appear on the bottom [Figure 1, 2]. The form of the defects on the upper central incisors testifies about the existence of restoration in the gingival third of the vestibular area [Figure 2].

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Figure 1. Inadequate composite dental fillings on tooth 23 and 24

Figure 2. Erosive changes in vestibular surfaces of upper central incisor
funds. On the incisal edges of the upper incisors, one can see worn sinusoidal form [Figure 1, 2], whereas the incisal edges of lower anterior teeth turned into a recessed striate surface [Figure 3, 5]. Furthermore, the existences of defects that match abrasion combined with erosive changes [Figure 4] appear in the vestibular gingival third area. The changes respond to cold and mechanical irritation of the toothbrush.

Figure 3. Worn incisal edge lower teeth

Figure 4. Abrasive erosive changes on the gingival third of the vestibular surfaces of lower anterior teeth

On teeth 23 and 24 one can observe visible fillings acting as grown [Figure 1]. The clinical examination showed the decreased vertical dimension of the occlusion and changes in the region of angles corresponding to angular cheilitis. The definitive restorations show great facets. The lack of teeth in the lateral region is of unknown etiological origin.

It has long been confirmed that there is no successful treatment without removing the causes of diseases. The first step of the therapeutic process was the identification of the etiologic factors and the introduction of the patient with them, as well as with all precautionary measures and the need to eliminate the cause of the diseases.

In this case the patient was advised to reduce the intake of food and liquids that are known to have erosive potential. He was also familiarized with the fact that it is better to intake the drinks with the straw and swallow them right away, rather than shaking the content in the mouth. The patient was advised to increase the intake of cheese, milk, almonds.

The patient was encouraged to use chewing gums as they lead to the increased salivary secretion. The patient was told to reduce the input of citrus fruit and berries, and consume plain water instead of mineral water, Fanta and Coca-Cola. Minimal consumption of pickled food is also recommended. Preventive measures focus on training the patient for adequate oral hygiene and change in nutritional habits. The Protocol on Oral Hygiene emphasizes the importance of brushing the teeth in the morning and after meals (at least three times a day) with a soft toothbrush, avoiding the use of abrasive paste and in accordance with the Bass brushing technique. The teeth should be brushed 30 minutes after waking up in the morning and after the consumption of acidic food and drinks.

The resistance of the enamel to the acid agents can be increased by the use of fluoride in the form of a solution, lozenge or varnish. Given the existence of bad breath from his mouth, firing over the heart accompanied by belching, which leads to a sense of relief, the patient was sent to the gastroenterologist since these symptoms correspond to gastroesophageal reflux. The second phase of the therapy procedure is reflected in the reconstruction of anatomical and morphological characteristics of teeth with composite, elimination of dental hypersensitivity and the establishment of adequate amount of occlusion. The elimination of dentinal hypersensitivity...
will be achieved by applying composites eroded surface, and using Colgate sensitive toothpaste.

The third step in the therapy will be taken after obtaining the results of Gastroenterologist and control of the implementation of precautionary measures. If the reflux disease is diagnosed, the patient will be advised to wear occlusal night guard. The problem of the partial edentulism will be solved with upper and lower removable partial denture.

Discussion

The formation of defects of dental erosion is caused by the decomposition of solid dental tissues due to the existence of a critical pH in the oral cavity after the due date of acidic foods, drinks or gastric contents. This value for the hard dental tissue amounts to pH = 5.5 and occurs during the saturation of such solutions (saliva, liquid component of dental plaque) with the mineral particles that make up the enamel. If the pH is above the critical value, it can lead to the precipitation, and if is below, the solution is unsaturated which can cause demineralization [2]. There are two main reasons for the dissolution of enamel acids. The first reason is that the acidic hydrogen ions react with the ionic product (OH) from hydroxyapatite (Ca_{10}(PO_{4})_6(OH)_2) and produce water. Thereby, the reaction has a bad influence on the consistency of products, which can further lead to the hydroxyapatite demineralization.

Another reason for the dissolution are the inorganic phosphates which occur in saliva and dental plaque fluid components in four different forms and as H_3PO_4, H_2PO_4^-, HPO_4^{2-}, PO_4^{3-}.

As pH has a direct impact on the proportion of these elements, the decrease of pH leads to the decrease of inorganic phosphate and demineralization. Non-ionized form of acids diffuses through interprysmatic space and dissolves the minerals below the surface layer, which leads to the mobilization of calcium and phosphate and the consequent increase in pH value of fluid within the salivary pellicle or saliva on the contact surface [3]. The process terminates if there is no new influx of acid. It is important to stress that the erosive changes are directly dependent on the number and duration of acid activity, and not on the type of acid and the ways of its maturities in the oral cavity.

Many studies suggest the connection of the occurrence of dental erosion with frequent consumption of soft drinks and other acidic foodstuff [4]. The current model of "healthy life" leads to the development of dental erosion because it involves the use of considerable amounts of citrus fruit and vegetables, as well as the intensive fluid intake which is reduced to the excessive intake – not of water, which is what our bodies need most, but the intake of soda / sparkling water, Coca-cola, juice from citrus fruits etc. [5]. We are familiar with the pH value of these favored drinks. PH value of lemon juice ranges 1.8 - 2.4; orange juice 2.8 - 4.0, Coca-Cola 2.7, juices from berries 3.2 - 3.6 [6]. Svi oni iskazuju kiselost koja je znatno niža od kritične vrednosti za demineralizaciju čvrstih zubnih tkiva, te nije čudo da dovode do razvoja dentalnih erozija. All of these express the acidity that is much lower than the critical value for the demineralization of hard dental tissues, thus, it is not surprising that they lead to the development of dental erosion. According to the medical history, the patient consumes "healthy life"; however, if he wants to preserve his tooth structure, he needs to reduce the consumption of the aforementioned.

If the dentist suspects of the gastric reflux patients, must be referred to further investigation, which includes endoscopic, histological and manometric methods to determine the function of the sphincter, the efficiency of peristalsis, presence of mucosal erosion and swallowing function [7]. Detection of dental erosion on the back teeth can be the first symptom of gastroesophageal reflux, and deceptive GERD.

Gastroesophageal reflux disease (GERD) is a chronic condition in which there is a return of gastric contents from the stomach into the esophagus. The correlation between GERD and dental erosion was first established 1971. In addition to the dental erosion, clinical gastroesophageal reflux is accompanied by the feeling of sour taste in the mouth, chest pain, epigastric pain, burping, bad breath, and difficulty in swallowing, especially hot drinks. Latent reflux disease implies the existence of gastric reflux without the above mentioned subjective symptoms. Dental erosion can also occur in the mouth of patients who have undiagnosed latent reflux disease, thus the dentist can relate the erosion of unknown etiology to the possible presence of deceptive GERD [7]. Erosive
esophagitis, Barrett’s esophagus, and lung abscesses. Laringopharyngitis can be considered to be the possible complications of gastric disease. Barrett esophagus (metaplasia of gastric mucosa with intestinal metaplasia in the esophagus) is a premalignant condition that developed by 10% of patients with GERD. For this reason, the diagnosis of gastroesophageal reflux is of great importance in the prevention of premalignant lesions of esophagus.

Reconstruction of eroded areas should be done with the use of adhesive composite systems with the highest power setting (14), with mandatory elimination of etiologic factors. Otherwise, the destruction of dental tissue continues.

**Conclusion**

In accordance with the data from the dietary history and the fact that changes are mainly localized on vestibular surfaces of teeth, we can conclude that in this case, the etiology factor is of exogenous origin. Unpleasant symptoms such as: bad breath, burning in the area heart, burping and the existence of cup recesses in the projection peak cusp of lower molars also show endogenous etiology in the form of gastroesophageal reflux.

This case shows how inadequate diet with excessive intake of acidic foods and beverages and the use of soft drinks leads to the occurrence of dental erosion. The intensity of the loss of tooth structure increases if GERD appears. Therefore, the correction of dietary habits and introducing the patient with the preventive measures are one of the first steps that need to be taken if exogenous factors are to be eliminated. The identification and further steps are of great importance for the definite diagnose and treatment of the disease and early detection of potentially malignant changes in gastroesophageal region.

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Retrospective Analysis of Intoxication Patients Admitted to Intensive Care Unit: Evidence Based Management vs Personal Experience

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Abstract

Objective: Intoxication is a major problem in emergency departments (ED). Patients shall either be hospitalized or discharged after ED care. This decision requires a thorough evaluation of the patients’ risk of mortality and cost effective approach. Aim of this study was to define characteristics of our poisoning patients and appropriateness of their hospitalization decisions to ICU.

Methods: Adult patients hospitalized to ICU following admittance to ED within three years period with acute poisoning were retrospectively enrolled. Demographics, poisoning data, former psychiatric history, ICU follow up information, outcome at hospital discharge and in the first 28 days and predominant pathological clinical findings were recorded.

Results: Our study group accounted for 3.6 per 1000 ED visits and 16.6% of ICU admittance. Mean age of the patients was 30.2±12.83 years, F/M ratio was 2.2 and 48.9% of the patients were married. Foremost encountered substances were psychoactive drugs (39.4%). 94.2% of our patients were suicidal and 39.8% of them used two or more agents. Intubation and mechanical ventilation was performed for 14 patients (5.1%), mean duration for intubated follow up was 7.07 days. Only two patients with caustic ingestions were dead (0.8%). Mean hospitalization period was 4.78±8.77 days and mean ICU bed use was 2.62±3.18 days. Total hospitalization duration was ≤48 hours 198 (72.3%) patients and > 48 hours in 76 patients (27.7%).

Conclusion: We speculate that, high rates of early discharge from ICU may support the necessity of a solid ICU admission criterion.

Key Words: Intoxication, Emergency Department, Intensive Care

Introduction

Intoxication constitutes one of the major patient groups in the emergency department (ED) practice, rates of poisoning related ED visits vary between 0.2 to 9.3 visits per 1000 population in the literature.²³ Although the causes of poisoning and the source of the poison are considered to be important in patient management, main factors guiding the basics of care in the ED are general status of the patient, vital signs, and the properties of substance or drug, if accessible. Confirmation of the patient history and adequacy of the physical examination affect the early prognosis of the patient along with the physician’s clinical experience.

Following the initial evaluation of the patient in the emergency department, patient will either be hospitalized or discharged. This decision requires a thorough evaluation of the patients’ probable risk of mortality and cost effective approach at the same time. Inaccurate discharge decisions or hospitalization of a patient requiring intensive care in medical wards may increase mortality. On the other hand unnecessary hospitalization causes escalated health care costs and inappropriate occupation of the invaluable intensive care unit (ICU) beds. In the literature there is a wide range of len-
Length of stay (LOS) both in ICU or ward beds. While Reiniluoto et al and Özköse et al stated a LOS shorter than 24 hours, Haennsen et al has given a range of 1 to 175 days of hospitalization.4,5,6

Due to the needs of and opportunities provided by our hospital, most of the acute poisoning patients are transferred to our tertiary level ICU following their primary care in our ED. Aim of this study is to define the characteristics of our poisoning patients admitted to the ICU and inquire the appropriateness of ICU transfers of these patients after the completion of ED care, based on their length of stay in the ICU and the criteria proposed by Krenzelok et al in 1996, and reviewed by Mokleshi et al in 2003 (Table 1).7,8


<table>
<thead>
<tr>
<th>Criteria</th>
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<tbody>
<tr>
<td>- Respiratory depression (PaCO₂ &gt; 45mmHg)</td>
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<tr>
<td>- Emergency intubation</td>
</tr>
<tr>
<td>- Seizures</td>
</tr>
<tr>
<td>- Cardiac arrhythmia (second- or third-degree atrioventricular block)</td>
</tr>
<tr>
<td>- Systolic BP &lt; 80 mmHg</td>
</tr>
<tr>
<td>- Unresponsiveness to verbal stimuli</td>
</tr>
<tr>
<td>- Glasgow coma scale score &lt; 12</td>
</tr>
<tr>
<td>- Need for emergency dialysis, hemoperfusion, or ECMO*</td>
</tr>
<tr>
<td>- Increasing metabolic acidosis</td>
</tr>
<tr>
<td>- Pulmonary edema induced by toxins (including inhalation) or drugs</td>
</tr>
<tr>
<td>- Hypothermia or hyperthermia including neuroleptic malignant syndrome</td>
</tr>
<tr>
<td>- Tricyclic or phenothiazine overdose manifesting anticholinergic signs, neurologic abnormalities, QRS duration &gt; 0.12s, or QT &gt; 0.5s</td>
</tr>
<tr>
<td>- Body packers and stuffers</td>
</tr>
<tr>
<td>- Concretions caused by drugs</td>
</tr>
<tr>
<td>- Emergency surgical intervention</td>
</tr>
<tr>
<td>- Administration of pralidoxime in organophosphate toxicity</td>
</tr>
<tr>
<td>- Antivenom administration in Crotalidae, coral snake, or arthropod envenomation</td>
</tr>
<tr>
<td>- Need for continuous infusion of naloxone</td>
</tr>
<tr>
<td>- Hypokalemia secondary to digitalis overdose (or need for digoxin- immune antibody Fab fragments)</td>
</tr>
</tbody>
</table>

*ECMO= extracorporeal membrane oxygenation.

### Methods

The patients hospitalized to ICU following admittance to the Department of Emergency Medicine at a tertiary care hospital within a three years period with history or symptoms regarding acute poisoning with drugs or other chemicals were retrospectively enrolled. Patients younger than 17 years old were seen and admitted in the pediatric ED and ICU respectively and therefore not included to our study. On the other hand, patients whose exposures could not be defined were excluded from the study.

The study design was approved by the local Ethics Committee. Records and demographics of the intoxication patients hospitalized to reanimation unit were collected by authors and transferred to a standardized data abstract sheet from electronic database systems of our hospital (Corteks® and Enlil®) and ICU patient archives, which kept records of the poisoning patients. Epicrisises of the patients and ICU follow up sheets were inquired retrospectively using appropriate ICD10 codes for intoxication to analyze for clinical parameters and therapeutical aspects. Demographic data included age, gender, date and time of the visit, marital status, and vital signs on admission. Former psychiatric and suicidal history of the patient, if any, was also questioned. The data about poisoning included route and source of exposure, characteristics of the exposed poison, and whether the intoxication was intentional or not. Follow up information during hospitalization included ICU LOS, duration of invasive mechanical ventilation, total hospital LOS in case the patients were transferred to the psychiatry ward, predominant clinical findings of the patients, outcome at hospital discharge and in the first 28 days. Predominant pathological clinical findings of the patients were classified as neurologic (focal or generalized seizures, headache, altered level of consciousness), psychiatric (agitation, delirium, depression), cardiac (bradycardia, tachycardia, arrhythmias, hypotension), and respiratory problems (dyspnea, permanent tachypnea, apnea, aspiration).

Patients were classified into either of two groups due to their length of stay in the ICU as over 48 hours or less. Patients requiring invasive mechanical ventilation and hemodynamic monitorization in both groups were also emphasized.
Statistical analysis was performed using the ‘16.0’ version of the ‘SPSS for Windows’ software package. Study variables were described by means, standard deviations, and percentages. Correlations of variables with group I and group II were analyzed by chi-square and Fisher’s exact test. P values of less than 0.05 were considered statistically significant.

Results

There were 282 patients hospitalized to ICU following admittance to the Department of Emergency Medicine between January 2007 and December 2009 with history or symptoms regarding acute poisoning. 8 of these patients were excluded from the study due to incomplete data. Poisoning patients were approximately 1.1% of the total ED admissions at the same time period, whether they were admitted to the ICU or not. Our study group of 274 patients, which is limited to ICU hospitalizations, accounted for 3.4 per 1000 ED visits and 16.6% of total ICU admittance.

Mean age of the patients was 30.21 (16-81, SD: 12.83), 86 of the patients (31.4%) were male with an overall F/M ratio of 2.2 and 134 of the patients were married (48.9%). We clustered the patients in age groups into decades such as 16-25, 26-35, 36-45 and older than 45. Proportional dispersion was as 49.6%, 24.8%, 12.4% and 13.1% respectively. There was a statistically significant female dominancy in the 16-25 (F/M= 106/30) and 26-35 (F/M= 48/20) age groups (p<0.0001). Poisoning related visits (Table 2) were more common in winter (n=95, 34.7%), followed by spring (n=74, 27.0%), summer (n=57, 20.8%) and autumn (n=48, 17.5%).

Substances those have been exposed to or used by the patients included antidepressants/antipsychotics other than tricyclic antidepressants (TCAs) (n=57, 20.8%), TCAs (n=51, 18.6%), organophosphates (n=43, 15.7%), multiple/mixed drugs (n=35, 12.8%), non steroidal anti-inflammatory drugs and paracetamol (n=22, 8%), antiepileptic drugs (n=19, 6.9%), cardiovascular drugs (n=15, 5.5%), super warfarins (n=11, 4%), carbamates (n=7, 2.6%), carbon monoxide (n=7, 2.6%), caustic chemicals (n=5, 1.8%) and antihistamines (n=2, 0.7%). Oral route established 94.2% of all cases (n=258), 12 patients inhaled the toxin, two were exposed via skin (0.75%) and two patients injected (one intravenous and one intramuscular) the chemicals (0.75%). There were no alcohol or illicit drug related intoxications in our patient group.

Table 2. Characteristics of patient group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>%</th>
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<tbody>
<tr>
<td>Mean Age (±SD)</td>
<td>30.21±12.83</td>
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</tr>
<tr>
<td>Age Groups</td>
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<tr>
<td>16-25</td>
<td>136</td>
<td>49.6</td>
</tr>
<tr>
<td>26-35</td>
<td>68</td>
<td>24.8</td>
</tr>
<tr>
<td>35-45</td>
<td>34</td>
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<tr>
<td>&gt; 45</td>
<td>36</td>
<td>13.1</td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
</tr>
<tr>
<td>Female / Male</td>
<td>188 / 86</td>
<td>68.6 / 31.4</td>
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<tr>
<td>Marital status</td>
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</tr>
<tr>
<td>Married</td>
<td>134</td>
<td>48.9</td>
</tr>
<tr>
<td>Single</td>
<td>125</td>
<td>45.6</td>
</tr>
<tr>
<td>Divorced/Widowed</td>
<td>15</td>
<td>5.5</td>
</tr>
<tr>
<td>GCS</td>
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<td></td>
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<tr>
<td>15</td>
<td>45</td>
<td>81.8</td>
</tr>
<tr>
<td>13-14</td>
<td>9</td>
<td>16.4</td>
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<tr>
<td>&lt; 13</td>
<td>1</td>
<td>1.8</td>
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<tr>
<td>Season</td>
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<tr>
<td>Winter</td>
<td>95</td>
<td>34.7</td>
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<tr>
<td>Spring</td>
<td>74</td>
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<tr>
<td>Summer</td>
<td>57</td>
<td>20.8</td>
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<tr>
<td>Autumn</td>
<td>48</td>
<td>17.5</td>
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<tr>
<td>Symptom</td>
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<tr>
<td>Neurologic</td>
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<td>Psychiatric</td>
<td>94</td>
<td>34.3</td>
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<tr>
<td>Cardiac</td>
<td>31</td>
<td>11.3</td>
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<tr>
<td>Respiratory</td>
<td>18</td>
<td>6.6</td>
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<tr>
<td>Intubated (±SD)</td>
<td></td>
<td></td>
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<tr>
<td>Mean Duration</td>
<td>7.07±12.62</td>
<td></td>
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<tr>
<td>LOS (±SD)</td>
<td></td>
<td></td>
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<tr>
<td>ICU</td>
<td>2.62±3.18</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>4.78±8.77</td>
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</table>

Patients with intentional exposures constituted 94.2% of our patients (n= 246). While 146 of these patients (60.2%) attempted suicide using only one drug or chemical, 98 of the patients (39.8%) used two or more agents. Within the same group there were 98 patients (39.8%) with a previous psychiatric history, 31 (12.6%) of these patients previously intended to commit suicide at least once and 64 (65.3%) of them used their own prescribed medica-
tions for their intention. Use of psychiatric medications were frequent in this group, 56 of the patients (57.1%) used TCAs or other antidepressants/antipsychotics. In our study group there were no patients intoxicated with alcohol or recreational drugs.

The signs and symptoms of the patients were evaluated, the dispersion was as follows; neurological symptoms in 114 patients (41.6%), psychiatric symptoms in 94 patients (34.3%), cardiac symptoms in 31 patients (11.3%), and respiratory problems in 18 patients (6.6%). Intubation and mechanical ventilation was performed for 14 patients (5.1%). Mean duration for intubated follow up was 7.07 days (2-50, SD: 12.62) with one exceptional patient of 50 days of intubated and 5 days of non-intubated follow up at the ICU (Table 2).

Following their ICU care, 240 (87.6%) of the patients were discharged directly from the reanimation unit and 32 of the patients (11.7%) were transferred to psychiatry ward. Only 2 of the patients (0.7%) were dead during the ICU follow up, both had intentional caustic ingestions and died at their third day due to profound intractable acidosis which did not respond to conventional haemodilaysis. Our search at the database showed that while 257 of the 272 patients (94.5%) discharged from the ICU had no recurrent visits, neither to ED nor psychiatry clinic, seven (2.6) patients had visited psychiatry clinic and eight of the patients (2.9%) were still hospitalized at psychiatry ward at the end of the first 28 days.

The appropriateness of ICU hospitalizations was inquired using the criteria for admission of the poisoned patients to ICU (Table 1). 15 (5.5%) of the 274 patients could not be evaluated due to missing data regarding these criteria. Of the remaining 259 patients, 164 (59.9%) had no matching

| Table 3. Comparison of Group I and Group II by study variables |
|-----------------|-----------------|-----|---|        |
|                 | Group I (n=198) | Group II (n=76) | P   | 95 %CI  |
| Male (%)        | 61 (30.8%)      | 25 (32.9%)      | 0.9 | -10.27 to 14.47 |
| Mean Age (±SD)  | 29.3±12.68      | 31.2±13.24      | 0.3 |         |
| Death (%)       | 0 (0%)          | 2 (2.6%)        | 0.1 | -0.98 to 6.18 |
| Marital status (%) |            |                |    |         |
| Married         | 98 (49.5)       | 36 (47.4)       | 0.9 | -11.11 to 15.31 |
| Single          | 89 (44.9)       | 36 (47.4)       | 0.8 | -10.69 to 15.69 |
| Divorced/Widowed| 11 (5.6)        | 4 (5.2)         | 0.9 | -5.53 to 6.33 |
| Exposure Route (%) |            |                |    |         |
| Oral            | 192 (97)        | 66 (86.8)       | 0.003* | 2.23 to 18.17 |
| Inhalation      | 5 (2.5)         | 7 (9.2)         | 0.04* | -0.15 to 13.55 |
| Other           | 1 (0.5)         | 3 (3.9)         | 0.1 | -1.06 to 7.86 |
| Exposure Purpose (%) |            |                |    |         |
| Intentional     | 184 (92.9)      | 62 (81.6)       | 0.01* | 1.88 to 20.72 |
| Unintentional   | 14 (7.1)        | 14 (18.4)       | 0.01* | 1.88 to 20.72 |
| Exposed Drugs (%) |            |                |    |         |
| TCAs            | 38 (19.2)       | 13 (17.1)       | 0.8 | -7.99 to 12.19 |
| Organophosphate | 25 (12.6)       | 18 (23.7)       | 0.04* | 0.48 to 21.72 |
| Analgesics      | 18 (9.1)        | 4 (5.3)         | 0.4 | -2.64 to 10.24 |
| Non-TCA psychoactive drugs | 40 (20.2) | 16 (21.1) | 0.9 | -10.50 to 10.70 |
| Carbamates      | 6 (3)           | 1 (1.3)         | 0.7 | -1.78 to 5.18 |
| Cardiovascular  | 13 (6.6)        | 2 (2.6)         | 0.3 | -0.98 to 8.98 |
| Superwarfarins  | 6 (3)           | 5 (6.6)         | 0.3 | -2.47 to 9.67 |
| Caustics        | 3 (1.5)         | 2 (2.6)         | 0.9 | -2.86 to 5.06 |
| CO              | 4 (2)           | 3 (3.9)         | 0.6 | -2.87 to 6.67 |
| Antiepileptics  | 16 (8.1)        | 3 (3.9)         | 0.3 | -1.58 to 9.98 |
| Antihistamines  | 2 (1)           | 0 (0)           | 0.9 | -0.4 to 2.4 |
| Mixed/Combined  | 27 (13.6)       | 9 (11.8)        | 0.8 | -6.88 to 10.48 |
|                 |                 |                 | *p < 0.05 |
criteria. 60 of the patients (21.9%) were recorded to be unresponsive to verbal stimuli, 14 (5.1%) had a GCS value less than 12, 6 patients (2.2%) had symptomatic TCA intoxication, other 6 (2.2%) had seizures, 4 (1.5%) had a PaCO$_2$ lower than 45 mmHg, 2 patients (0.7%) needed emergency intubation, 2 patients had Pralidoxime administered, and only one patient was recorded to have a systolic pressure below 80 mmHg (0.4%).

While only 22.6% of the patients with no positive criteria were hospitalized for more than 2 days, 34.7% of the patients with at least one positive criteria were hospitalized for more than 2 days (p=0.33).

Mean total hospital length of stay was 4.78 days (1-65, SD: 8.77) and mean ICU length of stay was 2.62 days (1-50 days, SD: 3.18). Total hospital length of stay was 48 hours or less in 198 (72.3%) patients (group I) and over 48 hours (group II) in 76 patients (27.7%). Characteristics of the groups are presented in Table 3.

**Discussion**

Poisoning is a common and sometimes life-threatening health issue for our country, as it is for the world.1,2 Our hospital is the foremost medical center in our territory. Therefore virtually all serious intoxication patients somehow end up in our facility. In this study we retrospectively analyzed the demographics, ICU care period and the early follow up of our intoxication patients.

Islambulcihar et al found that, a total of 5.4% of all admitted cases were poisoning related in their study.6 On the other hand, Hanssen et al declared a rate of 1.8 per 1000 and Lee et al found 4.2 per 1000 poisoning related visits to ED.8,10 The 2007 Annual Report of the American Association of Poison Control Centers’ National Poison Data System (NPDS) informed a rate of 8.1 per 1000 population.9 Turkish Statistical Institute stated the crude suicidal rates for years 2007 and 2008 among Turkey as 3.98 and 3.96 per 1000 population respectively.12 Due to the specific formation of our patient population, we studied with a more purified group. In our study, hospitalization rates of poisoning patients to ICU was higher than Sen- can and colleagues’ study where they had 13.4% (n=121) intoxication patient admittance rate to their ICU in a three years period.13 We speculate that, the high ICU hospitalization rate in our study may be associated with tertiary care provided in our hospital and lack of appropriate long term care in other regional hospitals.

Although there are different reports in literature, female to male (F/M) ratios range between 0.9 to 3.0.1,2,6,10,14 Our F/M ratio was 2.2, which is within this range and similar to Ayoglu and colleagues’ 2.1.15 A significantly high proportion of our patients were younger than 25 years old and patients younger than 35 years old constituted more than a half of our patients. This finding is also supported by the literature.6,9,10,13

In a mid-eastern study, majority of suicidal exposures were revealed to be young married females, and authors connected this dispersion to familial problems, failure in love affairs and economical problems.6 On the contrary, in their study conducted in Chicago, USA, Khliifi et al found that 69.3% of their patients were singles; compared to 12.3% married patients.16 In our study there was no statistically significant difference between married and single patient groups. This may be due to some specific and general stressors probably affecting each group equivalently: while loneliness and lack of attachment may be considered as specific reasons for single people; child issues (not having one or problems about children they have) and extramarital affairs might be examples for married people, and negative socio-economical movements in the society may motivate both married and single people to attempt suicide.

Islambulcihar et al reported that the most of the poisoning patients in their series were admitted in spring and least in autumn.6,9-11 Although most of the patients in our study were hospitalized in winter, lowest poisoning related admission rate in our study was also found in autumn. Unemployment and poverty are frequent stressors in our agricultural territory, where summer is the most occupied season and winter is the least. We believe incoccupation and availability of pesticides are the main reasons underlying our patients’ seasonal dispersion. Also, Turkey is a country which highly benefits from the sun in the summer days, lack of sunshine in the winter days may cause predisposition to depression.

In the literature the list of exposed poisons varies. McCaig et al declared that one fifth of the ex-
posed drugs in their study group were unspecified, 8.2% were analgesics and 5.4% were psychotropic agents. A study from Oman also indicated analgesics as the most frequent cause of specific drug poisoning. On the other hand there are many studies revealing psychoactive drugs, including benzodiazepines and other sedative-hypnotics, as the foremost exposed drug group. Psychoactive drugs (TCA’s, other antidepressants and antipsychotics) were the most frequently exposed toxic substances in our study, followed by pesticides (organophosphates and carbamates). Among all TCAs was the most common drug, just as it was in the 11 years experience of Unverir and colleagues. In our country, TCAs are easy to reach even without a prescription, cheap to buy and available to create a serious and life-threatening situation. We believe these are the main reason of this frequent tendency to this drug. The second most common substances of exposure were organophosphates and carbamates. Since apricot cultivation is the main source of income in our territory, organophosphates and carbamates can easily be obtained and found in many houses. Our results support that either intentional or unintentional poisoning may easily occur due to readily approachable substances.

In 1987, Hawton et al reported that, 40–50% of the medically serious suicide attempts had one previous attempt, 12–20% are likely to attempt again within a year, and 12–16% had a history of 5 or more previous attempts. A history of previous psychiatric problems was evident in about forty percent of our suicidal patients, and more than ten percent of these patients had at least one more previous suicide attempt. Aoygul et al and Unverir et al reported similar incidences in their studies. We also found that, patients on psychoactive medication used their own drugs, mostly TCAs, for their intentions. A study mainly focused on antidepressant poisonings in Turkey also showed that TCAs were the drug of choice for self-harm.

Many routes of exposure for poisoning including ingestion, injection, inhalation and even rectal/vaginal routes are reported. In our study oral route constituted the majority of exposures. Oral medications and chemicals are easy to reach and apply. Therefore, it is not surprising that oral route is the major way of substance intake when one intents to commit suicide. In the literature, suicidal intoxications are the most encountered types of poisoning, and most attempters use only one drug or chemical. A vast majority of our patients were exposed to the poison deliberately, with the intention to commit suicide, and more than a half of them used only one substance for their intention. The number and content of exposed substances are important, because interactions of the co-ingested substances may cause more morbid and mortal effects due to adverse drug reactions (ADRs) than the main substance regardless of the intention.

Intubation and mechanical ventilation are necessary measures in case patients have low Glasgow Coma Scale (GCS) scores, respiratory failure, and serious carbon monoxide poisoning and status epilepticus. The need for intubation varies about 4-21.2%. Definitive airway measures were deemed necessary for five percent of our patients, which is within this range. In our study, intubated patients were found to have a longer ICU LOS (> 2 days) as might have been expected.

Death rate during our 3 years experience, which is less than one percent, is rather pleasing. In the study by Lee et al, 4.2% of all poisoning patients and 11.2% of hospitalized or transferred patients had a fatal progress. Depending on the study population, overall poisoning mortality in literature varies between 0.01% and 6.9% worldwide, and 0% and 14% in Turkey. We believe, despite a relatively high rate of intubated patients, our low mortality rate is probably associated with the high proportions of patients with an ICU LOS less than 2 days, which may indicate unnecessary ICU hospitalization and our low mortality rates may be due.

The indications for ICU admission of the poisoned patient are debated in the literature. Brett et al proposed a list of 8 situations for ICU admission and denoted that no ICU interventions were deemed necessary for patients who did not represent PaCO2 > 45 mmHg, need for endotracheal intubation, toxin-induced seizures, cardiac arrhythmias, QRS duration > 0.12 s, systolic BP < 80 mm Hg, second- or third degree atrioventricular block, and unresponsiveness to verbal stimuli no ICU interventions. Others enlarged the list to 19 items. The retrospective nature of our study is a major limiting factor in our efforts to validate the appropriateness of our patients admission decisions to ICU.
The data we were able to reach thru the patients’ charts showed that more than half of our patients were admitted to our ICU without a proper indication, probably only via common sense.

A vast majority of our patients were discharged directly from ICU, and those except this group were transferred only to psychiatric ward. In the 28th day, only eight of the patients (2.9%) were still hospitalized at the psychiatry ward and only seven (2.6%) patients had visited psychiatry clinic following their disposition. Although more than 90% of our patients were poisoned intentionally, only about a ten percent of them required in-patient psychiatric treatment. This finding probably indicates the impulsive nature of the suicide attempts.

The total hospitalization period in our study is longer than the literature suggests. Reiniluoto et al stated 94% of their patients had a LOS shorter than 24 hours, and Özköse et al reports a mean LOS of 0.7 days. The contradistinction of our results reflects the differences between the patient groups. Our study group is consisted of patients who were already entitled for ICU hospitalization, which therefore did not include self discharges from ED and ward hospitalizations of probably shorter periods, unlike the aforementioned studies. On the other hand, while poisoning with organophosphates, and unintentional exposures are found to be significantly related with longer hospitalization, intentional exposures and oral route of exposure to poison are related with total hospital LOS ≤ 48 hours. We could not find any data regarding the factors affecting total hospital length of stay in the literature.

**Conclusion**

The high rate of patients discharged from ICU within 48 hours may indicate unnecessary hospitalization to ICU. Although we did not measure the effects of the poisoning patients to the health care costs, ICU beds are far more expensive than regular ward beds. Unless a criterion is used for ICU hospitalizations of poisoning patients, cost effective management may not be warranted. Our results suggesting that impulsive suicidal attempts are more common in our territory, also supports the necessity of a solid criterion for ICU admissions.

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Liver slices are the optimal model for mimicking apoptosis activation in vitro

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Abstract

Tissue slicing enables sectioning of tissue with metabolically active cells. The cutting of untreated rat and human liver tissues has been optimized recently to produce uniformly thick tissue slices of 200 μm. There are no reports in the literature about the optimal slice thickness to study induction of apoptosis. Therefore, the objective of our study was to determine the optimal slice thickness that would enable performing a uniform pharmacological treatment as well as reproducible slicing. The native slices of liver from 80 μm to 200 μm are suitable for studies of biochemical pathways and metabolism, however, only the slices of 80 μm are a reliable model for apoptosis triggering in liver. These slices are the best model for studying apoptosis initiation in liver, since the pathways of apoptosis triggering are changed in the primary liver cells (hepatocytes) due to stress during their isolation. Tissue slices are important in vitro models for studying metabolism and may become useful for evaluation of medical procedures in cell therapies and regenerative medicine.

Key words: Liver slices, apoptosis, organotypic culture, caspase activity

1. Introduction

Cell cultures, especially those of primary cells, are important models for studying biochemical and physiological phenomena. The primary cell cultures of isolated hepatocytes are being used as models to study drug metabolism or drug effects on liver; the human primary hepatocytes are believed to be the closest model for studying metabolism in vitro [1]. Nevertheless, there are some metabolic changes that occur upon culturing of primary hepatocytes. It was shown recently that the pathway of apoptosis triggering in primary hepatocytes differs from the one within the intact tissue [2]. The preapoptotic cell stress response, which occurs as a consequence of stress during hepatocytes’ isolation, makes even the best possible cellular model, the primary hepatocytes, unsuitable for studying apoptotic pathways in the intact tissue.

Tissue slicing is a new technique, which enables sectioning of a tissue with metabolically active cells. It was used for the production of organotypic slice cultures, mainly of the brain [3-5]. Cutting the intact soft tissues is generally more challenging than producing brain slices, however, more and more tissues are being successfully used in research, like pancreatic [6, 7], heart [8], lung [9], breast [10] and liver tissues [11, 12]. For example, the tissue slices were used for studying electrophysiology of pancreatic beta cells [6], evaluation of gene therapy vectors in pancreatic tissue [7] and lungs [10], for pharmacological drug testing in heart [8], lungs [9] and liver [10]. Therefore, tissue slices are good models for studying intracellular biochemical pathways. Tissue slices are useful also in molecular medicine; e.g. they were used for evaluation of gene therapy vectors in human pancreatic tissue cells already [7].

Liver is one of the soft tissues’ organs which are generally pre-treated with gelatine or low melting point agarose to enable cutting thin homogeneous slices [10, 12]. Recently the cutting of untreated rat and human liver tissues has been optimized to produce uniformly thick tissue slices of 200 μm [11]. There are no references in the literature about the optimal slice thickness for the study of apoptosis triggering. Therefore, the objective of our study was to determine the optimal slice thickness for the study of apoptosis triggering. Slices of 80 μm, 100 μm and 200 μm thickness survive in cul-
tecture with only background levels of caspase activity. We determined that the 80 μm liver slices are optimal since they enable reproducible apoptosis triggering by staurosporine (STS) with the highest activity of caspase 3. Equal treatment also triggers apoptosis in 100 μm and 200 μm slices, however, apoptosis seems to be induced in a smaller proportion of cells, presumably only in cells located in the outer most layers of the tissue sample. Therefore, 80 μm liver slices are a reliable model for apoptosis triggering in tissue and can mimic intact tissue better than the primary cells.

2. Material and Methods

All basic chemicals and materials were purchased from Sigma (Taufkirchen, Germany) and Merck (Darmstadt, Germany) if not stated otherwise.

Preparation of tissue slices

Liver was isolated from adult male rats (Wistar- Hannover, 200-300 g), which were treated as for the isolation of primary hepatocytes [13]. Isolated liver were placed into William’s medium E with penicillin and streptomycin (50 U/ml, each), insulin (0.1 U/ml) and 1 μM hydrocortisone hemisuccinate. Off-cuts of about 1 cm3 were sliced further with Leica VT1200 S vibrating blade microtome (Leica Microsystems GmbH, Wetzlar, Germany) in an ice cold William’s E medium as described above. The tissue slices were collected into a 24-well tissue culture plate (Nunc, Roskilde, Denmark) containing the William’s E medium as described above. The tissue slices were collected into a 24-well tissue culture plate (Nunc, Roskilde, Denmark) containing the William’s E medium and incubated with or without 1 μM STS for 6 hours in a humidified atmosphere with 95 % air and 5 % CO2 at 37 °C.

Measurement of caspase activity

The tissue slices were homogenized in cell culture lysis buffer (Promega, Madison, WI, USA). Protein concentrations were determined by BCA-TM Protein Assay Kit as described by the supplier (Pierce, Thermo Scientific, Rockford, USA). The activities of caspase-3 were deduced from formation of luminescent substrates by using Caspase-Glo 3/7 Assay (Promega, Madison, WI, USA) according to the supplier’s protocol. Each sample contained 20 μg of protein.

Statistical analyses

Percentage of relative activity of caspase-3 in a sample was calculated by dividing the luminescence values of treated or untreated cells with the average of luminescence values of untreated cells from each independent experiment. The data from at least tree independent experiments were plotted by Sigma Plot 11.0 (Systat Software, San Jose, CA, USA). Statistical analyses were done by Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA); Kruskal-Wallis rank sum test was used to compare more than 2 groups unequal variances). When indicated, post hoc analyses were performed by Dunnett T3. We considered values of samples as statistically significant when $P<0.01$.

3. Results and Discussion

Fresh liver without pre-treatment was cut into 50 μm, 80 μm, 100 μm and 200 μm thick slices. The 50 μm slices were not uniformly thick even by visual inspection and shedding of cells into the surrounding medium was observed during cutting. Therefore these slices were not used any further. Other slices appeared uniformly cut, which was deduced from visual inspection and by reproducible levels of cleavage of caspase-3 substrate DEVD even after 6 hours incubation (Figure 1, untreated). The incubation of untreated controls for 6 hours after isolation enables to control the quality of slices, since the damaged cells would likely die of apoptosis, as can be observed in some primary hepatocytes immediately after isolation [2]. The similar level of DEVDase activities in the STS-treated slices of 100 μm and 200 μm also supports that these slices were uniformly cut. This agrees with the published results, in which the 200 μm slices were confirmed to be uniformly thick by re-slicing and measurement by confocal micros-
copy [11]. In conclusion, the native slices of liver from 80 µm to 200 µm are suitable for studies of biochemical pathways and metabolism.

The DEVDase activity was measured on induced and control tissue slices cultured for 6 hours after isolation. The STS was added to appropriate samples immediately after isolation. Although the resulting DEVDase activity can be detected sooner after the induction of apoptosis by STS, it was determined previously that this activity reaches its maximum at about 6 hours from STS treatment [2].

The levels of DEVDase activity are higher in STS treated samples compared to their uninduced controls by about 50 to 400 % (Figure 1). These differences are statistically significant among all samples (P=3.5 x 10^-8, Kruskal-Wallis rank sum test). The levels of DEVDase activity also differ between the slices of different thickness. There is no statistically significant difference between the treated and untreated samples in slices of 100 µm and 200 µm (P=0.033 and P=0.219, respectively, Dunnett T3 post hoc test). On the contrary, there is a sharp increase of DEVDase activity indicating caspase-3 activation in 80 µm slices upon the treatment with STS (P=0.009, Dunnett T3 post hoc test). We propose that this increase is due to the uniform induction of caspase-3 activity throughout the 80 µm tissue slice, as opposed to the activation of only outermost layers of cells in the thicker slices.

Apoptosis was induced by exposing the liver slices of 80, 100 and 200 µm to 1 µM staurosponine for 6 hours (STS). The caspase activity of tissue slices was inferred from increased luminescence of the cleaved caspase-3 substrate (DEVD. aminoluciferin). Each sample contains data from 3 independent tissue isolations; from each isolation there are at least 3 parallel tissue samples for each group. DEVDase activity of STS treated tissue slices differ significantly among the samples (P=3.5 x 10^-8, Kruskal-Wallis rank sum test). DEVDase activity among untreated and STS treated samples of 80 µm is highly significant (P=0.009, Dunett T3 post hoc test).

Conclusion

Tissue slicing is a powerful tool for studying the processes as they occur in vivo. Even soft tissues like the liver can be cut into slices without pre-treatment; these slices are thin enough to enable uniform pharmacological treatment. Only the slices of 80 µm are useful as a model for studying apoptosis activation in liver, which is of importance, since the primary hepatocytes do not mimic the intact tissue for this purpose. Therefore, liver tissue slices are the best known in vitro model for studying liver metabolism so far. They may be useful for evaluation of medical procedures in cell therapies and regenerative medicine.

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Abstract

In this paper, we present a multiresolution scheme to detect stellate lesions in mammograms. Multiresolution analysis is used to analyze the images at different frequencies with different resolutions. First we removed the noise from mammograms using Multiresolution analysis and then we detected tumors. Then, using the Embedded Zerotree Wavelet (EZW) algorithm, we compressed denoising mammographic image and showed that by applying the algorithm to detect tumors in the compressed image we obtained the same results as in the case of non-compressed images. Experimental results obtained from the mammographic images of patients recorded in the Clinical Center in Kragujevac, show that using multiresolution algorithm can be detected tumors of different sizes.

Key words: Multiresolution analysis of mammograms, Discrete Wavelet Transformation, Embedded zerotree wavelet, Breast cancer.

Introduction

Mass noncommunicable diseases, such as cancer, are the leading cause of death worldwide [1]. Breast cancer is the most common cancer among women in the world. Rehabilitation in oncology is mentioned in the mid 60’s of the 20th century, when, due to quantitative and qualitative achievements in oncology, first of all in breast oncology, the need for complete or maximally possible functional training of the patients with malignant diseases had occurred [2].

Breast cancer screening with mammography has been shown to be effective for preventing breast cancer death. All women who belong to the age group 40 – 64 should be included in a regular screening program every year. This research included women between 40 and 64. On the first mammographic examination one screens mediolateral and craniocaudal mammograms [3]. However mammography screening can be harmful to women. One of the major problems is anxiety or lack of peace of mind in mammography screening [4].

The anatomy of the breast is very complex [5]. Each breast contains between 15 to 20 lobes that are connected to the nipple through a complex structure of converging ducts. Each lobule consists of 10 to 100 terminal duct lobular units, the areas where breast cancer originates. Therefore, many hospitals breast cancer screening programs using mammography have been started to detect cancers as early as possible.

An important development that may help to improve the performance in breast cancer screening as well as clinical practice is computer aided diagnosis (CAD). Mammograms have to be digitized before automated methods can search them for abnormalities. It is hoped that CAD can help to decrease the number of errors, both false negatives (malignant cases that were not recalled) and false positives (cases that are recalled unnecessarily). Software can help searching for suspicious signs, or could help classifying lesions or microcalcifications in benign or malignant types.

Important work was done to transform the mammogram in such away that it can be printed or examined on a monitor optimally [6, 7]. The dark area near the skin line can be enhanced and the pectoral muscle can be filtered out, largely reducing the intensity range in the mammogram [8, 9]. Good contrast will be available in the whole area of interest, both in the pectoral area as well as near the skin line.
If the mass is surrounded by a radiating pattern of spicules, it is called a stellate lesion. Not all tumors have a central mass, especially lobular carcinomas are often only detectable due to an architectural distortion of the breast tissue. However, in practice a whole spectrum of appearances from lesions without a central mass, lesions with both a mass and spicules to lesions without any spiculation is found.

The central mass is a more or less circular bright region with a diameter between 5 mm and 5 cm. Sometimes a mass looks very much like normal glandular structure, and is only detectable due to asymmetry between the left and right breasts. These approaches perform some kind of image subtraction, and can also be used to detect temporal changes when a mammogram is compared with an older mammogram of the same breast. Matching two breasts is a complicated procedure because there is only an approximate correspondence between the normal tissue in the two breast. The aim of this investigation is detection of such stellate patterns without relying on the presence of a central mass.

When a mass is surrounded by spicules, it is likely to be malignant. Many stellate lesions are easier to detect by their spicules than by their central mass, and for architectural distortions it is the only sign. The presence of spiculations or a more diffuse stellate appearance in mammograms is almost pathognomonic of breast cancer. Therefore, the detection of stellate lesions is very important in the characterization of breast cancer. Unfortunately, it is also very difficult. A stellate lesion has an irregular center with ill-defined borders radiating spicules that may extend from several millimeters to centimeters in size. Features usually are local and extracted within a certain neighborhood [10]. In many cases the stellate pattern of spicules is the most important sign.

Dealing with noise in mammograms is very important for microcalcification detection algorithms. A major problem with denoising images by filtering is that filtering leads to blurring of the image. Hence filtering can be practically used only to remove noise which is out of the band of frequencies of the signal. The wavelet decomposition of a signal not only indicates the frequency content of the signal, but also the temporal or spatial position of that component. Hence, by working in the wavelet domain, it should be possible to reduce the blurring of image edges which occurs in image denoising by filtering [11].

Denoising, the task of removing or suppressing uninformative noise from signals, is an important part of many signal or image processing applications. Wavelet transform, which was put forward by the mathematicians at first, is a signal processing technique. The Wavelet technique can be applied to various fields such as applied mathematics, signal processing, sound and picture compressing techniques [12]. Wavelets are common tools in the field of signal processing. The popularity of wavelets in denoising is largely due to the computationally efficient algorithms as well as to the sparsity of the wavelet representation of data. By sparsity we mean that majority of the wavelet coefficients have very small magnitudes whereas only a small subset of coefficients have large magnitudes. This small subset contains the interesting informative part of the signal, whereas the rest of the coefficients describe noise and can be discarded to give a noise-free reconstruction [13].

The best known wavelet denoising methods are thresholding approaches. In hard thresholding all the coefficients with greater magnitudes than the threshold are retained unmodified as they are thought to comprise the informative part of data, while the rest of the coefficients are considered to represent noise and set to zero. However, it is reasonable to assume that coefficients are not purely either noise or informative but mixtures of those. In soft thresholding the coefficients with magnitudes smaller than the threshold are set to zero, but the retained coefficients are also shrunk towards zero by the amount of the threshold value in order to decrease the effect of noise assumed to corrupt all the wavelet coefficients [13]. The drawbacks of thresholding schemes are that they may lead to discontinuities in the wavelet coefficient values at the threshold values (in case of hard thresholding), and that they direct thresholding schemes do not take into account inter-scale correlations [11].

Wavelet image coding has been fertile area of research in the image processing community in recent years particular in relation to image compression. It does not only provide a good compression result, but it is also suitable for progressive
transmissions and provides a multi resolution capability. However, applying the wavelet transform on images for compression alone does not reduce the amount of data to be compressed, since it may remove some of the redundancy and decorrelate the neighbour pixels [14].

We used intensity adjustment to improve an image. Intensity adjustment is an image enhancement technique that maps an image's intensity values to a new range.

An image lacks contrast when there are no sharp differences between black and white. Brightness refers to the overall lightness or darkness of an image. In this process, pixel values below a specified value are displayed as black, pixel values above a specified value are displayed as white, and pixel values in between these two values are displayed as shades of gray. The result is a linear mapping of a subset of pixel values to the entire range of grays, from black to white, producing an image of higher contrast. The following figure shows this mapping. Note that the lower limit and upper limit mark the boundaries of the window, displayed graphically as the red-tinted window [15].

![Figure 1. Relationship of pixel values to display range](image1.png)

Given that we process a monochrome picture, reinforcing the contrast will be seen more clearly stellate lesions on mammograms. Figures 2. and Figure 3. shows that the stellate lesions clearly observed in the picture with enhanced contrast. Figure 2. represents the mammographic image before increase contrast.

Figure 2. Mammographic image before increase contrast

Figure 3. Mammographic image after increase contrast

Figure 3. Mammographic image after increase contrast

**Materials and Methods**

**Multiresolution analysis**

Multiresolution analysis is designed to give good time resolution and poor frequency resolution at high frequencies and good frequency resolution and poor time resolution at low frequencies. This approach makes sense especially when the
signal at hand has high frequency components for short durations and low frequency components for long durations.

The continuous wavelet transform was developed as an alternative approach to the short time Fourier transform to overcome the resolution problem. The wavelet analysis is done in a similar way to the STFT analysis, in the sense that the signal is multiplied with a function, (it the wavelet), similar to the window function in the STFT, and the transform is computed separately for different segments of the time-domain signal.

The continuous wavelet transform is defined as follows:

\[
\text{CWT}_x^\psi(\tau, s) = \frac{1}{\sqrt{|s|}} \int x(t) \psi\left(\frac{t - \tau}{s}\right) dt \quad (1)
\]

As seen in the above equation, the transformed signal is a function of two variables, \(\tau\) and \(s\), the translation and scale parameters, respectively. \(\psi(t)\) is the transforming function, and it is called the mother wavelet.

The term translation is used in the same sense as it was used in the STFT; it is related to the location of the window, as the window is shifted through the signal. This term, obviously, corresponds to time information in the transform domain. However, we do not have a frequency parameter, as we had before for the STFT. Instead, we have scale parameter which is defined as \(1/\)frequency. The term frequency is reserved for the STFT.

The parameter scale in the wavelet analysis is similar to the scale used in maps. As in the case of maps, high scales correspond to a non-detailed global view (of the signal), and low scales correspond to a detailed view. Similarly, in terms of frequency, low frequencies (high scales) correspond to a global information of a signal (that usually spans the entire signal), whereas high frequencies (low scales) correspond to a detailed information of a hidden pattern in the signal (that usually lasts a relatively short time).

In today’s world, computers are used to do most computations. It is apparent that neither the FT, nor the STFT, nor the CWT can be practically computed by using analytical equations. It is therefore necessary to discretize the transforms. First consider the discretization of the scale axis. Among that infinite number of points, only a finite number are taken, using a logarithmic rule. The most common value for the base of the logarithm is 2 because of its convenience. If 2 is chosen, only the scales 2, 4, 8, 16, 32, 64, etc. are computed. The time axis is then discretized according to the discretization of the scale axis. Since the discrete scale changes by factors of 2, the sampling rate is reduced for the time axis by a factor of 2 at every scale.

Expressing the above discretization procedure in mathematical terms, the scale discretization is \(S = S_0^j\), and translation discretization is \(\tau = k S_0^j \tau_0\) where \(S_0 > 1\) and \(\tau_0 > 0\). When discretized dilation and translation replace the continuous wavelet function:

\[
\psi_{\tau, s} = \frac{1}{\sqrt{s}} \psi\left(\frac{t - \tau}{s}\right) \quad (2)
\]

obtain the discretized wavelet function:

\[
\psi_{j, k}(t) = \frac{S_0^{-j}}{S_0^{-j}} \psi (S_0^{-j} t - k \tau_0) \quad (3)
\]

Although the discretized continuous wavelet transform enables the computation of the continuous wavelet transform by computers, it is not a true discrete transform. As a matter of fact, the wavelet series is simply a sampled version of the CWT, and the information it provides is highly redundant as far as the reconstruction of the signal is concerned. This redundancy, on the other hand, requires a significant amount of computation time and resources. The discrete wavelet transform (DWT), on the other hand, provides sufficient information both for analysis and synthesis of the original signal, with a significant reduction in the computation time.

The main idea is the same as it is in the CWT. A time-scale representation of a digital signal is obtained using digital filtering techniques. Recall that the CWT is a correlation between a wavelet at different scales and the signal with the scale (or the frequency) being used as a measure of similarity. The continuous wavelet transform was computed by changing the scale of the analysis window, shifting the window in time, multiplying by the signal, and integrating over all times. In the discrete case, filters of different cutoff frequencies are used to analyze the signal at different scales.
The signal is passed through a series of high pass filters to analyze the high frequencies, and it is passed through a series of low pass filters to analyze the low frequencies.

The resolution of the signal, which is a measure of the amount of detail information in the signal, is changed by the filtering operations, and the scale is changed by upsampling and downsampling (subsampling) operations. Subsampling a signal corresponds to reducing the sampling rate, or removing some of the samples of the signal.

The DWT analyzes the signal at different frequency bands with different resolutions by decomposing the signal into a coarse approximation and detail information. DWT employs two sets of functions, called scaling functions and wavelet functions, which are associated with low pass and highpass filters, respectively. The decomposition of the signal into different frequency bands is simply obtained by successive highpass and lowpass filtering of the time domain signal. The original signal $x[n]$ is first passed through a halfband highpass filter $g[n]$ and a lowpass filter $h[n]$. After the filtering, half of the samples can be eliminated according to the Nyquist’s rule, since the signal now has a highest frequency of $p/2$ radians instead of $p$. The signal can therefore be subsampled by 2, simply by discarding every other sample. This constitutes one level of decomposition and can mathematically be expressed as follows:

$$y_{\text{high}}[k] = \sum_n x[n] \cdot g[2k - n] \quad \text{and} \quad y_{\text{low}}[k] = \sum_n x[n] \cdot h[2k - n]$$

where $y_{\text{high}}[k]$ and $y_{\text{low}}[k]$ are the outputs of the highpass and lowpass filters, respectively, after subsampling by 2.

This decomposition halves the time resolution since only half the number of samples now characterizes the entire signal. However, this operation doubles the frequency resolution, since the frequency band of the signal now spans only half the previous frequency band, effectively reducing the uncertainty in the frequency by half. The above procedure, which is also known as the subband coding, can be repeated for further decomposition.

At every level, the filtering and subsampling will result in half the number of samples (and hence half the time resolution) and half the frequency band spanned (and hence double the frequency resolution) [16].

The difference between this transformation and Fourier transformation is that it is known time of occurrence frequency. However, the time during which these frequencies will have a resolution which depends on the level at which they appear. If the main information signal contained in very low frequencies, their temporal location will not be very accurate, because only a few samples used to represent the signals at these frequencies. This algorithm provides good temporal resolution for high frequencies and good frequency resolution for low frequency signals. Frequencies that are not highlighted in the original signal will have very low amplitude and that part of the signal obtained by the discrete wavelet transformation can be discarded without significant loss of information, which significantly reduces the amount of data.

In the case of two-dimensional signals such as images, in this way is formed multiresolution pyramid, shown in Figure 4. At each higher level is kept image two times less resolution than it was at the previous level and image details needed for the reconstruction of the signal.

![Multiresolution pyramid](image)

Figure 4. Multiresolution pyramid

Multiresolution representation of images in each level of decomposition consists of a discrete image approximation at lower resolution, and three image detail. Approximation corresponds to part of the spectrum which is obtained by lowpass filtering (LP) in both directions in the frequency plane. One of the image detail is obtained with horizontal LP and vertical highpass filtering (HP), the second vertical LP and horizontal HP filtering,
while the diagonal detail images obtained by HP filtering in both directions. Multiple repeat leads to images with worsening resolution, which corresponds to a pyramidal decomposition, Figure 5.

![Pyramidal decomposition of image](image)

**Figure 5. Pyramidal decomposition of image**

Filtering images using wavelet transformation

An image is often corrupted by noise during its acquisition or transmission. The de-noising process is to remove the noise while retaining and not distorting the quality of the processed image. The traditional way of image de-noising is filtering. A lot of research about non-linear methods of signal de-noising has been developed. These methods are mainly based on thresholding the Discrete Wavelet Transform (DWT) coefficients, which have been affected by additive white Gaussian noise [17].

The general wavelet denosing procedure is as follows:

- Apply wavelet transform to the noisy signal to produce the noisy wavelet coefficients to the level which we need.
- Select appropriate threshold limit at each level and threshold method (hard or soft thresholding) to best remove the noises.
- Inverse wavelet transform of the thresholded wavelet coefficients to obtain a denoised signal.

The thresholding techniques are simple non-linear techniques that eliminate all the subband coefficients that their magnitude is under a certain threshold. The remaining coefficients remain either unaffected, which is called hard thresholding or modified, which is called soft thresholding. The soft thresholding techniques eliminate the coefficients with magnitude less than the pre-specified threshold and shrink the rest of them. The reconstruction of the “clean” image, after the thresholding process, is performed with the inverse wavelet transform. The quality of the reconstructed image, which will contain some noise and may be distorted, is measured either subjectively by an optical evaluation or objectively by the Signal to Noise Ratio [17].

The de-noising algorithms, which are based on thresholding, suggest that each coefficient of every detail subband is compared to a threshold level and is either retained or killed if its magnitude is greater or less respectively. The approximation coefficients are not submitted in this process, since on one hand they carry the most important information about the image and on the other hand the noise mostly affects the high frequency subbands [17].

The type of the threshold is either hard or soft. Figure 6 indicates the two types of thresholding, which can be expressed analytically as follows.

Hard threshold: \[ y = \begin{cases} x & |x| > T \\ 0 & |x| < T \end{cases} \] \hspace{1cm} (5)

Soft threshold: \[ y = \text{sign}(x)(|x| - T) \] \hspace{1cm} (6)

where \( x \) is the input signal, \( y \) is the signal after threshold and \( T \) is the threshold level.

![Threshold types](image)

**Figure 6. Threshold types**

(a) Original signal;
(b) Hard;
(c) Soft
The hard type does not affect the coefficients that are greater than the threshold level, whereas the soft type causes shrinkage to these coefficients [17].

For de-noising we used Haar wavelet. Figure 7 shows Haar wavelet's scaling function \( \phi(t) \) and mother wavelet function \( \psi(t) \).

\[
\begin{align*}
\phi(t) &= \phi(2t) + \phi(2t - 1) \\
\psi(t) &= \phi(2t) - \phi(2t - 1)
\end{align*}
\] (9)

The Haar transform is the simplest of the wavelet transforms. This transform cross-multiplies a function against the Haar wavelet with various shifts and stretches, like the Fourier transform cross-multiplies a function against a sine wave with two phases and many stretches. The Haar transform can be thought of as a sampling process in which rows of the transform matrix act as samples of finer and finer resolution.

Image de-noising is executed by applying a two-level wavelet decomposition, wavelet Haar, and the threshold of 44.5.

In Figure 8 shows the original mammography images and the estimated mammography images.

\[
\begin{align*}
\phi(t) &= \phi(2t) + \phi(2t - 1) \\
\psi(t) &= \phi(2t) - \phi(2t - 1)
\end{align*}
\]

The Haar wavelet's mother wavelet function \( \psi(t) \) can be described as:

\[
\psi(t) = \begin{cases}
  1 & 0 \leq t < 1/2 \\
  -1 & 1/2 \leq t < 1 \\
  0 & \text{otherwise}
\end{cases}
\] (7)

Its scaling function \( \phi(t) \) can be described as:

\[
\phi(t) = \begin{cases}
  1 & 0 \leq t < 1 \\
  0 & \text{otherwise}
\end{cases}
\] (8)

The Haar wavelet has several notable properties:

1. Any continuous real function can be approximated by linear combinations of \( \phi(t), \phi(2t), \phi(4t), \ldots, \phi(2^k t), \ldots \) and their shifted functions. This extends to those function spaces where any function therein can be approximated by continuous functions.

2. Any continuous real function can be approximated by linear combinations of the constant function, \( \psi(t), \psi(2t), \psi(4t), \ldots, \psi(2^k t), \ldots \) and their shifted functions.

3. Wavelet/scaling functions with different scale \( m \) have a functional relationship:

\[
\phi(t) = \phi(2t) + \phi(2t - 1) \\
\psi(t) = \phi(2t) - \phi(2t - 1)
\]

Many methods for setting the threshold have been proposed. The most time-consuming way is to set the threshold limit on a case-by-case basis. The limit is selected such that satisfactory noise removal is achieved. For a Gaussian noise, if we apply orthogonal wavelet transform to the noise signal, the transformed signal will preserve the Gaussian nature of the noise, which the histogram of the noise will be a symmetrical bell-shaped curve about its mean value. From theory, four times the standard deviation would cover 99.99% of the noise. Therefore, we could set the threshold be 4.5 times the standard deviation of the wavelet-transformed signal to remove the Gaussian noise in the signal.

We have known that the wavelet transform is constituted by different levels. The maximum level to apply the wavelet transform depends on how many data points contain in a data set. One factor
that affects the number of level we can reach to achieve the satisfactory noise removal results is the signal-to-noise ratio in the original signal.

Image data compression using discrete wavelet transformation

Compressions based on wavelet transform are the state-of-the-art compression technique used in medical image compression. For medical images it is critical to produce high compression performance while minimizing the amount of image data so the data can be stored economically. The wavelet-based compression scheme contains transformation, quantization, and lossless entropy coding [18].

Modern radiology techniques provide crucial medical information for radiologists to diagnose diseases and determine appropriate treatments. Since more and more medical images are in digital format, more economical and effective data compression technologies are required to minimize mass volume of digital image data produced in the hospitals [18].

A measure of achieved compression is given by the compression ratio (CR) and the Bit-Per-Pixel (BPP) ratio. CR and BPP represent equivalent information. CR indicates that the compressed image is stored using CR % of the initial storage size while BPP is the number of bits used to store one pixel of the image. For a grayscale image the initial BPP is 8. For a truecolor image the initial BPP is 24, because 8 bits are used to encode each of the three colors (RGB color space) [15].

The challenge of compression methods is to find the best compromise between a low compression ratio and a good perceptual result.

Typically, compression scheme can be categorized into two major categories: lossless and lossy compressions. Lossless image compression can be achieved if the original input image can be perfectly recovered from the compressed data while lossy image compression cannot regenerate the original image data. Lossy image compression, however, is able to maintain most details of the original image that is useful for diagnosis. The precise detail preservation of an image is not usually strictly required because the degraded part of the image is often not visible to a human observer. But the lossy image compression is not very commonly used in clinical practice and diagnosis because even with a slight data loss, it is possible that physicians and radiologists missed the critical diagnostic information that could be a decisive element for the diagnosis of a patient and the following treatment [18].

Medical image compression based on wavelet decomposition has become a state-of-the-art compression technology since it can produce notably better medical image results compared to the compression results that are generated by Fourier transform based methods such as the discrete cosine transform [18].

In general, there are three essential stages in a transform-based image compression system: transformation, quantization, and lossless entropy coding. Figure 9 depicts the encoding and decoding processes in which the reversed stages are performed to compose a decoder. The only different part in the decoding process is that the de-quantization takes place and it is followed by an inverse transform in order to approximate the original image. The purpose of transformation stage is to convert the image into a transformed domain in which correlation and entropy can be lower and the energy can be concentrated in a small part of the transformed image. Quantization stage results in loss of data because it reduces the number of bits of the transform coefficients. Coefficients that do not make significant contributions to the total energy or visual appearance of the image are represented with a small number of bits or discarded while the coefficients in the opposite case are quantized in a finer fashion. Such operations reduce the visual redundancies of the input image. The entropy coding takes place at the end of the whole encoding process. It assigns the fewest bit code words to the most frequently occurring output values and most bit code words to the unlikely outputs. This reduces the coding redundancy and thus reduces the size of the resulting bit-stream [18].
1. Transformation

Wavelet transform exploits both the spatial and frequency correlation of data by dilations (or contractions) and translations of mother wavelet on the input data. It supports the multiresolution analysis of data i.e. it can be applied to different scales according to the details required, which allows progressive transmission and zooming of the image without the need of extra storage. Another encouraging feature of wavelet transform is its symmetric nature that is both the forward and the inverse transform has the same complexity, building fast compression and decompression routines. The implementation of wavelet compression scheme is very similar to that of subband coding scheme: the signal is decomposed using filter banks. The output of the filter banks is downsampled, quantized, and encoded. The decoder decodes the coded representation, up-samples and recomposes the signal [19].

Details of multiresolution analysis are explained above.

2. Quantization

The embedded zerotree wavelet (EZW) is an effective algorithm employed in quantization stage. The EZW algorithm was one of the first algorithms to show the full power of wavelet-based image compression. At a given compression ratio in bit rate, EZW is able to achieve the best image quality and encode the image so that all lower bit rate encodings are embedded at the beginning of the final bit-stream. An embedded coding is a process of encoding the transform magnitudes that allows for progressive transmission of the compressed image. Zerotrees are a concept that allows for a concise encoding of the positions of significant values that result during the embedded coding process. The EZW algorithm is based on four key concepts: 1) a discrete wavelet transform or hierarchical subband decomposition, 2) prediction of the absence of significant information across scales by exploiting the self-similarity inherent in images, 3) entropy-coded successive-approximation quantization, and 4) universal lossless data compression which is achieved via adaptive arithmetic coding [20].

In the EZW’s algorithm, the information on which the coefficients are significant is generated and then encoded via quantization. The significance map determines whether a DWT coefficient is to be quantized as zero or not. A wavelet coefficient $x$ is considered insignificant with respect to a given threshold $T$ if $|x| \leq T$. Otherwise a coefficient is called significant. Since the wavelet decomposition has the hierarchical structure in which each coefficient can be related to a set of coefficients that is at the next finer resolution level, a tree structure depicted in Fig. 5 can be defined as the concept of descendants and ancestors. The coefficient at the coarse scale is called the parent, and all coefficients corresponding to the same spatial location at the next finer scale of similar orientation are called children. For a given parent, the set of all coefficients at all finer scales of similar orientation corresponding to the same location are called descendants. Similarly, for a given child, the set of coefficients at all coarser scales of similar orientation corresponding to the same location are called ancestors. Figure 10 shows that parents must be scanned before children. Also note that all positions in a given subband are scanned before the scan moves to the next subband [20].

Given a threshold $T$ to determine whether or not a coefficient is significant, a coefficient $x$ is said to be an element of a zerotree root (ZRT) for the threshold $T$ if itself and all of its descendents are insignificant with respect to the threshold $T$. An element of a zerotree for threshold $T$ is a Zerotree root if it is not the descendant of a previously found zerotree root for threshold $T$, i.e., it is not predictably insignificant from the discovery of a zerotree root at a coarser scale at the same thre-
shold. For the case which not all the descendants are insignificant, the coefficients are encoded as isolated zero (IZ). For encoding a significant coefficient, the symbol POS and NEG are used. Therefore, given a threshold $T$, the wavelet coefficients can be represented by the four symbols: zerotree root (ZRT), isolated zero (IZ), positive significant (POS) and negative significant (NEG) [18].

To perform the embedded coding, successive-approximation quantization (SAQ) is applied. SAQ is related to bit-plane encoding of the magnitudes. The SAQ sequentially applies a sequence of thresholds $T_0, \ldots, T_{N-1}$ to determine significance, where the thresholds are chosen so that $T_i = T_{i-1}/2$. The initial threshold $T_0$ is chosen so that $|X_j| < 2T_0$ for all transform coefficients $x_j$ [20].

During the encoding (and decoding), two separate lists of wavelet coefficients are maintained. At any point in the process, the dominant list contains the coordinates of those coefficients that have not yet been found to be significant in the same relative order as the initial scan. The subordinate list contains the magnitudes of those coefficients that have been found to be significant. For each threshold, each list is scanned once. During a dominant pass, coefficients with coordinates on the dominant list, i.e., those that have not yet been found to be significant, are compared to the threshold $T$, to determine their significance, and if significant, their sign. This significance map is then zerotree coded. Each time a coefficient is encoded as significant, (positive or negative), its magnitude is appended to the subordinate list, and the coefficient in the wavelet transform array is set to zero so that the significant coefficient does not prevent the occurrence of a zerotree on future dominant passes at smaller thresholds. A dominant pass is followed by a subordinate pass in which all coefficients on the subordinate list are scanned and the specifications of the magnitudes available to the decoder are refined to an additional bit of precision. More specifically, during a subordinate pass, the width of the effective quantizer step size, which defines an uncertainty interval for the true magnitude of the coefficient, is cut in half. For each magnitude on the subordinate list, this refinement can be encoded using a binary alphabet with a “1” symbol indicating the lower half. The string of symbols from this binary alphabet that is generated during a subordinate pass is then entropy coded. Note that prior to this refinement, the width of the uncertainty region is exactly equal to the current threshold. After the completion of a subordinate pass the magnitudes on the subordinate list are sorted in decreasing magnitude, to the extent that the decoder has the information to perform the same sort. The process continues to alternate between dominant passes and subordinate passes where the threshold is halved before each dominant pass. [15].

In the decoding operation, each decoded symbol, both during a dominant and a subordinate pass, refines and reduces the width of the uncertainty interval in which the true value of the coefficient (or coefficients, in the case of a zerotree root) may occur. The reconstruction value used can be anywhere in that uncertainty interval. The encoding stops when some target stopping condition is met, such as when the bit budget is exhausted. The encoding can cease at any time and the resulting bit stream contains all lower rate encodings [20].

![Figure 10. Scanning order of the subbands for encoding a significance map](image-url)

3. Entropy coding

The output symbol stream is an input to an entropy encoder to complete the last stage of the compression without adding distortion. The lo-
ssless entropy encoding process replaces the symbol stream produced in the quantization stage with a sequence of binary codewords which is called a bit stream. The probability of the corresponding symbol is proportional to the length of a codeword. The smallest possible number of bits that is required to represent a symbol sequence can be defined as the entropy of the symbol source:

$$H = - \sum_i p_i \log_2 p_i$$  \hspace{1cm} (10)

Here the $p_i$ is the probability of the $i$th symbol. In the optimal case, the sum of the probability $\sum_i p_i$ would be equaled to 1 and the $i$th symbol would be $- \log_2 p_i$. We can define the entropy as the expected length of binary code over all possible symbols [18].

### Image compression results

For image compression we use the Haar wavelet and EZW algorithm. Key parameter for image compression using EZW algorithm is number of loops that growing give a better picture and a lower compression ratio. Figure 11 compares the original image and image compressed using the EZW algorithm, 6 loop and Haar wavelet.

![Figure 11. The original image and image compressed using EZW algorithm (6 loops) and Haar wavelet](image1)

Compression ratio and the ratio of bit-pixels are very small, which is good, but picture quality is very poor. For this reason it is necessary to increase the number of loops. If instead of the 6 loops use the 9 loop compression ratio and the ratio of bit-pixel is a small increase in benefit quality of compressed images but the quality of compressed images is still unsatisfactory, as shown in Figure 12.

![Figure 12. The original image and image compressed using EZW algorithm (9 loops) and Haar wavelet](image2)

Satisfactory compression ratio, satisfactory bit-pixel ratio and satisfactory image quality is obtained using the EZW algorithm with 12 loop and the Haar wavelet, shows Figure 13.

![Figure 13. The original image and image compressed using EZW algorithm (12 loops) and Haar wavelet](image3)

### Results and discussion

The basic idea is to detect breast tumors using image parameters which are reliably known to contain tumor. Necessary data about the image that contains the tumor and by which we detect a tumor are the central pixel coordinates and latitude and longitude (expressed by the number of pixels) of images representing the tumor. From the image we know that contains a tumor, we extract the tumor that have the following parameters: mean pixel intensity value is 166.7601 and the standard deviation is 32.9223. Based on these values we are looking for parts of the uncultivated image to detect the tumor with the approximate values of the same parameters. Standard deviation
and mean pixel intensity value we calculated as the following.

Let \((i; j)\) be the spatial location in the mammogram at row \(i\) and column \(j\); \(u_{ij}\) be the pixel brightness at \((i; j)\). For each \(M\)-by-\(N\) image, the standard deviation is the square root of the variance and is given by the following equation:

\[
\sigma = \sqrt{\frac{\sum_{i=1}^{M} \sum_{j=1}^{N} (u_{ij} - \mu)^2}{M \times N - 1}} \quad \text{(11)}
\]

where \(\mu\) is the mean of the input matrix \(u\) and is given by the following equation:

\[
\mu = \frac{\sum_{i=1}^{M} \sum_{j=1}^{N} u_{ij}}{M \times N} \quad \text{(12)}
\]

After removing the noise on both images, to calculate the values of the parameters that characterize the tumor, we separated the tumor with a known location. Figure 14 shows previously processed image that is image which is used for detecting tumors.

Figure 14. Mammography image with detected tumor which is used for detection of other tumors

Figure 15 shows the tumor which is detected on mammography image from Figure 14.

After extracting the known tumor, we calculated mean pixel intensity value and standard deviation of that known tumor. Then we defined the width and height for part of image whose parameters are checked, here it is 100 pixels for both, and tolerances for mean pixel intensity value and standard deviation. Then, on the image where we need to detect tumor, we search for part size 100x100 with mean pixel intensity value and standard deviation which are approximately equal to the mean pixel intensity value and standard deviation of known tumor, taking into account the tolerances. Figure 16 shows the result of that search, original mammography breast image without increased contrast, with detected tumors.

Figure 15. Tumor which is used for detecting other tumors

Figure 16. Mammography image with detected tumors
Figure 17 shows the part of mammographic image with enhanced contrast, that is tumor detected in Figure 16.

![Figure 17. Tumor detected in Figure 16](image)

The results of detecting tumor in mammogram before and after compression of mammogram are very similar, almost the same. The reason is the fact that the values of standard deviation and mean pixel intensity of image part which represent the tumor, before compression and the value of standard deviation and mean pixel intensity of image part which represent the tumor, after compression, almost the same. As we said, before compression, image part which represent the tumor has the following parameters: mean pixel intensity value is 166.7601 and the standard deviation is 32.9223. After compression, mean pixel intensity value and the standard deviation of image part which represent the tumor are 166.6986 and 32.1573, respectively.

**Conclusion**

Discrete wavelet transformation analyzes the image at different frequency bands, different resolutions, decomposing the image in a coarse approximations and detailed informations. Using two-dimensional wavelet analysis images can be effectively compressed without sacrificing image quality. Using two-dimensional wavelet analysis we can effectively remove noise from the image. The problem of developing algorithms to detect tumors is the inability of a competent testing due to lack of high-quality database of digital images.

In this paper we presented an automatic method of detecting breast cancer, comparing the standard deviation and mean pixel intensity of two mammograms of which is the one reliably known to contain tumor, and for others it is not known. Initial results that we obtained show that it is possible to compress mamograms using multiresolution mammogram analysis and EZW algorithm and the result of detection of the tumor did not change compared to the detection of tumors in uncompressed mammograms. In future work, we will try to improve the technique of automatic detection of tumors by comparing standard deviation of gradient orientation histogram and the standard deviation of the folded gradient orientations of the two mammograms.

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Abstract

Background: Deaths occurring in hotels are the result of several causes like asphyxia due to fire, intoxication and multiple traumas on the body.

Methods: In this study, 28359 death records of the Council of Forensic Medicine, Istanbul, Turkey between 1 January 2000 and 1 January 2008 were analyzed retrospectively and 76 cases of deaths identified in hotels were included. Data about these cases were evaluated regarding age, sex, marital status, occupation, nationality, history, scene investigation and autopsy findings and cause of death.

Results: The mean age of the cases was 39.69±4.32 (range between 1 and 78 years). Sixty (78.9%) males and 16 (21.1%) females with a male/female ratio of 3.75 were recorded. Nationality was Turkish in 58 (76.3%) cases and foreign in 18 (23.7%) cases. Autopsy was performed in 75 (98.7%) cases.

Conclusion: We concluded that safety measures targeting detailed booking and health records at entry to hotels, improving security measures against firearms at airports, correcting deficiencies in basic facilities like electricity and ensuring barricades and lifeguards at the beaches and pools and implementing legal arrangements like physicians at place of work could lower death rates.

Key Words: Hotel, death, autopsy, scene investigation

Introduction

Individuals with limited housing options might also live in motels, sometimes with rent subsidised by welfare agencies. These housing situations can be important indicators of socioeconomic deprivation beyond that which can be determined on the basis of income alone [1]. Homeless and marginally housed individuals living in shelters, rooming houses, or hotels have significantly higher mortality rates than individuals with incomes in the lowest fifth of the distribution [2]. Compared with the entire cohort, life expectancy was shorter by 13 years for men and eight years for women living in shelters; 11 and nine years, respectively, for those living in rooming houses; and eight and five years, respectively, for those living in hotels [3].

Many excess deaths were attributable to diseases related to alcohol and smoking and to violence and injuries, much of which might have been related to substance misuse. There were also many excess deaths related to mental disorders and suicides. Other research suggests that expanding the implementation of recent innovations in supported housing programmes for people with addictions and mental illness could be instrumental in reducing the number of excess deaths [4]. The most common methods of suicide for the Manhattan nonresidents were long fall, hanging, overdose, drowning, and firearms; the most common locations included hotels and commercial buildings [5]. Between 1978 and 1997 the Institute of Legal Medicine of the Hannover Medical School examined 17 fatal autoerotic deaths. One of the them were found in a hotel room [6].

Hotels are economic, social and socially controlled establishments that provide paid lodging and meals, usually on a short-term basis to be preferred by physical components like structure, technical equipment, comfort and service conditions and moral components like social value and quality of service and staff [7]. Hotels are used by national and international guests with travel and business purposes. Service is provided by hotel employee.

Deaths occurring in hotels are the result of several causes like asphyxia due to fire, intoxication and multiple traumas on the body [8-15].
The aim of this study was to determine the causes of deaths in hotels to investigate hotel neglect and deficiencies in preventable deaths and to discuss possible precautions.

Methods

All cases submitted to the First Specialization Board of the Council of Forensic Medicine, Ministry of Justice between 1 January 1999 and 1 January 2008 were reviewed. The First Specialization Board is an official expert commission and serves as a supreme board in Turkey. Cases are submitted to this board by the courts from all over the country demanding a more detailed examination and a final conclusion. The Board consists of a general surgeon, a cardiovascular surgeon, a neurosurgeon, a gynecologist, an internist, a cardiologist, a hematologist, an immunologist, a pathologist and three forensic specialists. This Board evaluates the whole material in the files and tries to determine the cause of death and prepares a final report. The final reports are detailed in origin and preserved by the Council of Forensic Medicine.

In this study, 28359 death records of the Council of Forensic Medicine, Istanbul, Turkey between 1 January 1999 and 1 January 2008 were analyzed retrospectively and 76 cases of deaths identified to have occurred in hotels were included. Data about these cases were evaluated regarding age, sex, marital status, occupation, nationality, history, scene investigation and autopsy findings and cause of death.

Results

The mean age of the cases found death in hotels and evaluated by the First Specialization Board of the Council of Forensic Medicine was 39.69±4.32 (range between 1 and 78 years). Sixty (78.9%) males and 16 (21.1%) females with a male/female ratio of 3.75 were recorded. Data about age and sex are shown in Table 1.

Marital status was recorded as married in seven, single in eight and unknown in 61 (80.3%) cases and eighteen (23.7%) of them were recorded as living alone, 21 (27.6%) not living alone and in 37 (48.7%) cases no information was available.

Nationality was Turkish in 58 (76.3%) cases and foreign in 18 (23.7%) cases. Information about occupation was missing in 62 (81.5%) cases; the remaining were four students, three retired, three self-employee, three hotel personnel and one tourist guide.

Location of cases were hotel room in 68 (89%), hotel bathroom in three, hotel toilet in two, hotel disco in one, hotel beach in one, hotel pool in one and in front of the hotel building in one.

Main findings at scene investigations consisted of medications used in chronically heart diseases, asthma and diabetes, alcoholic beverage, oriental tobacco, cigarettes, numerous sedatives and narcotic drugs and related empty boxes, eight syringes, knives, bloodstains on the bed sheets, sofa bed, toilet seat edges and carpets, a rope hanging from the ceiling, short barrel guns shells and cartridges, suicide letters, a hammer, damaged and loose electricity conduits, pieces from the corpses in sacks in the rooms or bathrooms and messiness. A dead

Table 1. Distribution of the cases according to age groups and sex

<table>
<thead>
<tr>
<th>Years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>11-20</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>6.6</td>
</tr>
<tr>
<td>21-30</td>
<td>10</td>
<td>2</td>
<td>12</td>
<td>15.7</td>
</tr>
<tr>
<td>31-40</td>
<td>19</td>
<td>6</td>
<td>25</td>
<td>32.9</td>
</tr>
<tr>
<td>41-50</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>21.1</td>
</tr>
<tr>
<td>51-60</td>
<td>8</td>
<td>3</td>
<td>11</td>
<td>14.5</td>
</tr>
<tr>
<td>61-70</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>71-</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>60 (78.9)</td>
<td>16 (21.1)</td>
<td>76 (100.0)</td>
<td>100.0</td>
</tr>
</tbody>
</table>
person with an intravenous serum in the arm and a syringe with dissolved heroin and a piece of lemon in the background can be seen in Figure 1,2.

Figure 1. A dead person with an intravenous serum in the arm is seen

Figure 2. The same dead person with a syringe with dissolved heroin and a piece of lemon in the background is seen.

Wounds in 1-1.5 cm diameter at the chest region of the clothing probably due to firearms and plaster bandage on the leg of one case were observed. Deposits of gunpowder were detected at ballistic investigation of the wounds. Examination of the guns demonstrated that one was handmade and each of them was in running order.

Upon autopsy examination performed in 75 (98.7%) cases no signs of external trauma could be detected in 52 cases. In nine cases, decay of the body resulted in insufficient data for differential diagnosis about external trauma. Two cases with foamy fluids in the mouth and nose were recorded.

Entrance and exit wounds in the skull and chest were detected in cases with findings in accordance with firearms injuries (Figure 3,4). X-ray evaluation before autopsy of the case deceased after gunshot injury on the neck revealed pellets compatible with hunting gunshot (Figure 3). In cases with findings indicating trauma and electric shock (Figure 5), crater-like lesions collapsed in the center and raised in the margins, superficial skin and subcutaneous lesions, blunt wounds, ecchymosis and hematoma were identified (Figure 6).
In a female case, a woman is found dead, a plastic bag covering her head, face and neck so that it prevents her from breathing. Hands and arms with legs and foot are tied to her back with a rope which is also tied to her neck, which is called swine rope (Figure 7).

In a female case lacerations in vulva and vagina and in the posterior fornix and cervix were noticed. In one case, the head and the body were separated reflecting traumatic injury and both arms and legs were rid apart from the trunk with their ends released.

In three cases, signs of head injury, in four cases injection marks on the back of the hand and in the antecubital fossa were observed.

In five cases, scar on the arms and abdomen were detected possibly resulting from razor blade wounds. Macroscopic results upon autopsy examination of the internal organs illustrated decay in nine cases while assessment was impossible but signs about trauma to the bony structure were not detected.

One case with incised wounds on the chest revealed stab wounds in the lung and heart at autopsy examination. Autopsy examination in one case of death from hanging demonstrated subcutaneous ecchymosis in soft tissue under the posterior region of the neck showing an ascending pattern of ecchymosis and fracture of the hyoid cartilage.

In four cases skull bone fractures, subarachnoid hemorrhage, brain tissue damage, cervical bone fracture and spinal cord injury, and neck vessel-nerve bundle injury were detected in concordance with gunshot injuries. In one of these cases a hammer was found beside the corps (Figure 6,8,9). In one case, fracture of the rib, lung and heart injury were detected probably due to firearm injuries. In another case with a severe damaged corpse, the head was separated at the 3. and 4. cervical vertebra. In one case, fractures in the skull bones, subarachnoid hemorrhage, and damage of the brain tissue, rib fractures and lung contusion were recorded in accordance with fall from height.

Macroscopic findings were as follows: Brain examinations revealed edema, congestion, paleness, and hyperemia, elimination of the sulcus, flattening of the gyrus, epidural, subdural and subarachnoid and intracerebral hemorrhage and skull

Figure 6. Crater-like lesions collapsed in the center and raised in the margins, superficial skin and subcutaneous lesions, blunt wounds, ecchymosis and hematoma were seen in an old woman

Figure 7. Hands and arms with legs and foot are tied to her back with a rope which is also tied to her neck, called swine rope

Figure 8. A hammer was found beside two corps
bone fractures in some cases while in some cases macroscopic pathology was absent.

Figure 9. Blunt wounds of superficial skin in the skull, ecchymosis, hematoma and fracture were seen in an old man

Macroscopic pathology upon examination of the heart exposed hypertrophy, aneurisms, minimal to severe obstructive atherosclerotic changes in the coronary arteries, minimal to diffuse whitish color changes in the myocardium in some cases while no changes were observed in some.

Examination of the lungs showed macroscopic pathological changes like edema, increase in weight, and hyperemia in some cases while no changes were recorded in some. Examination of the liver resulted in findings like stasis, macrovesicular fattening and hyperemia. Life-threatening macroscopic pathological changes could not be detected in other internal organs.

The following findings were noticed in microscopic evaluation of the organs and tissues of the cases: three cases with putrefaction showed autolysis at histological examination. Thrombosis in the lungs was observed in the case with the plaster bandage on the leg. Histological examination was missing in the files of cases where the cause of death was due to firearms and knives.

Additional findings included hyperemia-hemorrhage and epidural, subdural and subarachnoid hemorrhage in the brain, hypertrophy and fibrosis, chronic hypoxic changes and fibrous tissue bands at the myocardium and myocardial fibers, atherosclerotic changes resulting in 30%-100% obstructive lesions in the coronary arteries, edema, hyperemia and intra-alveolar hemorrhage in the lungs, microvesicular fattening in the liver, chronic pyelonephritic changes in the kidney and thermal changes in the skin.

Upon chemical examination of tissue, blood, urine and gastric content of the corpses no toxic, hypnotic and narcotic substances could be detected in 45 (59.%) cases while in 18 (23.7%) cases ethanol, naproxen, diclofenac, amitripty-

### Table 2. Cause of death and distribution of gender

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firearm injury</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>6.6</td>
</tr>
<tr>
<td>Intoxication</td>
<td>8</td>
<td>5</td>
<td>13</td>
<td>17.1</td>
</tr>
<tr>
<td>Existing heart disease</td>
<td>25</td>
<td>1</td>
<td>26</td>
<td>34.2</td>
</tr>
<tr>
<td>Undetermined</td>
<td>16</td>
<td>4</td>
<td>20</td>
<td>26.5</td>
</tr>
<tr>
<td>Electric shock</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Generalized trauma due to fall from height</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Blunt head trauma</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>Pulmonary emboli due to femur fracture</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Mechanical asphyxia due to hanging</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Drowning (pool)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Sharp injuries</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Hypovolemic shock due to sexual trauma</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Drowning (sea)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Total (%)</td>
<td>60 (78.9)</td>
<td>16 (21.1)</td>
<td>76 (100.0)</td>
<td>100.0</td>
</tr>
</tbody>
</table>
lin, benzodiazepam, barbiturate and carboxyhemoglobin was identified although serum concentration of these substances were below toxic or fatal levels. In 13 cases, serum levels of ethanol, doxilamine, sertaline, codeine, heroin, morphine, amitriptilin were toxic and reported as the cause of death.

Heart disease was the cause of death in 26 (34.2%) cases. Cause of death could not be determined in 20 (26.3%) cases and in nine of them a decay of the corpse was reported. In addition, causes of death were reported as intoxication in 13 cases, firearm injury in five cases, electric shock in two cases, fall from height in one case, blunt head trauma in three cases, pulmonary emboli due to femur fracture in one case, mechanical asphyxia resulting from hanging in one case, mechanical asphyxia due to drowning in a pool and in the sea in two cases, separation of the head with a sharp instrument in one case and hypovolemic shock after sexual intercourse in one case. Cause of death and distribution of gender are shown (Table 2).

The serum levels of ethanol, doxylamine, sertaline, codeine, heroin, morphine, and amitriptilin were found fatally high in 13 (17.1%) cases after chemical examination. Association of cause of death and chemical examination results showed statistically significance (Table 3).

**Discussion**

Death can occasionally occur in hotels during accommodation [8-17]. Hotel fires have resulted in death for many people. Three major hotel fires have occurred in Turkey. A fire in the Hotel Washington in Istanbul has caused 36 deaths and 59 injured in 1983, a fire in Hotel Tözbe in Istanbul has caused 18 deaths and 41 injured in 1993 and an incendiary fire in the Hotel Madimak in Sivas has caused 37 deaths [8,9]. Our cases are not related with these major fires or raised fires due to our database foundation date behind these events. We are not sure how many of these cases were sent for autopsy to our council.

However, a national regulation on fire was instituted in our country after these major fires. This regulation aims to assure preventative or protective measures that are needed as a result of a risk assessment and necessary training, organization and inspection in order to safeguard lives and property in the event of a fire in all kinds of buildings, enterprises and constructions owned by institutional, private and real persons [18].

In 34.2% of our cases the First Specialization Board of the Council of Forensic Medicine reported a condition of “existing disease”. This condition is used in cases where results of scene investigations, witness statements and clinical and autopsy findings are missing or confusing and the cause of death is undeterminable or in cases where

<table>
<thead>
<tr>
<th>Substance</th>
<th>Fires</th>
<th>Intoxication</th>
<th>Disease</th>
<th>Undetermined</th>
<th>Drowning</th>
<th>Trauma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxylamine</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Sertaline</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Laroxil (Amytriptilin)</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Barbiturate in the urine</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Codeine</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Heroin</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Morphine</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Naproxen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Carboxy-hemoglobin (COHb)</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Benzodiazepam derivates</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>13</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>31(40.8%)</td>
</tr>
</tbody>
</table>
other causes of death were excluded. These cases are considered as natural deaths. For example, in persons with an illness like heart disease with the likelihood of sudden death, a record of previous diseases during check-in could help to give information to the health personnel in case of an emergency or in case of a missing person.

Medical point of view requires the distinction of trauma or intoxication while regarding the law the distinction of accident, murder and suicide is important in a case of death. This enables an opportunity for judicial bodies.

The cause of death was undetermined in 26.5% of our cases. In these cases, where the cause of death could not be linked to a consequence of trauma, intoxication or existing disease, a termination of “undetermined cause and mechanism of death” is reported by our Council. Decay of the corps or indistinct pathology is the reason for this result in most of these cases. Problems in identifying cause of death in our country are mainly due to a lack of standard procedure and deficiencies in practice.

The percentage of deaths, which are due to guns, varies greatly in different parts of the world, mainly due to availability of weapons. There is a substantial and growing literature on the epidemiology of firearm related deaths. Many of these directly address the issue of the impact of gun control on death rates [19-24]. The cause of death in 6.6% of the cases was the result of handgun injury and within these cases; four Turkish and two foreign nationalities were recorded.

Stricter firearm legislation and control of guns similar to airport security measures or proper storing of firearms at stopover places are required, which might help to reduce unnecessary deaths and injuries related with firearms.

The cause of death was the result of electric shock in two cases emphasizing the importance of control on old electricity wirings and replacements in regular periods.

An adult case was found dead in the sea after resting on the beach. Besides, a three years old child with foreign nationality accommodating with his grandmother was drowned in the pool. Mothers, fathers or young attendants energetic enough to keep in step with them should supervise children.

Carbon monoxide (CO) poisoning at hotels, motels, and resorts was described [25,26]. The cause of death in 13 (17.1%) of our cases of intoxications was related to medications but carbon monoxide poisoning was not reported. Extraordinary cases such as body-packing as cause of unexpected sudden death may occur everywhere, the circumstances being uncharacteristic. The cases demonstrate, for example, different localities such as a private home, a motorway service area, a hotel room and a backyard [27]. Intoxication due to cocaine was not detected between our cases but body-packing syndrome in one case was reported as the cause of death in Turkey [28].

On July 25, 2000, around 4.30 pm, a Concorde airplane with 109 people on board, 96 of who were of German nationality, crashed onto a hotel situated near the town of Gonesse. The accident resulted in 113 deaths (100 passengers, 9 crew members, and 4 hotel employees) and six were injured [29]. Mass deaths due to airplane accident or fire were not reported as causes of death in our series. As a result, travel health advisers should include advice concerning personal safety abroad and tourist authorities should endeavor to promote and advocate for tourism safety [11].

Hotel entry records should be detailed and information about existing diseases and medications used should be recorded for every tourist at check-in in order to prevent deaths at stopover places. Security precautions similar to the airports at the entrance at stopover places could be suggested considering firearms. Safety measures like periodic control of infrastructure and technical equipment by independent institutions to ensure renewal of old wiring and restoration of aged buildings, in addition to precautions like barricades and lifeguards at the beaches and pools are important. As a result, we believe that in addition to safety measures in parts of the hotels serving for amusement and relaxation, employing experts in their fields and legislative measures ensuring health care service for employees and customers could result in a decrease in injuries and deaths.
References


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Abstract

Introduction: The main methods for root canal working length determination are radiographic and electrometric. The digital radiography obtained through intrabuccal sensors represents technological progress that allows quantitative analysis and measurement of working length.

Aim: The aim of this study was to investigate the difference in values of the accurate root canal working length and the working length determined by digital radiography and to test if the digital radiography is a reliable method for working length determination.

Materials and methods: Nineteen extracted human teeth without endodontic treatment and with well preserved coronal and radicular structures were selected - 11 lower incisors and 8 lower canines. Teeth length (TL1) was measured by a millimeter ruler. Coronal access to the teeth was obtained with a round bur and high speed handpiece. The actual working length (WL1) was obtained by introducing a #15 K file in the canal until it appeared at the apical foramen. The length was verified on endometer. The digital images were obtained for every tooth sample. The canal working length (WL2) and the length of the tooth (TL2) were measured in the Kodak dental imaging software.

Mean value and standard deviation were calculated and statistical analysis was performed by student t-test.

Results: Average working length in lower incisors was 21,68 mm, and average working length in lower canines 21,93 mm. Statistical analysis has shown no statistical difference between the accurate root canal working length and the working length obtained by digital radiography. It was also found that there is no statistical difference between the tooth length and working length measured by digital radiography (p>0,05).

Conclusion: There is no significant difference between the accurate root canal working length and the working length measured by digital radiography. Digital radiography is a reliable method for working length determination.

Key words: Working length, digital radiography, tooth length

Introduction

The establishing of accurate root canal working length is one of the most critical steps of endodontic therapy. Cleaning, shaping and obturation of the root canal system cannot be accomplished accurately unless the working length is determined precisely. The generally accepted methods of working length determination are radiographic and electrometric [1,2].

In endodontics, conventional film-based radiography is an important resource for diagnosis, transoperative procedures and treatment control [3]. However, diagnostic X rays are the largest man made source of radiation exposure to the general population [4]. The digital radiography obtained through intrabuccal sensors rather than radiographic films represents technological progress that allows qualitative and quantitative analysis of all stages of endodontic therapy. Regarding radiographic estimation of endodontic working length, direct digital imaging provides measurement tools that facilitate the definition of the apical limit of root canal instrumentation. Moreover, there is substantial reduction in image processing time with the acquisition of digital radiography. Hence, the...
clinical procedures are performed more quickly, with reduced radiation. Further, in digital radiography, the application of various image enhancement modalities improves the accuracy of endodontic file lengths. The software program provides a sequence of straight lines for evaluation of root canal working length [5].

The aim of this study was to investigate the difference in values of accurate root canal working lengths and working lengths determined by digital radiography.

**Materials and methods**

Nineteen extracted single rooted human teeth, without endodontic treatment and with well preserved coronal and radicular structures, were selected - 11 lower incisors and 8 lower canines. The criteria for tooth selection also included the accessibility of the apical foramen with a #0.08 K file (Dentsply-Maillefer, Ballaigues, Switzerland). The teeth have been cleaned after extraction and stored until used in saline solution under the temperature of 4ºC. Tooth length (TL1) was measured by a millimeter ruler. Access openings were made with a high speed handpiece and a round diamond bur (Figure 1). An endodontic K file #0.15 was introduced into the canal until it appeared at the apex (Figure 2).

Working length was determined (WL1, accurate working length) for each tooth using hand K file # 0.15 and the endometer. The file was introduced into the canal to a depth until the tip of the instrument appeared in the region of the apical foramen and the rubber stopper of the file has been brought into contact with the coronal reference point (incisal edge of the tooth). The length of the file for each canal was checked using an endometer. In this way the accurate working length (WL1) was measured (Figure 3).
An endodontic file #15 was introduced in the canal until it appeared at the apex and at this point digital radiography was taken. File was placed in the root canal in the same position as in determining the WL1. Kodak RVG 5100 digital radiography sensor of universal size, Kodak X-ray generator (the generator voltage 70 kVp and 7 mA current strength) and the remote control to activate the software and sensors were used. Dental imaging was performed by placing the tooth with a file in the canal, along the active (radio-sensitive) sensor surface (Figure 4). Generator as a source of X-ray was set at a distance of 20 cm in relation to the object (tooth). Exposure time was set to 0.05 seconds.

In this way 19 rendgenographic digital images were obtained (Figure 5). These images were stored and processed in Kodak dental imaging software. In this program radiographic working length (WL2) and the tooth length (TL2) were measured. Radiographic working length was measured using a millimeter ruler (Figure 6). Two points were marked, one on the rubber stopper and the other on the top of the file. Tooth length was also measured with a millimeter ruler from the most coronal point till the most apical point of the tooth (Figure 7).

Mean value and standard deviation were calculated for WL1, WL2, TL1 and TL2. The difference between WL1 and WL2 and the difference between the WL1 and TL1 were tested with the
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Student t-test. Statistical significance was set at the 5% level of error (p<0.05).

**Results**

The mean values of the accurate tooth length (TL1), Rtg tooth length (TL2), the real working length (WL1) and the working length measured by digital radiography (WL2) are shown on Table 1. Percentage of the morphological groups of teeth was: incisors 58% and canines 42% (Figure 8).

The mean value of the real working length (WL1) was 21.68 mm and mean radiographic working length (WL2) was 22.33 mm, for the incisors (Figure 9).

The mean value of the real working length (WL1) was 21.93 mm and the mean radiographic working length (WL2) was 22.56 mm, for the canines (Figure 10).

**Table 1. Mean working lengths and tooth lengths for incisors and canines**

<table>
<thead>
<tr>
<th>Tooth type</th>
<th>WL1</th>
<th>WL2</th>
<th>TL1</th>
<th>TL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incisors</td>
<td>21.68 (±0.72)</td>
<td>22.33 (±0.60)</td>
<td>22.00 (±0.52)</td>
<td>(±0.59)</td>
</tr>
<tr>
<td>Canines</td>
<td>21.93 (±1.65)</td>
<td>22.56 (±1.86)</td>
<td>22.25 (±1.73)</td>
<td>(±1.85)</td>
</tr>
</tbody>
</table>

*values are in millimeters
Mean tooth length (TL1) of the lower incisors was 22 mm and mean tooth length of the lower canines was 22.25 mm (Figure 11 and 12).

![Figure 11. Mean working length (WL1) and tooth length (TL1) for incisors]

![Figure 12. Mean working length (WL1) and tooth length (TL1) for canines]

Statistical analysis has shown no significant difference between the accurate working length of teeth and the value of working length measured by digital radiography (WL1 and WL2). It was also found that there was no significant difference between the values obtained for the length of teeth and the working length measured by digital radiography (p > 0.05).

**Discussion**

Determination of working length represents one of the key stages of endodontic therapy in order to provide high quality preparation and obturation of the canal, creating the preconditions for the successful outcome of the entire therapy. In order to assess the approximate working length at the beginning of therapy, it is helpful to have information about the average length of the teeth that belongs to the corresponding morphological group. The accurate determination of working length is made by radiographic or electrometric method after canal trepanation.

Tests have shown that the length of the tooth does not have to coincide with the length of the canal and the working length for the root canal preparation, for a variety of anatomical variations or root canal curvatures [6].

One of the aims of this investigation was checking the correspondence between the average teeth length measured on the x-ray dental digital photography and working length of teeth. The results confirmed that there was no significant difference between the tested values. These matching values were obtained probably because of the choice of morphological groups and types of teeth used in this study. It is considered that single rooted anterior teeth, such as lower incisors and canines which are used in this study, have less complicated canal anatomy in relation to the posterior multi rooted teeth [7]. Therefore it would be good to extend the research to other morphological groups of teeth, especially molars, where larger deviations of tested values could be expected.

The literature states that the average length of lower incisors is 21.7 mm, and the average length of lower canine 25.6 mm [8]. In this survey an average length of the lower incisors was 22 mm and 22.25 mm of lower canines. The difference in the literature data concerning the length of the canines, can be attributed to the limited number of samples used in this study, so it would be desirable to test the results on a larger sample.

It is believed that radiography represents a very reliable method for determining the working length, but still during the recording process, an image distortion could happen to some extent, and thus a discrepancy between the values of canal length measured on digital photography, and the real value of the length of the root canal.

According to some studies, radiographic method is unreliable for the working length determination due to image distortion, and because of overlapping of anatomical structures, film radio contrast and subjective interpretation of the clinician [9,10,11].
In this study, slightly higher values for radiographic working length were obtained, comparing to the accurate working length of root canals. The difference between these values is in the domain of 0 to 1.2 mm, but is not statistically significant. It is possible that such results were obtained because the testing was done in vitro on extracted teeth where it was possible to provide optimal conditions for the x-ray recording, in terms of distance between the object from the tube. On the other hand, in vivo conditions, there is a danger of patient’s moving at the time of exposure and the possibility of superimposition of other anatomical details that may affect the deviation between measured and real values.

Some studies show that the value of working length obtained by conventional and digital radiography is higher than the real working length of root canal [5].

In other studies it is stated that digital radiography is better compared to conventional, in cases where the working length was measured with instruments of a larger diameter [12,13], and when measured in single rooted teeth [14,15]. On the other hand, some studies have shown the superiority of conventional radiography compared to digital, where instruments with smaller diameters have been used [16]. A detailed comparison of these results is very difficult due to the use of various digital systems in each study.

**Conclusion**

According to the results of this study, it can be concluded that there is no statistically significant difference (p<0.05) between the accurate working length of root canal and working length measured by digital radiography. Digital x-ray imaging is a reliable method for determining the working length of root canals.

**References**

4. Đedić S, Pranić N: Lung cancer risk from exposure to diagnostic x rays, HealthMED, 2009; 3(3): 307-313


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Quality Of Life Of Patients With Tuberculosis

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Department of Nursing, Faculty of Health Sciences, Atatürk University, Erzurum, Turkey.

Abstract

Setting: This study was conducted at the Erzurum Nihat Kitapçı State Hospital and Aziziye Research Hospital of Atatürk University.

Objective: This study was conducted to determine the status of life quality of the patients with tuberculosis and the factors influencing it.

Design: The data was collected by interviews with tuberculosis patients using an inventory of quality of life and patient information form.

Results: It was found that age, gender, educational status, monthly income and occupation of the patients had an impact on the quality of life (p<0.05). It was also found that the status of the disease, familiar, neighborhood and friendship relations after onset of the disease influenced the quality of (p<0.05).

Conclusion: To increase the quality of life of the patients with tuberculosis, the patients need to be informed about the disease, the importance of completing the whole therapeutic course, and accepting the disease; and they need to be cared and treated by the health professionals specialized in that area during and after treatment period.

Key words: Quality of life, tuberculosis, nursing.

Introduction

Tuberculosis is still an important health problem worldwide and in our country. According to the World Health Organization (WHO) report on global tuberculosis published in 2008, the number of new tuberculosis cases expected in 2006 is 9.2 million (139/100 000); estimated case prevalence is 14.4 million; estimated number of multi-drug-resistant tuberculosis cases is 0.5 million.¹ In our country, according to the report published by Directorate of Struggle Against Tuberculosis in 2007, 20 535 patients were diagnosed with tuberculosis in 2005 and 91.3% of them were new cases. The rate of pulmonary tuberculosis is 73%.² The tuberculosis incidence was found to be 17.60 per 100 000 population in the Eastern Anatolian region.³

Tuberculosis is a common and social disease. These two situations may direct the tuberculosis disease towards a process that cannot be expressed easily and makes the person be isolated from social relationships when it is recognized.⁴⁻⁶

Treatment requires prolonged therapy (at least 6 months) with potentially toxic drugs that can lead to adverse reactions in a significant number of patients. Also, among foreign-born patients, there is considerable social stigma associated with active TB, leaving the individual feeling shunned and isolated from their friends and families. Diagnosis of TB also leads to depression and anxiety. Finally, among Aboriginal and marginalized inner-city populations, there is a lack of knowledge regarding the disease process and its treatment, which may contribute to feelings of helplessness and anxiety.⁷

In general, studies of TB have focused on outcomes such as mortality and biologic markers of cure. However, there has been increasing interest in the patient’s perspective of disease, health, and medical care; and quality of life (QOL) is recognized as a key outcome.⁸

Clinically, the burden of TB extends beyond its acute presentation. However, there has been little attention given to the burden of the illness and treatment or to quality of life (QOL) of people with TB.⁹

According to the definition established by the World Health Organization, the “quality of life” is the individual’s perception of where he/she stands in the life with the cultural and moral system under which he/she lives relating to the individual’s purposes, prospects, standards and interests.
to the new philosophy of health care, to increase the quality of life of the patients, nurses should consider the quality of life under holistic (wholeness) approach basing on the mutual respect and collaboration. The studies performed by nurses using the quality of life scales intend to obtain information about the health status of large groups and to implement the results to establish healthcare policies.

In the literature, as a result of the studies with different scales and methodological implementations, it was proved that the quality of life of the patients with tuberculosis decreased to various degrees, namely, tuberculosis negatively affected the quality of life.7,9–13

In our country, there were few studies about the quality of life of the patients with tuberculosis.3,9,14 One of the goals of healthcare professionals is to help the individual until he or she will be able again to take care of himself/herself as soon as possible, to make the individual meet his/her own needs and to maximize the quality of life of the individual throughout these processes.

This study was performed to determine the status of the quality of life of TB patients and the factors having an impact on which.

Materials and methods

This descriptive study was performed to determine the quality of life and related factors of patients with TB patients. This study was conducted at the Erzurum Nihat Kitapçı State Hospital and Aziziye Research Hospital of Atatürk University in the period between 5th of May and July 2009. These hospitals are the largest hospitals located in the Eastern Anatolian region.

Patients hospitalized for at least 1 month were included in the study to be able to evaluate negative effects of the illness process. Patients who had known respiratory co-morbidity other than TB or any known and diagnosed chronic illness, which may affect QOL were excluded from the study. The study was completed with 79 TB patients who were older than 18 years old, were able to comprehend the questions and agreed to participate in the study. Data were obtained by interviewing with the patients face to face, by researchers. Each interview lasted 10–15 minutes, on the average.

Data were collected using two instruments: a QOL instrument and patient identifiers form.

QOL instrument: QOL, developed by Greenley and Greenberg15 in 1994. The validity and reliability of this inventory for Turkish populations were studied by Şimşek16. This is a Likert type self-administered scale with 24 items including ‘living situation’, ‘finances’, ‘leisure’, ‘family relations’, ‘social life’, ‘health’ and ‘access to health care’. Respondents are asked to indicate which item describes how they have been thinking for the past 6 months. Responses are scored 1–5, with 5 the most optimistic response. Scores can range from 24 to 120, where higher scores indicate higher QOL levels. It has good internal consistency and test–retest reliability, and concurrent validity has been established. Cronbach alpha coefficient was found to be .75 for this study.

Patient Identifiers Form: 1) social and demographic characteristics such as age sex, marital status, educational level, family income; 2) changes in social milieu after the illness, such as relations with neighbors, relations with friends, spirituality and working conditions; and 3) the illness (the status of relapsed or new onset).

Before the start of the study, approval was obtained from the hospital’s institutional review board. All study participants gave informed consent.

Data Analysis: The data were analyzed using the SPSS statistical package, version 11.5. Descriptive statistics included percentages, standard deviations and means. To assess descriptive features of the patients, percentage distributions and medians were used; medians were used to determine the scale points; to assess the status of being influenced of the scale points by certain variables, parametric (t test) and nonparametric (Kruskall Wallis and Mann Whitney U) tests were used; to evaluate the correlation, Pearson’s correlation test was used; and to determine the internal consistency of the scale items, Cronbach alpha coefficient was calculated.17

Results

Table 1 shows the means of the quality of life points by descriptive features of the patients. The mean age of the patients was 35.29±15.81 years.
It was found that of the patients, 53% were women, 53% were graduated from primary school, 55% were married, 67% had poor monthly income, 83% had health insurance.

Table 1. Characteristics of Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>18-27</td>
<td>35 (44)</td>
</tr>
<tr>
<td>28-37</td>
<td>9 (11)</td>
</tr>
<tr>
<td>38-47</td>
<td>10 (13)</td>
</tr>
<tr>
<td>&gt; 48</td>
<td>25 (32)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>42 (53.2)</td>
</tr>
<tr>
<td>Male</td>
<td>37 (46.8)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>42 (53)</td>
</tr>
<tr>
<td>High school</td>
<td>31 (39)</td>
</tr>
<tr>
<td>University</td>
<td>6 (8)</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>35 (44.3)</td>
</tr>
<tr>
<td>Married</td>
<td>44 (55.7)</td>
</tr>
<tr>
<td><strong>Satisfaction from income</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (32.9)</td>
</tr>
<tr>
<td>No</td>
<td>53 (67.1)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
</tr>
<tr>
<td>Freelance</td>
<td>22 (27.8)</td>
</tr>
<tr>
<td>Retired</td>
<td>12 (15.2)</td>
</tr>
<tr>
<td>Homemaker</td>
<td>29 (36.7)</td>
</tr>
<tr>
<td>Working</td>
<td>16 (20.3)</td>
</tr>
<tr>
<td><strong>Health insurance</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>66 (83.5)</td>
</tr>
<tr>
<td>No</td>
<td>13 (16.5)</td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
</tr>
<tr>
<td>Village</td>
<td>42 (53.2)</td>
</tr>
<tr>
<td>City</td>
<td>37 (46.8)</td>
</tr>
<tr>
<td><strong>Duration of disease</strong></td>
<td></td>
</tr>
<tr>
<td>New case</td>
<td>83.5 (66)</td>
</tr>
<tr>
<td>Relepsa case</td>
<td>16.5 (13)</td>
</tr>
</tbody>
</table>

Given the association between the quality of life and age, gender, educational status, marital status, occupation and monthly income of the patients, it was found that the difference between them was statistically significant (p<0.01). When the relationship between the quality of life and the living area, social insurance was evaluated, the difference was found to be statistically insignificant (p>0.05).

It was found that 83.5% of the patients were new cases and after the disease onset, there were no changes in familiar relations (74.7% of the patients), in neighborhood-friendship relations (75.9% of the patients), in religious beliefs (79.7% of the patients), in relations with the spouse (82.3% of the patients), in workplace relations (75.9% of the patients).

The difference between the quality of life and familiar relations, neighborhood-friendship relations after the disease onset was found to be statistically significant (p<0.05 and p<0.05, respectively). The difference between the quality of life and the status of religious beliefs, relations with the spouse, changes in working life seen after the disease onset was found to be statistically insignificant (p>0.05).

**Discussion**

As a result of the study, the mean quality of life point was found to be 85.58±13.53. As a consequence, we can say that the quality of life of the patients ranked at moderate level.

As a result of the studies in the literature which were performed using different scales and methodological implementations, it was proved that the quality of life of the patients with tuberculosis decreased to various levels and the disease of tuberculosis negatively influenced the quality of life of the individuals in physical, emotional and social aspects. In many other studies, it was reported that tuberculosis had an impact on general well-being, body perception, mental health, physical and social role functions, all other quality of life aspects, and moreover, led to further problems such as social labeling, solitude, troubles resulted from drug use, prolonged therapy, sexual malfunctions, loss of income, and fears.

It was found a highly significant relationship between the ages of the patients and the quality of life. In this study, it was found that the mean scores of the quality of life of the patients increased as the age became advanced. This result correlates with Taşkın, but not with Duyan. We can recommend further studies with larger patient populations to elicit this matter.

Comparison of the means of QOL points by gender revealed that the mean QOL points of female patients were higher than that of men. The results of the study performed by Taşkın et al. correlate with the present study, however, Ünal...
Table 2. Relationship between socio-demographic characteristics and quality of life

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-27</td>
<td>81.89±13.28</td>
<td>KW: 9.177 p&lt;0.01</td>
</tr>
<tr>
<td>28-37</td>
<td>80.78±9.98</td>
<td></td>
</tr>
<tr>
<td>38-47</td>
<td>86.80±14.73</td>
<td></td>
</tr>
<tr>
<td>&gt;48</td>
<td>92.0±12.57</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>89.31±13.24</td>
<td>t: -2.714 p&lt;0.01</td>
</tr>
<tr>
<td>Male</td>
<td>81.35±12.74</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school</td>
<td>85.02±12.11</td>
<td>KW: 13.852 p&lt;0.01</td>
</tr>
<tr>
<td>High school</td>
<td>82.32±13.22</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>106.33±4.23</td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
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<td></td>
</tr>
<tr>
<td>Single</td>
<td>82.43±14.25</td>
<td>t: -1.878 p&lt;0.01</td>
</tr>
<tr>
<td>Married</td>
<td>88.09±12.52</td>
<td></td>
</tr>
<tr>
<td><strong>Satisfaction from income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>92.81±15.71</td>
<td>MWU: 335.500 p&lt;0.01</td>
</tr>
<tr>
<td>No</td>
<td>82.04±10.81</td>
<td></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freelance</td>
<td>81.18±13.30</td>
<td>KW: 14.214 p&lt;0.01</td>
</tr>
<tr>
<td>Retired</td>
<td>97.42±10.80</td>
<td></td>
</tr>
<tr>
<td>Homemaker</td>
<td>86.86±11.17</td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>80.44±14.63</td>
<td></td>
</tr>
<tr>
<td><strong>Health insurance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>86.83±16.24</td>
<td>MWU: 286.500 p&gt;0.05</td>
</tr>
<tr>
<td>No</td>
<td>79.23±16.08</td>
<td></td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village</td>
<td>84.24±11.68</td>
<td>t: -0.940 p&gt;0.05</td>
</tr>
<tr>
<td>City</td>
<td>87.11±15.38</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Changes that occurred within the family environment and social environment after the illness and effects on quality of life

<table>
<thead>
<tr>
<th>Variables</th>
<th>n  (%)</th>
<th>Mean ± SD</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family relations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakened</td>
<td>6 (7.6)</td>
<td>77.33±4.03</td>
<td>KW: 8.950 p&lt;0.05</td>
</tr>
<tr>
<td>No change</td>
<td>60 (74.7)</td>
<td>84.17±13.05</td>
<td></td>
</tr>
<tr>
<td>Strengthened</td>
<td>14 (17.7)</td>
<td>95.07±13.96</td>
<td></td>
</tr>
<tr>
<td><strong>Relations with neighbours and friends</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakened</td>
<td>7 (8.9)</td>
<td>73.71±8.56</td>
<td>KW: 8.526 p&lt;0.05</td>
</tr>
<tr>
<td>No change</td>
<td>60 (75.9)</td>
<td>85.50±11.62</td>
<td></td>
</tr>
<tr>
<td>Strengthened</td>
<td>12 (15.2)</td>
<td>92.92±19.61</td>
<td></td>
</tr>
<tr>
<td><strong>Belief system</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>64 (81)</td>
<td>86.02±12.40</td>
<td>MWU: 414.0 p&gt;0.05</td>
</tr>
<tr>
<td>Strengthened</td>
<td>15 (19)</td>
<td>83.40±18.18</td>
<td></td>
</tr>
<tr>
<td><strong>Sexual relations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakened</td>
<td>9 (11.4)</td>
<td>81.33±12.55</td>
<td>KW: 3.390 p&gt;0.05</td>
</tr>
<tr>
<td>No change</td>
<td>65 (82.3)</td>
<td>86.17±12.91</td>
<td></td>
</tr>
<tr>
<td>Strengthened</td>
<td>5 (6.3)</td>
<td>79.40±13.84</td>
<td></td>
</tr>
<tr>
<td><strong>Relations within the work environment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weakened</td>
<td>24.1 (19)</td>
<td>85.53±17.62</td>
<td>MWU: 562.0 p&gt;0.05</td>
</tr>
<tr>
<td>No change</td>
<td>75.9 (60)</td>
<td>85.60±12.13</td>
<td></td>
</tr>
</tbody>
</table>
et al. found lower scores of quality of life in women. Duyan et al. in a study, did not find any relationship between gender and the quality of life. Women and men have different roles in family and society. Loss of role resulted from the disease and affection process differ by gender. That is an important factor influencing the quality of life. It was thought that high quality of life of female patients in this study was resulted from the fact that most of women included in the study were housewives and assumed less social roles than men did. Furthermore, we can recommend performing studies about the clarification of the relationship between gender and quality of life.

The mean quality of life points of the patients graduated from academy was found to be high. This result is in accordance with the results of the studies performed by Duyan et al., Taşkın et al., and Ünalan et al. The individual becomes less affected by external factors as the level of education gets higher, and consequently his/her quality of life becomes higher. It can be argued that this result may be related to the fact that the patients with higher education level use proper methods of coping.

Although the mean point of quality of life of married patients was higher than that of single patients, the difference was found to be statistically insignificant. Duyan et al. found the mean point of quality of life of single patients to be higher. It can be thought that the result obtained from this study may be related to better social support provided for married patients.

The mean point of quality of life of the patients having proper monthly income was found to be higher than that of other group and the difference was found to be statistically significant. This result is consistent with the results of the studies performed by Duyan et al., Taşkın et al., Zautra et al. and Lehman et al. It is thought that the facts that the patients feel good, cope better with the problems resulted from the disease and are able to maximize their life standards with the increased income influence the results in that manner.

As a result of the study, the mean point of quality of life of unemployed patients was lower than that of other groups, the difference between the groups was found to be statistically significant. Working in a job remarkably contributes to overcome the financial loss resulted from the disease and treatment, to establish again self-esteem of the individual, to make the life more satisfying and meaningful providing social support. Poverty is very important for the widespread occurrence of tuberculosis. It has been reported that as long as poverty remains, the widespread dissemination of tuberculosis cannot be prevented, and therefore life standard and quality of life are entirely influenced.

The relationship between social insurance and quality of life of the patients was found to be statistically insignificant. The results of this study were found to be consistent with the results of the study performed by Taşkın et al. The fact that healthcare institutions cover the cost of the treatment of tuberculosis patients might have impressed the results in that manner.

Although the patients living in urban areas had higher average points of QOL, the difference between the groups was found to be statistically insignificant. The result achieved by this study was found to be consistent with the results of the previous study. It can be thought that the fact that the patients living in urban centers reach easily to healthcare institutions may have an impact on the results.

The average points of quality of life of new cases were found to be higher than that of relapsed patients. This result was consistent with the study conducted by Duyan et al. It can be thought that disease recurrences lead to despair in the patients, and consequently influence the results in this way.

The average points of quality of life of the patients whose family relations were disrupted after the onset of the disease were lower than that of the patients defining that their relations were not changed or became stronger; the difference was found to be statistically significant. The result of this study was consistent with the results of previously performed studies. We can say that there is a favorable correlation between the support of family members and quality of life.

The average points of quality of life of the patients indicating a decrease in neighborhood and friendship relations were found to be lower. Chronic diseases like tuberculosis negatively influence the status of physical health of the patients as well as social, psychological and economic well-being.

The result of the study was found to be similar to the previously performed study.
Of the patients, 15% defined that their religious beliefs became stronger after the disease. Taşkın et al.,3 in a study previously performed, reported that religious beliefs became stronger in 23.3% of the patients. Hansel et al.,10 reported that religion and spiritualism played an important role for the approach of tuberculosis patients to the disease and having tuberculosis disease remarkably increased their level of spiritualism.

Although the difference between the average points of quality of life of the patients by the changes seen in sexual relations with the spouse after the disease onset was found to be statistically insignificant, average point of quality of life of the patients having troubles in their sexual lives was found to be lower. Taşkın et al.,3 Duyan et al.,13 found that the patients experienced a decrease in the frequency of sexual relationship after the disease had lower points of quality of life as well.

The average points of QOL of the patients defining that their working life relations became stronger were found to be higher. Taşkın et al.,3 Duyan et al.,13 reported that the patients having a decrease in the working relations after the disease onset had lower points of quality of life.

Conclusions

In the light of the results obtained from the study, to increase the quality of life of the patients with pulmonary tuberculosis, we think that it is necessary to provide information to the patients about the disease, the importance of completing the whole course of the treatment and complying with the disease; to ensure the patients to be cared and treated by healthcare professionals specialized in this area; to offer proper information and support to the patients and their families; to make all healthcare professionals be aware of the factors influencing the quality of life.

References


Abstract

Introduction: The majority of studies published so far on bacterial vaginosis (BV) have not been consistent in associating BV and cervical dysplasia. The aim of this study was to determine the correlation between BV and cervical intraepithelial neoplasia (CIN), and between BV and degree of severity of these changes on the uterine cervix.

Materials and Methods: The study included sexually active women who were referred by gynecologists to colposcopic examination. Based on histopathological results, the examinees were divided into three groups: 41 women with confirmed CIN changes; 30 women without precancerous and cancerous changes in the cervix and 29 women with histopathologically confirmed invasive cervical cancer. Microbiological testing of samples from the genital tract included direct microscopic smears, wet and stained, and inoculation of culture medium, testing for *Chlamydia trachomatis* and genital mycoplasmas.

Results: BV was present among 18 (43.9%) women with cervical intraepithelial neoplasia and 13 (44.8%) women with invasive cancer, which in both cases meant statistically significantly higher frequency compared to women without precancerous and cancerous cervical changes, who had confirmed bacterial vaginosis in 3 (10.0%) cases ($p = 0.003$). The risk of finding the precancerous changes on the cervix was 8.36 (1.89 to 37.04) times higher in patients with BV than in women without that infection.

Conclusion: BV was significantly more common in women with the finding of precancerous changes in the cervix, and was not associated with severity of histopathological changes in the cervix.

Key words: Bacterial vaginosis; Precancerous changes; Cervical intraepithelial neoplasia

Introduction

Bacterial vaginosis (BV) is a non-inflammatory, infectious syndrome described in the vaginal pathology. Clinically, it is polymicrobial, primarily anaerobic infection with subacute course, which is followed by homogenous secretions adherent to vagina, odor and poor leukorrhea. Dysuria may be present and external vaginal itching or redness may be seen. Many women with bacterial vaginosis do not have any signs or symptoms [1].

Microbiologically, BV is defined as a disruption in the balance of bacterial morphotypes, overgrowth of a large number of anaerobic and microaerophilic bacteria, Gram-positive cocci, *Mycoplasma* spp. and reduction in the number of *Lactobacillus* spp. In women with BV, lactobacilli are absent or severely reduced, while concentrations of other bacteria are generally increased 100- to 10000-fold over normal levels [2-6].
The human papillomavirus (HPV) is the major etiologic agent in the development of cervical cancer, while BV and other genital infections are considered to increase the risk of cervical cancer [7-9]. The majority of infected women are found to heal spontaneously with no ill effects, probably due to a competent immune response. However, if woman develops persistent infection, the cervix is prone to the transformation of cells and development of precancerous changes [10].

The majority of studies published so far on bacterial vaginosis (BV) have not been consistent in associating BV and cervical dysplasia. Marrazzo et al., as well as many other researchers, found that BV causes inflammatory changes of the cervix and that chronic inflammation of the cervix with BV may be considered as one of the mechanisms for the development of malignant changes in the cervix [11-14]. Co-factors that are also associated with an increased risk of cervical intraepithelial neoplasia (CIN) and cervical cancer are smoking, poor nutritional status, hormonal factors (age of women at first childbirth), use of hormonal contraception, having sex at an early age (less than 16 years) and a large number of sexual partners, race, multiple births, low socioeconomic status. Older women have increased risk of CIN and cervical cancer, as well as women with sexually transmitted infection (STI), such as infection with bacteria Chlamydia trachomatis (C.trachomatis) and protozoa Trichomonas vaginalis (T.vaginalis) [7, 15, 16].

The aim of this study was to determine the correlation between BV and CIN, and between BV and degree of severity of these changes on the uterine cervix.

Materials and Methods:

The study enrolled sexually active women who were primarily examined by gynecologists and then referred to colposcopic examination from May 2005 to January 2007 for a biopsy and further histopathological examination. Ethical approval was obtained from the local ethics committee. Written, informed consent was taken from the patients for participation in the study.

Based on histopathological results (results of biopsy), the participants were divided into three groups:

- The target group: 41 women with histologically confirmed CIN 1, CIN 2 and CIN 3.
- The negative control group: 30 women without precancerous and cancerous cervical changes.
- The positive control group: 29 women with histologically confirmed invasive cervical cancer.

Samples were taken from women examined by speculum for microbiological examination:

- Two high vaginal swabs were taken from each woman for bacteriological examination;
- Vaginal swab from the posterior vaginal fornix which was immediately after taking suspended in 1 ml of normal saline solution and incubated at 37°C in order to identify the protozoa T. vaginalis by using the wet mount technique;
- Two cervical swabs for bacteriological examination;
- Endocervical swab for detection of genital mycoplasmas was collected using a Dacron endocervical swab;
- Endocervical swab for detection of C.trachomatis was collected using a Dacron endocervical swab [17].

Microbiological testing of samples from the genital tract included direct swabs for wet and stained smears for microscopy, and inoculation of culture medium, testing for C. trachomatis and genital mycoplasmas.

The wet preparation method was used for the diagnosis of trichomoniasis, bacterial vaginosis, and candidiasis in women. BV diagnosis was based on the presence of the "clue cells", which are vaginal epithelial cells covered with numerous tiny rods and coccobacilli. If the swabs from women with bacterial vaginosis are positive for Mobiluncus spp., the abundance of highly motile bacteria can be observed on the microscopic preparation.

Gram-stained vaginal smears were used for the direct cytobacteriological examination. Gram-stained vaginal smear enables observing the epithelial cells, and at the same time provides the insight into the number and presence of leukocytes and
microbial flora. "Clue cells" in the Gram-stained vaginal smear are covered by masses of Gram-negative bacilli and cocciobacilli. BV was diagnosed also by using the Nugent score, a semi quantitative scoring system to evaluate bacterial morphotypes on the direct Gram stain of vaginal fluid [17]. The increased number of polymorphonuclear leukocytes (PMN) in the DMP indicates the presence of inflammatory reaction.

Different microbiological culture media were used for the inoculation of vaginal and cervical swabs. For the isolation of aerobic Gram-positive bacteria (Columbia agar), differential medium for isolation of Enterobacteriaceae (Mac Conkey), a selective medium for the isolation of Gardnerella vaginalis (Blood agar: a selective medium with 5% Human Blood), enriched medium for the selective isolation of Neisseria gonorrhoeae (Thayer Martin Medium with selective supplements) and medium for the isolation of yeasts of the genus Candida (Sabouraud's Dextrose Agar). Medium (Mac Conkey agar) for Enterobacteriaceae was incubated at 37°C and examined after 24 hours. Other culture media were incubated in an atmosphere of high relative humidity and a CO2-enriched atmosphere for 48 hours. Microorganisms cultivated from positive culture media were identified by using standard microbiological methods [17].

Confirmation of BV diagnosis was done by the growth of G. vaginalis in pure culture or by its presence in relatively large numbers compared to the other bacteria on the selective medium with 5% Human Blood. Colonies of G. vaginalis are easy to isolate on the solid medium. They are round, transparent, smaller than 0.5 mm in diameter, surrounded by discrete weak β-hemolysis. On the Gram-stained smear using a plate culture of G. vaginalis, we observed Gram-negative or Gram-labile short rods [17].

Diagnosis of genital mycoplasmas was performed with the commercially available Mycoplasma IST 2 Kit (bioMérieux, France), by means of which the genital Mycoplasma were identified and quantified; simultaneously antibiotic susceptibility tests were performed.

C. trachomatis infection was diagnosed by direct immunofluorescence using commercial Fluorotect Chlamydia test (Omega Diagnostics Ltd, Scotland, United Kingdom). In women with histopathologically verified invasive cancer, testing for C. trachomatis was not performed, due to inability to get adequate samples for testing (presence of blood on the swab).

Biopsy samples were taken from colposcopically suspect changes of the cervix (white-colored after the application of acetic acid, mosaic, irregular vascularization) with a Q-tip or hook; the depth of biopsy specimens was about 3 mm. The cervical biopsy results were reported as without precancerous and cancerous changes in the cervix, as CIN 1 = mild dysplasia; CIN 2 = moderate dysplasia; CIN 3 = severe dysplasia or ICC = invasive cervical cancer [18].

The participants completed a questionnaire prepared for the research purposes. A questionnaire was a series of questions posed to women to obtain statistically useful information about sexual behavior and socio-demographic data (date of birth, age of first sexual intercourse, number of sexual partners, frequency of sexual activity, presence of sexually transmitted diseases, number of childbiths, age of first childbirth, use of hormonal contraceptives and hormonal drugs, data on vaginal washing practices, kinds of lower genital tract symptoms, education level, nationality, smoking status).

Quantitative statistical analysis was performed using SPSS version 10.0 and the StatCalc function of Epi Info software package version 6th.

The Student t-test was used to compare the mean values of numerical symbols; comparison of the frequency of the descriptive characteristics among groups was performed using Mantel-Haenszel Chi square test, and in cases where expected frequencies were less than five - Fisher's exact probability test for testing the zero hypothesis. The assessment of correlation between each individual risk factor and the finding of precancerous changes in the cervix was carried out using a logistic regression analysis. As the threshold value for declaring statistical significance was estimated error of less than 5% (p <0.05) in all analyses. The results of statistical analysis are shown in tables.

Results

On average, women in the group with invasive cervical cancer were 50.28 ± 9.17 years old, and they were significantly older than women in the test group with precancerous changes in the cer-
vix (43.95 ± 9.27 years old and p-value = 0.019), and they also were older than women without precancerous and cancerous changes on the cervix (41.97 ± 10.89 years old and p value = 0.007) (Table 1). The difference in age between women with precancerous changes in the cervix and women without precancerous and cancerous changes in the cervix was not statistically significant.

Bacterial vaginosis was found in 18 (43.9%) women with precancerous changes in the cervix and 13 (44.8%) women with invasive cervical cancer, which in both cases meant that the frequencies of women with BV was significantly higher in both groups compared to the group of women without precancerous and cancerous changes in the cervix, where BV was confirmed only in 3 (10.0%) cases (p = 0.003) (Table 2). It is very important that BV in our research was not proven as a co-infection with other tested infectious agents in combination.

*Chlamydia trachomatis* was confirmed in 24 (58.5%) cases in the test group, while the percentages of females with this infection was significantly lower in women without precancerous and cancerous changes in the cervix (30.0% and p = 0.029). The frequencies of infection with other bacteria did not differ significantly among the groups (p >0.05) (Table 3).

In women with precancerous changes of the cervix, yeasts of the genus *Candida* were not found.

Table 1. Age of patients by groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Examinees</th>
<th>Comparison between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with CIN (I)</td>
<td>with Ca (II)</td>
</tr>
<tr>
<td>A</td>
<td>43.95</td>
<td>50.28</td>
</tr>
<tr>
<td>SD</td>
<td>9.27</td>
<td>9.17</td>
</tr>
<tr>
<td>Min</td>
<td>26.00</td>
<td>31.00</td>
</tr>
<tr>
<td>Max</td>
<td>63.00</td>
<td>63.00</td>
</tr>
</tbody>
</table>

A: arithmetic mean; SD: standard deviation; Min: minimal value; Max: maximal value; CIN: cervical intraepithelial neoplasia; Ca: invasive cervical cancer

Table 2. Association between bacterial vaginosis and CIN

<table>
<thead>
<tr>
<th>BV</th>
<th>Examinees</th>
<th>Comparison between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV present no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV present no. (%)</td>
<td>18   (43.9%)</td>
<td>13  (44.8%)</td>
</tr>
<tr>
<td>BV absent no. (%)</td>
<td>23    (56.1%)</td>
<td>16   (55.2%)</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; Ca: invasive cervical cancer; BV: bacterial vaginosis

Table 3. Association between other bacteria and CIN

<table>
<thead>
<tr>
<th>Findings</th>
<th>Examinees</th>
<th>Comparison between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with CIN (I)</td>
<td>with Ca (II)</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>24 (58.5%)</td>
<td>...</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>6 (14.6%)</td>
<td>4 (13.8%)</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>1 (2.4%)</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>6 (14.6%)</td>
<td>6 (20.7%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5 (12.2%)</td>
<td>3 (10.3%)</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; Ca: invasive cervical cancer; n.s.: statistically not significant difference
The frequencies of infection caused by the protozoa *Trichomonas vaginalis* did not differ significantly between the groups (p >0.05) (Table 4).

Eight (53.3%) women had bacterial vaginosis among 15 women with CIN 1; seven (50.0%) had bacterial vaginosis among 14 women with CIN 2; and three (25.0%) had bacterial vaginosis among 12 women with CIN 3. The incidence of bacterial vaginosis did not differ significantly between the groups with various severity of cervical dysplasia (p >0.05) (Table 5).

### Table 4. Association between *Candida* spp. and *Trichomonas vaginalis* and CIN

<table>
<thead>
<tr>
<th>Finding</th>
<th>Examinees with CIN</th>
<th>Examinees without CIN and Ca</th>
<th>Comparison between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp.</td>
<td>1 (3.4%)</td>
<td>3 (10.0%)</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>2 (4.9%)</td>
<td>2 (6.9%)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; Ca: invasive cervical cancer; n.s.: statistically not significant difference

The assessment of association between bacterial vaginosis and CIN

### Table 5. Association between bacterial vaginosis and severity of CIN

<table>
<thead>
<tr>
<th>CIN</th>
<th>BV present no. (%)</th>
<th>BV absent no. (%)</th>
<th>Comparison between CIN 1, CIN 2 and CIN 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>low-grade</td>
<td>8 (53.3%)</td>
<td>7 (46.7%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>moderate-grade</td>
<td>7 (50.0%)</td>
<td>7 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>high-grade</td>
<td>3 (25.0%)</td>
<td>9 (75.0%)</td>
<td></td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; BV: bacterial vaginosis; n.s.: statistically not significant difference; CIN 1: low grade CIN; CIN 2: moderate grade CIN; CIN 3: high grade CIN

### Table 6. Assessment of association between bacterial vaginosis and CIN

<table>
<thead>
<tr>
<th>Microbiological results</th>
<th>OR</th>
<th>Limits of 95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>The upper</td>
<td>The lower</td>
</tr>
<tr>
<td>BV present</td>
<td>7.040</td>
<td>1.838</td>
<td>26.955</td>
</tr>
<tr>
<td>BV absent</td>
<td>0.142</td>
<td>0.037</td>
<td>0.544</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; OR: odds ratio; CI: confidence interval; BV: bacterial vaginosis

Univariate logistic regression analysis revealed also a significant relationship between the development of precancerous changes in the cervix and other possible factors such as the following: the presence of *Chlamydia trachomatis*, a positive family history of cancer, irregular visits to gynecologist, having six or more than six sexual partners in patient’s lifetimes, having sex seven and more times per week, being 26 or older at the first childbirth, the use of contraceptive pills, having a low educational level, and smoking 11–20 cigarettes per day.

Multivariate analysis identified the four risk factors which significantly influence the development of precancerous changes in the cervix: bacterial vaginosis, irregular visits to gynecologist, use of contraceptive pills and having low education level (Table 7). The final multivariate logistic regression model showed that, if the other risk factors that contribute to development of CIN can be controlled, BV increases the risk for development of precancerous changes in the cervix about 8.36 times (1.89 to 37.04).

The model that includes as independent variables the listed risk factors and the regression constant explains the change of risk for development of CIN in our sample by 49.2% (The model was assessed by coefficient of determination, R² = 0.492).

The frequencies of irregular visits to gynecologist, use of contraceptive pills and having low education level did not differ significantly among
the groups in women with BV (p >0.05). The compared frequencies of the listed factors among women with bacterial vaginosis and without it did not significantly differ in study groups. This result showed that irregular visits to gynecologist, use of contraceptive pills and low education level influence the development of precancerous changes in the cervix apart from bacterial vaginosis.

Discussion

In 1863, the great pathologist Rudolf Virchow formulated the hypothesis that transformation of cells occurs areas affected with chronic infection. He explained this as a consequence of tissue injury, inflammation and increased cell proliferation during chronic infection [19, 20]. Schwebke et al. in their study by univariate analysis pointed out a significant linkage between BV and cervicitis and between the use of metronidazole gel and resolution of cervicitis [21]. Plasma cell endometritis in women with symptomatic bacterial vaginosis is very common, and it is not associated with vaginal and cervical infections by other bacteria. These results suggest a possible link between BV and non-chlamydial and non-gonococcal infection of the upper genital tract in women [22]. BV causes cervical inflammatory epithelial changes, and cervical chronic inflammation in women with BV could be considered as a possible mechanism that causes potentially malignant cellular changes anywhere on the cervix [11, 12, 14]. By univariate analysis, Lehtovirt et al. revealed that BV was significantly associated with increased risk of developing CIN [9].

In our study, bacterial vaginosis was found in 18 (43.9%) women with CIN and in 13 (44.8%) with invasive cervical cancer, which in both cases was statistically significantly higher compared to the group of examinees without precancerous and cancerous changes of cervical biopsy specimen, in which bacterial vaginosis was confirmed only in 3 (10.0%) cases (p = 0.003). The risk for development of CIN was 7.04 (1.84 to 26.95) times higher in women with bacterial vaginosis than in women without BV (p = 0.004). Univariate logistic regression analysis showed that BV is a statistically significant predictor for the precancerous changes in the cervix. The model for a multivariate regression separated BV as cofactor that increases the risk for development of precancerous changes in the cervix about 8.36 times (1.89 to 37.04) and three other factors as factors that significantly influence the development of CIN.

Our results are quite compatible with the results of Clomp et al. who found a correlation between cytologically diagnosed G. vaginalis infection and CIN [23]. They collected data from 800,498 Dutch asymptomatic women, participating in the Dutch national screening program. Clomp et al. showed that the prevalence of (pre) cancerous changes of cytologically diagnosed G. vaginalis infection was 0.6 per thousand women. The risk of (pre)cancerous changes was significantly higher in women with cytologically diagnosed G. vaginalis infection than in women with normal vaginal flora (Odds ratio (OR), 10.3, 95% confidence interval, 6.6 to 16.1).

Uthayakumar et al. found in their retrospective study performed in England that women with BV were at increased risk of CIN [24]. Diagnosis of bacterial vaginosis was based on the presence of the “clue cells” in the direct Gram stain preparation of vaginal fluid taken from the upper part of the vagina, positive amine test, pH over 4.5, presence of characteristics vaginal discharge. Our results

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>OR</th>
<th>Limits of 95% CI</th>
<th>Test and p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV</td>
<td>8.360</td>
<td>1.887 to 37.042</td>
<td>0.005</td>
</tr>
<tr>
<td>Irregular visits to gynecologist</td>
<td>3.380</td>
<td>1.032 to 12.256</td>
<td>0.048</td>
</tr>
<tr>
<td>Using oral contraceptives</td>
<td>19.476</td>
<td>2.048 to 185.242</td>
<td>0.010</td>
</tr>
<tr>
<td>Low level of education</td>
<td>11.089</td>
<td>1.181 to 104.100</td>
<td>0.035</td>
</tr>
<tr>
<td>The regression constant</td>
<td>0.205</td>
<td></td>
<td>0.004</td>
</tr>
</tbody>
</table>

CIN: cervical intraepithelial neoplasia; OR: odds ratio; CI: confidence interval; BV: bacterial vaginosis
are also consistent with the findings of Nam et al. who found that the incidence of CIN was significantly higher in the BV-present group (p = 0.043), however, no statistically significant relationship between BV and CIN was demonstrated by multivariate analysis (p=0.081) [25].

A retrospective, longitudinal, cohort study was performed as a part of the Dutch Cervical Screening Program, involving the population of 100,605 women, each of whom had 2 smears taken over a period of 12 years [26]. The dysbacteriotic cohort was significantly more likely to have low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) in their follow-up smear (OR, 1.85; 95% confidence interval [95% CI], 1.28-2.67 and OR, 2.00; 95% CI, 1.31-3.05, respectively) compared to women in the control group. The results obtained in this study are consistent with our results. In addition, Schiff et al. found similar results to ours: an association between the presence of the "clue cells" in vaginal wet mount and precancerous changes of the cervix [27].

We found the negative correlation between bacterial vaginosis and degree of cervical dysplasia in our study. Of 15 women with 8 (53.3%) had bacterial vaginosis and CIN 1, of 14 women 7 (50.0%) had BV and CIN 2, and of 12 women 3 (25, 0%) had BV and CIN 3. Nam et al. [25]. in their study found no correlation between BV and severity of precancerous changes of the cervix; this result is consistent with our results. Peters et al. also found in their work that BV has no effect on the severity of CIN, or on the mitotic index in cervical intraepithelial neoplasia [28]. Neuer et al. also did not find statistically significant difference between bacterial vaginosis and the grade of cervical dysplasia in their study [29].

Zbroch et al. found in their study that CIN 1 is more common in women with BV and in women with Chlamydia trachomatis infection than in women without these infections [30]. Discacciati et al. found that BV was similarly present among women of both their study groups: in 18% of women with SIL and in 12% of women without SIL. Their results were also similar when the grade of SIL was taken into consideration. BV was detected in 16% of women with low-grade SIL, and in 33% women with high-grade SIL when compared to the controls (12%) [8].

Platz-Christensen et al. investigated the relationship between the presence of clue cells in Papinicolaou-stained vaginal smears and CIN. They examined a total of 6.150 specimens and discovered that any grade CIN was more common among women with BV (p <0.001). The relative risk of having CIN 3/carcinoma in situ was 5.0 for the confidence interval in 95% of women with BV. Their conclusion was that BV can be associated with the development of CIN, ie. BV infection may be an independent factor or cofactor for HPV in the development of cervical precancerous changes [31]. However, not all scientists accept this correlation as statistically significant.

Peters et al. did not reveal a significantly higher prevalence of bacterial vaginosis in women with dysplasia in the cervix [28]. Boyle et al. showed that women with BV were not found to have CIN more frequently than women with normal vaginal flora, and the quantities of nitrosamines produced by women with BV did not differ significantly from women without BV [32]. Frege et al. found the opposite conclusion to ours in their study. In their investigation diagnosis of BV is based on four criteria: presence of clue cells, pH 4.5, positive amine test and increased vaginal discharge. The results of their study demonstrated that there is no significant correlation between CIN and BV (P <0.00005) [33].

Numerous studies were conducted to evaluate the correlation between bacterial vaginosis and CIN, however, their results were not consistent because the methods of investigation varied from study to study. Sensitivities and specificities vary for different diagnostic tests for bacterial vaginosis depending on several different diagnostic criteria which must be taken into account when comparing the results of various studies. Taking into consideration that there are many risk factors, it is necessary to examine all of them, especially the infection with HPV. Our study has potential limitations that must be considered in interpretation of results: lack of validation of interview data; our study did not involve the entire female population but only the patients referred to colposcopic examination for sampling material for pathohistological examination; a limitation of this study is also the small sample size. We reminded the study subjects at several points in the interview about the confidential nature of all data collected so as to
increase the likelihood of accurate responses about sexual behavior. We must bear in mind that the diagnosis of HPV infection has not been made. However, all mentioned limitations of this study leave room for further study, by using a larger sample and tests with high sensitivity and specificity.

In conclusion, based on the obtained results, we found that bacterial vaginosis was significantly more frequent in women with CIN and in women with invasive cervical cancer. We did not confirm neither correlation between BV and the severity of precancerous changes in the cervix, nor significant effect of BV on development of precancerous changes in the cervix apart from other confirmed risk factors.

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References


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Abstract

Background: It is important to promote emotional intelligence and leadership behavior of male nursing students to train nurses who have leadership skill to meet emotional requirements of their patients.

Aim: The study was planned in descriptive, correlative and relational style to determine the relationship between emotional intelligence and leadership behaviors of male nursing students.

Methods: Data was collected from male nursing students (n=87) who receive undergraduate education in a faculty of medical sciences. In data collection, a questionnaire form including 4 questions investigating the personal information of students, “Emotional Intelligence Scale” and “Leadership Behavior Description Scale” was used. Questionnaire forms were distributed to male nursing students in classroom in January 2010, and then collected back when they were completed. 87 out of total 130 students agreed to participate in the study. Participation rate was 67%. Collected data was analyzed in SPSS 11.5 by frequency, percentage distribution and Pearson correlation analyses.

Findings: As a result of the study, emotional intelligence level of male nursing students was found moderate in all sub-dimensions and they needed to improve their emotional intelligence level. On the other hand, students were determined to exhibit leadership behavior higher than moderate level. Furthermore, a positive relation was detected between the emotional intelligence level and leadership behavior of male nursing students.

Key Words: Male nursing students, nursing, leadership, emotional intelligence, Turkey.

Introduction

Emotions have important roles in understanding human nature, and interpreting thoughts, and behaviors. Emotions have been neglected in science world for long period of time, and wisdom have been given importance and identified with intelligence. Emotions were accepted human weaknesses and the expression of emotions was not found suitable for people like leaders who should have strong will (1,2). At present, modern psychology studies have reported that emotions are motivating and effective on behaviors, and give meaning and purpose; in addition, people have emotional intelligence (3,4,5).

The term of emotional intelligence was first used by Peter Salovey and John Mayer in 1990 (4). Salovey & Mayer defined emotional intelligence as an ability to understand and question through emotions, absorb emotions in opinions, and arrange emotions of others (4) Afterwards, Daniel Goleman defined emotional intelligence as self-motivation, progressing despite problems, postponing satisfaction by controlling impulses, arranging state of mind, disallowing problems to hinder reasoning and empathy, and also expressed that emotional intelligence was more important than cognitive intelligence (3).

With its increasing importance for both social life and working life of individuals, emotional intelligence directly affects the interpersonal relations in private and working lives, and thus affects the work performance (1,6,7). Previous studies reported that emotional intelligence increases the ability to solve problems (8,9), decreases desensitization the beginning of exhaustion and increases...
personal sense of achievement (10). I and facilitates coping with stress by reducing perceived stress (9,11). In addition, emotional intelligence is stated to positively affect the leadership behavior and accepted as one of the properties that strengthen leadership (12, 13,14,15).

Leaders should know and control their emotions, understand the emotions of their followers, cope with negative emotions, empathize with followers, motivate them and be open in social relations in order to affect their followers and make changes (1, 7, 12, 16, 17,18). Furthermore, leaders should be good listeners of their followers, respect their opinions, transform negative emotions to positive ones, be supportive, objective, trustable, inspierer, and should have emotional intelligence to effectively present leadership behaviors like good communication skill (7,19,20).

In many different studies investigating the relation between emotional intelligence and leadership behavior of managers (1, 14,19,21), university personnel (15), female nursing students (22), graduate students in faculty of management (23), bank staff (1) and soldiers (2,24), a positive relation was detected between emotional intelligence and leadership behavior, and emotional intelligence was found to positively affect the leadership behaviors.

Emotional intelligence is especially important for health staff to correctly understand the emotional requirements of patients and demonstrate convenient behaviors (25,26, 27). Nurses are expected to have emotional intelligence to effectively meet the health requirements of individuals who are ill and have complex emotions and to assume the leadership to provide a positive working environment (16, 17, 28, 29, 30).

Leadership behaviors slowly develop in nursing profession made, to a great extent, by females. It is reported that leadership ability of females cannot be adequately developed due to the perception of females weaker than male, females not improving their skills and abilities and the traditional roles and belief resulting from social identity of females. Certain behaviors like respect and obedience of females to males in patriarchal family-based Turkish society are also expected from nurses, which negatively affects the leadership in nursing (31). Leadership and success are generally ascribed to males, and males are inclined towards professions that could provide more strength and success, which all positively affect the leadership behavior of males (31,32).

Almost all of the nurses are females in Turkey as in the rest of the world. 14 male nurses were trained between 1963 and 1967 in Turkey; however, they could not find a job opportunity because men were not employed as a nurse according to the nursing law at the time. With the changes in Nursing Law in 2007, sex discrimination was outlawed and the obstacles for men to become nurse were removed. This development has aroused men’s interest for nursing and the number of male students in nursing schools has been increased (33). Positive approach of society for male nurses in the recent years (,33,34,35,36,37,38,39), and increasing interest of men for nursing profession indicate that male nurses will take a greater part in working life, and perhaps more effective leaders will appear.

It has not known yet whether male nursing students have certain skills desired in nursing profession like emotional intelligence and leadership behaviors. Evaluation of emotional intelligence and leadership behavior in male students and determination of missing points that should be developed later (24,28,40) will provide important data for male students to acquire these skills during their education, and to graduate as nurses with leadership ability to meet the emotional requirements of patients.

Methods

Aim and design: The study was planned in descriptive, correlative and relational style to determine the relationship between emotional intelligence and leadership behaviors of male nursing students.

Sample: The study population was composed of male students receiving education in a faculty of medical sciences, while all male nursing students were included in the scope of study without any sample selection.

Research questions:
- What is the emotional intelligence level of male nursing students?
- What are the leadership behaviors of male nursing students?
Is there a significant relationship between emotional intelligence and leadership behaviors of male nursing students?

**Instruments:** In data collection, a questionnaire form including 4 questions investigating the personal information of students, “Emotional Intelligence Scale” and “Leadership Behavior Description Scale” was used.

**Emotional Intelligence Scale** was developed by Hall (1999) and its Turkish adaptation and reliability of the scale for university students was made by Ergin (41). The scale was a 6-point Likert type scale (1: definitely disagree, 2: disagree, 3: partly disagree, 4: partly agree, 5: agree, 6: definitely agree) and composed of 30 items and 5 sub-dimensions (6 items on awareness of emotions, 6 items on administration of self emotions, 6 items on self motivation, 6 items on empathy and social skills).

There is no reversible statement in the evaluation of scale. The sub-dimension score was calculated by summing the scores of answers given to items constituting the sub-dimension. Evaluation of scale according to scores obtained from sub-dimensions is given in Table 1 (41).

Cronbach’s alpha coefficient of scale was found 0.84 by Ergin, while it was 0.92 in the present study (41).

**Leadership Behavior Description Scale** was developed by the researchers of OHIO State University and its Turkish adaptation and reliability was made by Ergun (42). This scale was developed to measure the leadership behaviors and general leadership skills of individuals. The scale was composed of 40 items and 10 sub-dimensions (possession, stimulating the mass, protecting membership, representation of organization, integrating purposes, organizing, top to bottom communication, recognition and production) and it was a 5-point Likert scale (4: always, 3: usually, 2: sometimes, 1: rarely, 0: never). There are 4 items in each sub-dimension of scale, and the scoring was reverse for 12th, 20th and 39th statements. Maximum 16 point can be obtained from sub-dimensions. Higher scores indicate that individual demonstrates leadership behaviors in the sub-dimension. Cronbach’s alpha coefficient of the scale was found 0.70 by Ergun (42), while it was 0.86 in the present study.

**Ethical consideration:** Necessary approvals were obtained from ethical committee and the institution in the study. Before data collection, all male nursing students were informed about the study, and data was collected from students who agreed/volunteered to participate in the study based on willingness.

**Data collection:** Questionnaire forms were distributed to male nursing students in classroom in January 2010, and then collected back when they were completed. 87 out of total 130 students agreed to participate in the study. Participation rate was 67%.

**Data analysis:** Collected data was analyzed in SPSS 11.5 by frequency, percentage distribution and Pearson correlation analyses.

**Results**

Considering the personal characteristics of male nursing students participating in the study, 40.2% are second year students, 37.9% are first year students, 66.7% are standard high school graduates, 57.5% live in east Turkey, and 54% live in metropolitan cities.

<table>
<thead>
<tr>
<th>Sub-dimensions of Scale</th>
<th>Quite strong (high)</th>
<th>A little bit improvement is needed (Normal)</th>
<th>Improvement is certainly needed (low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awareness of emotions</td>
<td>≥31</td>
<td>26-30</td>
<td>≤25</td>
</tr>
<tr>
<td>Management of self emotions</td>
<td>≥32</td>
<td>27-31</td>
<td>≤26</td>
</tr>
<tr>
<td>Self motivation</td>
<td>≥31</td>
<td>27-30</td>
<td>≤26</td>
</tr>
<tr>
<td>Empathy</td>
<td>≥31</td>
<td>26-30</td>
<td>≤25</td>
</tr>
<tr>
<td>Social skills (management of other’s emotions)</td>
<td>≥30</td>
<td>25-29</td>
<td>≤24</td>
</tr>
</tbody>
</table>
Table 2. The mean scores of male nursing students on sub-dimensions of Emotional Intelligence Scale (N=87)

<table>
<thead>
<tr>
<th>Sub-dimensions of Emotional Intelligence Scale</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>Comment</th>
<th>(Conclusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awareness of emotions</td>
<td>18</td>
<td>36</td>
<td>29.17</td>
<td>3.41</td>
<td>A little bit improvement is needed</td>
<td></td>
</tr>
<tr>
<td>Management of self emotions</td>
<td>11</td>
<td>36</td>
<td>26.60</td>
<td>4.87</td>
<td>Improvement is certainly needed</td>
<td></td>
</tr>
<tr>
<td>Self motivation</td>
<td>8</td>
<td>35</td>
<td>27.70</td>
<td>4.42</td>
<td>A little bit improvement is needed</td>
<td></td>
</tr>
<tr>
<td>Empathy</td>
<td>17</td>
<td>36</td>
<td>28.41</td>
<td>3.75</td>
<td>A little bit improvement is needed</td>
<td></td>
</tr>
<tr>
<td>Social skills (management of other’s emotions)</td>
<td>18</td>
<td>36</td>
<td>28.13</td>
<td>3.82</td>
<td>A little bit improvement is needed</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. The mean scores of male nursing students on sub-dimensions of leadership behavior description scale (N=87)

<table>
<thead>
<tr>
<th>Sub-dimensions of Leadership Behavior Description Scale</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possession</td>
<td>4</td>
<td>16</td>
<td>11.77</td>
<td>2.40</td>
</tr>
<tr>
<td>Stimulating the mass</td>
<td>4</td>
<td>16</td>
<td>10.86</td>
<td>2.20</td>
</tr>
<tr>
<td>Protecting membership</td>
<td>7</td>
<td>16</td>
<td>11.91</td>
<td>1.99</td>
</tr>
<tr>
<td>Representing the organization</td>
<td>5</td>
<td>15</td>
<td>10.90</td>
<td>2.27</td>
</tr>
<tr>
<td>Integrating purposes</td>
<td>5</td>
<td>16</td>
<td>10.88</td>
<td>1.92</td>
</tr>
<tr>
<td>Organizing</td>
<td>5</td>
<td>16</td>
<td>10.96</td>
<td>2.27</td>
</tr>
<tr>
<td>Top to bottom communication</td>
<td>6</td>
<td>15</td>
<td>10.78</td>
<td>1.84</td>
</tr>
<tr>
<td>Bottom to top communication</td>
<td>6</td>
<td>16</td>
<td>12.25</td>
<td>2.11</td>
</tr>
<tr>
<td>Recognition</td>
<td>5</td>
<td>15</td>
<td>11.19</td>
<td>2.23</td>
</tr>
<tr>
<td>Production</td>
<td>3</td>
<td>16</td>
<td>11.36</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Table 4. Relations between the sub-dimensions of Emotional Intelligence Scale and the sub-dimensions of Leadership Behaviors Description Scale of male nursing students

<table>
<thead>
<tr>
<th></th>
<th>Awareness of emotions</th>
<th>Management of self emotions</th>
<th>Self motivation</th>
<th>Empathy</th>
<th>Social skills</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r p</td>
<td>r p</td>
<td>r p</td>
<td>r p</td>
<td>r p</td>
</tr>
<tr>
<td>Possession</td>
<td>.39 0.000*</td>
<td>.31 0.003**</td>
<td>.35 0.001**</td>
<td>.29 0.006**</td>
<td>.41 0.000*</td>
</tr>
<tr>
<td>Stimulating the mass</td>
<td>.40 0.000*</td>
<td>.42 0.000*</td>
<td>.42 0.000*</td>
<td>.44 0.000*</td>
<td>.35 0.001**</td>
</tr>
<tr>
<td>Protecting membership</td>
<td>.44 0.000*</td>
<td>.44 0.000*</td>
<td>.33 0.002**</td>
<td>.44 0.000*</td>
<td>.45 0.000*</td>
</tr>
<tr>
<td>Representing the organization</td>
<td>.34 0.001**</td>
<td>.32 0.002**</td>
<td>.37 0.000*</td>
<td>.27 0.009**</td>
<td>.36 0.000*</td>
</tr>
<tr>
<td>Integrating purposes</td>
<td>.32 0.003**</td>
<td>.34 0.001**</td>
<td>.31 0.003**</td>
<td>.30 0.005**</td>
<td>.38 0.000*</td>
</tr>
<tr>
<td>Organizing</td>
<td>.37 0.000*</td>
<td>.39 0.000*</td>
<td>.28 0.007**</td>
<td>.42 0.000*</td>
<td>.38 0.000*</td>
</tr>
<tr>
<td>Top to bottom communication</td>
<td>.30 0.004**</td>
<td>.28 0.007**</td>
<td>.35 0.001**</td>
<td>.24 0.024**</td>
<td>.38 0.000*</td>
</tr>
<tr>
<td>Bottom to top communication</td>
<td>.43 0.000*</td>
<td>.44 0.000*</td>
<td>.47 0.000*</td>
<td>.47 0.000*</td>
<td>.41 0.000*</td>
</tr>
<tr>
<td>Recognition</td>
<td>.44 0.000*</td>
<td>.44 0.000*</td>
<td>.51 0.000*</td>
<td>.39 0.000*</td>
<td>.47 0.000*</td>
</tr>
<tr>
<td>Production</td>
<td>.37 0.000*</td>
<td>.39 0.000*</td>
<td>.38 0.000*</td>
<td>.29 0.005**</td>
<td>.54 0.000*</td>
</tr>
</tbody>
</table>

* p < 0.001    ** p < 0.05

Investigation of the mean scores of male nursing students on sub-dimensions of emotional intelligence scale (Table 2) demonstrated that the mean score of students was 29.17 ±3.41 in awareness of emotions, 26.60± 4.87 in managing self emotions, 27.70± 4.42 in self-motivation, 28.41± 3.75 in empathy, and 28.13±3.82 in social skills (administrating emotions of others). Emotional intelligence levels of students were found moderate in all sub-dimensions and their emotional intelligence should be improved.

Considering students’ sub-dimension scores of the leadership behavior description scale (Table 2)
3), the mean score was found 11.77±2.40 in possession sub-dimension, 10.86 ±2.20 in stimulating the mass, 11.91 ± 1.99 in protecting membership, 10.90 ± 2.27 in integrating purposes, 10.88 ± 1.92 in organizing, 10.78 ± 1.84 in top to bottom communication, 11.19 ± 2.23 in recognizing and 11.36 ± 2.35 in production sub-dimension. These results demonstrated that leadership behaviors of students were above the moderate level and positive.

The examination of the relationship between emotional intelligence scale sub-dimensions and leadership behavior description scale sub-dimensions indicates that (Table 4):

- there was a positive, weak, but significant relation between awareness of emotions, managing self emotions sub-dimensions and possession, stimulating the mass, protecting membership, representing the organization, integrating the purposes, top to bottom communication, recognition and production sub-dimensions (0.26 < r < 0.49, p < 0.05),
- there was a positive, weak but significant relation between self motivation, possession, stimulating the mass, protecting the membership, representing the organization, integrating the purposes, organizing, top to bottom communication and production sub-dimensions (0.26 < r < 0.49, p < 0.05), while there was a positive, moderate and significant relation with recognition sub-dimension (r=.51 , p < 0.001),
- there was a positive, weak but significant relation between empathy and bottom to top communication sub dimensions ( r=.24 , p=0.024), while there was a positive, weak but significant relation between possession, stimulating the mass, protecting membership, representing the organization, integrating purposes, organizing, bottom to top communication, recognition, and production sub-dimension (0.26 < r < 0.49, p < 0.05),
- there was a positive, weak but significant relation between social skills (administrating other’s emotions) and possession, stimulating the mass, protecting the membership, representing the organization, integrating purposes, organizing, bottom to top communication, and recognizing sub-dimensions (0.26 < r < 0.49, p < 0.05), while there was a positive, moderate and significant relation with production sub-dimension ( r=.54 , p < 0.001).

Discussion

Emotional intelligence and leadership are among the most important factors effective on behaviors, and they have been the objects of interest in health field and many studies have been implemented to determine the emotional intelligence and leadership behaviors of students in different fields of health. Studies on nursing students yielded results on female students. This study is especially important because this is the first study in both international and national fields concerning the emotional intelligence levels and leadership behaviors of especially the male nursing students.

Study results demonstrated that male nursing students had moderate level of emotional intelligence, and needed improvement. Although students were aware of their emotions, they were not adequate to control their emotions and motivate themselves, but they could manage other’s emotions better than their emotions. This situation indicates that students were aware of their own emotions but they could not manage their emotions, but they could better manage other’s emotions.

The finding that students had moderate level of emotional intelligence is compatible with literature. Emotional intelligence increases with age (18), but the students were in the beginning of twenties; therefore, their emotional intelligence levels were not sufficiently developed.

Similar to the findings of studies made on female nursing students in Turkey, students were determined to have moderate level of emotional intelligence and they needed to improve their emotional intelligence levels (26,43,44,45), and students were aware of their emotions and less successful in managing their emotions (26, 44,45). In another study made on female students in the same school, female students obtained their maximum scores in “ability to control self emotions” and “self motivation” sub-dimensions, while they obtained their minimum scores in “awareness of self emotions” and “empathy” sub-dimensions (43), which was different from the findings of the
present study. This situation suggests that sex factor affects on sub-dimensions of emotional intelligence and male and female students differ in sub-dimensions of emotional intelligence.

Different results were reported by studies in literature investigating the relationship between emotional intelligence and sex of different students. Some studies determined the general level of emotional intelligence higher in female students than male students (8,26), while some other studies reported that male student had higher emotional intelligence than female students (46,47). On the other hand, some other studies reported no significant difference between male and female students (6,9). In a study carried out on undergraduate and postgraduate students in different departments, it was determined that female students had higher scores in sub-dimensions of understanding their own emotions and other’s emotions and controlling their own emotions compared to male students.

Social roles and expectations in Turkish society could be said to be effective on good leadership characteristics of male nursing students. Social expectations from individuals are also expected in working life (31,48). Traditional role theory suggests that females and males are affected by their social roles while they perform leadership behaviors (48). In traditional Turkish society, administration and leadership roles are generally ascribed to men and male children are raised to have leadership properties like active, strong, decisive etc. (48). Due to this raising style, men assume these roles, and therefore, they demonstrate certain behaviors like possession, representing, independence, enforcing obedience, acquiring recognition, entrepreneurial spirit, and self-reliance and their leadership behaviors are better. In studies investigating the leadership behaviors of female students, they were found to have moderate level of leadership tendency (22) and they needed to improve their leadership skills (49), which supported the previous opinion.

According to the study findings, emotional intelligence level of male nursing students positively affects the leadership behaviors. These findings demonstrate that students will have better leadership behaviors if they improve their emotional intelligence levels. Male nursing students who are aware of other’s emotions, could emphasize, control both their and other’s emotions are expected to be better leaders.

Similarly, studies in literature determined positive and significant relations between emotional intelligence and leadership (2, 17) and emotional intelligence positively affects the leadership behaviors especially towards people (1) and transforming leadership behaviors (2). In the study of Duygulu et al. (22) investigating the relation between emotional intelligence and leadership behaviors, a significant relation was determined between job-oriented leadership behaviors and emotional intelligence.

**Conclusion**

Study results demonstrate that male nursing students have moderate level of emotional intelligence and need to improve them, but they show better leadership behaviors and their emotional intelligence levels positively affect the leadership behaviors. Accordingly, training curriculum of nursing programs should be arranged to promote emotional intelligence of students, and leadership behaviors.

**Study limitations and implications for future research**

This is the first study on male nursing students and implemented only on male students of one faculty of medical sciences. For this reason, future studies should be made on larger sample groups, which will provide more detailed information.

In addition, findings especially related to leadership are based on self evaluations of male students; therefore, it is suggested to evaluate these characteristics of students from different viewpoints.

**Acknowledgement**

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References


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Abstract

Purpose: In order to prevent resorption and collapse of the inner walls of the alveolar bone after the removal of cystic processes and to accelerate regeneration, rehabilitation, and creation of new bone tissue at the site of the enucleated process, biphasic calcium phosphate/poly-DL-lactide-co-glycolide composite was utilized.

Material and methods. The granules of calcium phosphate/DLPLG sterilized with x-rays and mixed with physiologic solution were implanted into the alveolar bone defects created by cystectomy. Enucleation of 60 cystic alveolar bone defects was performed in 60 patients aged 42-66 years. After enucleation of cystic defects of alveolar bone, in 30 patients BCP/PLGA composite was implanted into the defects, while in the remaining 30 cases, alveolar bone defects after cystectomy were left to heal spontaneously, without any implantation (biocomposite vs control group). Radiological methods were used to assess regeneration and reparation of the restored defects, while clinical assessment of healing was done based on the condition of the regenerated tissue.

Results. The results of clinical assessment indicated good flap closure and well formed regenerated tissue without central soft tissue depression after the removal of cystic processes and BCP/PLGA implantation into the osseous defects. While the results of radiological assessment indicated the presence of complete trabecular composition and increased alveolar bone density after BCP/PLGA implantation compared to poor trabecular composition and lower alveolar bone density in controls.

Conclusion. The study indicated that the above composite accelerated regeneration and rehabilitation of the alveolar bone, and creation of new bone tissue at the site of enucleated cystic process, and, thanks to its osteocunductive effect, can completely replace and renew lost alveolar bone. Synthetic BCP/PLGA composite belongs to the group of biomaterials able to facilitate and accelerate healing of the soft tissue without central depression.

Key words: BCP/PLGA composite, bony restoration, alveolar bone defects

Introduction

Alveolar bone can be viewed as the collection of all its anatomical, structural, and functional properties – the necessary elements for its proper functioning. The removal of any cystic and periodontal processes involves not only the disturbance of functional properties of the jaw segment, but it...
also initiates the mechanism of reorganization of all the supporting structures (connective elements and alveolar bone) (1, 2). After the removal of the above pathologic processes, bone tissue regeneration mechanisms are activated, starting from the bottom of the alveolar defect, continuing to the top of the alveolar ridge. The presence of connective tissue in the portion closest to the bone defect can introduce a competition in regeneration dynamics, which facilitates the withdrawal of connective tissue towards the inner portion of the defect. The phenomenon is more pronounced in the presence of cystic processes. Several studies have confirmed bone resorption of 4.0-4.5 mm after tooth extraction and removal of cystic and periapical processes, which presents a huge problem for implant placement, especially in the frontal region where alveolar bone morphology and size are very limited (3). Numerous materials and techniques have been used to prevent resorption and collapse of the inner alveolar bone walls. Autologous bone has been considered as an ideal material due to its osteogenetic ability and minimal immune reaction. Before the process of resorption and bone implant revascularization, osteoprogenitor cells differentiate into osteogenetic ones (4). Heterogenous is the second utilized bone implant type. It can be found in different forms as a cortical bone, demineralized, or heterogenous non-demineralized bone. Limitations related to the use of heterogenous bone lie in the potential of transferring viral infections (although the material is sterile), as well as in a difficult implant resorption, so that they can serve as membranes for osteoconductivity (5). Various experimental and clinical experiences have confirmed the potential of calcium sulphate as a bone implant which is non-toxic, completely resorbable, biocompatible, non-infectious (6, 7). Recently, tricalcium phosphate has been increasingly used to restore bone tissue defects after the removal of osseous pathologic processes (8, 9). Polymers have an important place in the resolution of problems of alveolar bone resorption (10). In some clinical studies replacement of alveolar bone during distraction osteogenesis has been observed using morphofunctional analyses (11). In advanced alveolar bone resorption within systemic osteoporosis, excellent results have been achieved using biostimulative biocomposites (12). The most important property of biphasic calcium phosphate is its chemical similarity to mineralized phase of the bone. Polymers are very important substrates for distribution of bioactive molecules. Synthetic BCP/PLGA composite belongs to the group of biomaterials which facilitate new bone formation (12, 13), and, thanks to its osteoconductive effect, is able to successfully replace bone tissue (13). Since BCP/PLGA has been shown to be an excellent osteoconductive biomaterial in the processes of osteoreconstruction, the composite has been evaluated in clinical studies.

The aim of these researches was radiological and clinical assessment of the outcomes of treatment for alveolar defects created after enucleation of cystic processes resored with BCP/PLGA composite. We assessed the impact of use of BCP/PLGA composite in the healing of soft tissues and replacement of lost bone tissue after the removal of cystic alveolar bone processes in our patients. The success of intervention was assessed analyzing the clinical and radiological criteria. Clinical criteria involved the observation of presence or absence of central depression and soft tissue in the defect center, as well as the presence of complete flap closure with formed strengthened tissue. Radiological assessment involved the analysis of orthopantomograms, spotting the presence or absence of radiotransparency in the treated region, as well as the measurement of density of bone structures with multidetector CT and creation of the new bone.

Material and methods

The clinical study of use of biocomposite materials in dentistry patients was approved by the Ethics Committee of the Dentistry Clinic in Niš on 21.10.2005.

A calcium phosphate gel was produced by the precipitation of calcium nitrate and ammonium phosphate in an alkaline medium (14, 15). Gels or granules of calcium phosphate were added into completely dissolved polymer. After solvent evaporation, the particles were dried at the room temperature for 24 hours (12, 13). Addition of non-dissolve (methanol) into the 3-component system of solvent-polymer-calciumphosphate ca-
uses its thermodynamic destabilisation. This induces sedimentation of polymer onto the calcium phosphate granules and their covering by polymer. The granules of calcium phosphate/DLPLG composite biomaterial of sizes 150-250 μm, were sterilized by x-rays (25kGy) before use.

The study enrolled patients aged 42 to 66 years. Enucleation of 60 cystic defects of the alveolar bone was done in 60 cases. In 30 cases, BCP/PLGA composite was implanted into the defect resulting from the enucleation of cystic defects (experimental group – I). In the other 30 patients the defects were left to heal spontaneously without biocomposite implants (control group – II). Out of 30 cystic defects in group I, 15 were situated in the upper jaw and 15 in the lower jaw alveolar bone. In controls, 15 defects were similarly situated in the upper jaw and 15 in the lower jaw alveolar bone. Diagnosis and classification of cysts was done based on orthopantomograms taken on the Rotograph Plus machine (Villa Sistemi Medici, MR 05, CEI-Bologna, Italy). Over each of the tomograms, a transparent thermostable foil for graphoscope was placed, with a printed 2 x 2 mm grid. Cyst borders were marked on the foil, and then measured.

Bone structure densities (measured in HU) after cystectomy were obtained by native scanning of the mandibles, with density measurement with multidetector CT machine Aquilion 64 CFX Toshiba. Scanning was done at 120 kV and 300 mA scan-gantry-rotation time 0.5, and helical pitch 53, with slide thickness of 3 mm. The analysis of scans was done in MPR reconstructions on a 512 x 512 matrix.

After clinical, radiological examination, diagnosis, and preoperative preparation of patients for local anesthesia (2% cystocain, ICN Galenika, Belgrade), surgical cyst enucleation was performed. The access incision remained on the bone support and not on the implant, at a distance from the bone defect. A high fornical incision through the movable mucosa was most commonly employed. After detection, the cystic saccus was enucleated in its entirety. Hemostasis was effectuated as usual: diathermy, striking the bone with the blunt part of the chisel. Sharp bone edges were flattened. Smooth walls of the cystic defect were perforated in several spots with a sterile drill.

BCP/PLGA composite granules were mixed with physiologic solution and introduced with an appropriate instrument, and then compressed to the defect walls to fill up the defect completely (without „dead spots“). After the implant introduction, the flap was returned and the wound closed using individual dense sutures.

Cystic processes sized on the average 5-10 cm² were monitored. Depending on the sites of cystic processes, three regions were analyzed: frontal (F), premolar region (P), and molar (M) region. Five positions were observed in the study (one in the defect center, two on the left and two on the right defect side) and analyzed based on clinical and radiological criteria (8, 16).

Clinical assessment criteria:
- Presence of central opening,
- Flap closure and soft tissue presence in the center,
- Flap closure with central depression and irregular edges,
- Complete soft tissue restoration, without any invagination, with total flap closure, and presence of well formed, augmented tissue.

Radiological assessment criteria:
- Radiotransparency in the treated zone,
- Absence of radiotransparency and restoration of the trabecular structure,
- Complete trabecular bone structure,
- Evaluation of density of bone structures.

Results

Figure 1a presents a cyst in the molar region of the alveolar mandibular bone in group (I). Figure 1b illustrates the defect after the removal of a cystic process of the alveolar mandibular bone in group (I) filled with BCP/PLGA composite. Figure 1c depicts intense osteogenesis of the molar region of the alveolar mandibular bone in group (I) 12 weeks after cyst enucleation and BCP/PLGA composite implantation – new trabecular bone is formed, very similar to the tissue of the untreated, healthy bone.
Figure 1a. A cyst in the molar mandibular region

Figure 1b. Cyst enucleation and BCP/PLGA implantation into the defect

Figure 1c. Presence of the trabecular structure 12 weeks after BCP/PLGA implantation

Figure 2a. A cyst in the frontal mandibular region

Figure 2b. Cyst enucleation without BCP/PLGA implantation

Figure 2c1. Spontaneous healing of the cystic defect with scarce trabecular structure (image with a measurement grid and measurement fields)

Figure 2c2. Spontaneous healing of the cystic defect with scarce trabecular structure (image without any measurement grid and without measurement fields)

Figure 3a presents control, native MSCT scan (axial section) of the defect resulting after the re-
moval of a cystic process of the molar region of mandibular alveolar bone in group (I), 6 weeks after cyst enucleation and BCP/PLGA composite implantation. Trabecular structure of the alveolar bone was present, with the focus of intense osteogenesis. Figure 3b depicts control, native MSCT scan of the mandible (axial section), with intense osteogenesis of the molar region of alveolar bone in group (I) 12 weeks after BCP/PLGA composite implantation, where new trabecular bone similar to the tissue of untreated, healthy bone was present, with partly more intense structure similar to the mandibular cortex. Figure 3c is a VR 3D reconstruction of the mandible 12 weeks after BCP/PLGA composite implantation, where the contour of the mandible did not reveal the presence of defect.

Clinical healing criteria 12 weeks after BCP/PLGA composite implantation are shown in Tables 1 and 2. The criteria from Table 1 indicated complete soft tissue restoration (4 sites in the frontal, 3 sites in premolar, and 3 sites in molar maxillary region), without invagination and with total flap closure and presence of well formed augmented tissue in 10 treated cystic defects of the maxilla (66.7%) out of 15 treated overall. Only in 5 treated cystic defects (33.3%) out of 15 treated (1 site in the frontal, 2 sites in premolar, and 2 sites in molar maxillary region) there was flap closure with central depression and irregular edges in group (I). In controls – group (II) – in 9 out of 15 treated cystic defects of the maxilla (60%) there was flap closure with soft tissue in the center (3 sites in the frontal, 3 in premolar, and 3 in molar region of the maxilla). In 6 cystic defects of the maxilla (40%) out of 15 treated (2 sites in the frontal, 2 sites in premolar, and 2 sites in molar maxillary region) there was central soft tissue depression with irregular edges during flap closure.

Clinical healing criteria in Table 2 indicated complete soft tissue restoration (3 sites in the frontal, 4 sites in premolar, and 4 sites in molar region) without invagination and with total flap closure and presence of well formed augmented tissue in 11 treated cystic defects of the mandible (73.4%) out of 15 treated in total. In only 4 treated cystic defects (26.6%) out of 15 (2 sites in the frontal, 1 site in premolar, and 1 site in molar mandibular region) there was flap closure with central depression and irregular edges in group (I). In controls – group (II)
In 9 out of 15 treated cystic defects (60.0%) of the mandible, there was flap closure with soft tissue presence in the center (3 sites in the frontal, 3 sites in premolar, and 3 sites in molar mandibular region). In 6 cystic defects of the mandible (40%) out of 15 treated in total (2 sites in the frontal, 2 sites in premolar, and 2 sites in molar mandibular region) there was central soft tissue depression with irregular edges during flap closure.

The results of clinical assessment indicated that BCP/PLGA composite 12 weeks after intervention led to more rapid consolidation and complete regeneration of soft tissues, effective wound healing, and total flap closure, with the presence of well formed, augmented tissue. Insufficient tissue tropism was almost nonexistent in the defects treated with BCP/PLGA composite in experimental group (I). The results of clinical assessment in controls (II), where the alveolar defects of the maxilla and mandible were left to heal spontaneously, indicated slower flap closure, with central depression and soft tissue in the center with irregular edges.

Tables 3 and 4 present radiological assessment criteria for alveolar defect healing after enucleation of cystic processes in experimental (I) and control group (II). Radiological assessment criteria

Table 1. Results of the clinical assessment criteria for the alveolar defects of the maxilla 12 weeks after cyst enucleation.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group (I)</th>
<th>Control group (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maxilla</td>
<td>Maxilla</td>
</tr>
<tr>
<td>Number of defects</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Frontal region (F)</td>
<td>4d 1c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Premolar region (P)</td>
<td>3d 2c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Molar region (M)</td>
<td>3d 2c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Clinical criteria in %</td>
<td>66.7 33.3</td>
<td>60.0 40.0</td>
</tr>
</tbody>
</table>

Note. a – Presence of central opening; b – Flap closure and presence of central soft tissue; c – Flap closure with central depression and irregular edges; d – Complete soft tissue restoration without invagination, with total flap closure and presence of well formed augmented tissue.

Table 2. Results of the clinical assessment criteria for the alveolar defects of the mandible 12 weeks after cyst enucleation.

<table>
<thead>
<tr>
<th></th>
<th>Experimental group (I)</th>
<th>Control group (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mandible</td>
<td>Mandible</td>
</tr>
<tr>
<td>Number of defects</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Frontal region (F)</td>
<td>3d 2c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Premolar region (P)</td>
<td>4d 1c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Molar region (M)</td>
<td>4d 1c</td>
<td>3b 2c</td>
</tr>
<tr>
<td>Clinical criteria in %</td>
<td>73.4 26.6</td>
<td>60.0 40.0</td>
</tr>
</tbody>
</table>

Note. a – Presence of central opening; b – Flap closure and presence of central soft tissue; c – Flap closure with central depression and irregular edges; d – Complete soft tissue restoration without invagination, with total flap closure and presence of well formed augmented tissue.

Table 3. Results of radiologic criteria for the assessment of healing of alveolar defects of the maxilla

<table>
<thead>
<tr>
<th></th>
<th>Experimental group (I)</th>
<th>Control group (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mandible</td>
<td>Mandible</td>
</tr>
<tr>
<td>Number of defects</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Frontal region (F)</td>
<td>4C 1B</td>
<td>3A 2B</td>
</tr>
<tr>
<td>Premolar region (P)</td>
<td>4C 1B</td>
<td>4A 1B</td>
</tr>
<tr>
<td>Molar region (M)</td>
<td>4C 1B</td>
<td>2A 3B</td>
</tr>
<tr>
<td>Radiologic criteria in %</td>
<td>80.0 20.0</td>
<td>60.0 40.0</td>
</tr>
</tbody>
</table>

Note. A – Presence of radiotransparency; B – Absence of radiotransparency and restoration of trabecular structure; C – Complete trabecular structure of the bone.
Radiological assessment, compared to clinical, showed that the sites after cystectomy and BCP/PLGA implantation showed optimal clinical results, as confirmed by the x-rays showing complete trabecular structure of the bone in 93.4% of group (I) patients, in contrast to the sites left untreated with BCP/PLGA composite – group (II) – where in some spots radiotransparency was still conspicuous, and in other spots the presence of bone trabeculae was scarce and poorly visible, with marked absence of radiotransparency. Twelve weeks after cyst enucleation and BCP/PLGA implantation, trabecular bone was more mature and present in higher percentage, indicating intense osteogenesis and creation of new bone tissue, very similar to the tissue of untreated, healthy bone.

Radiological assessment of density of bone tissue of defects in the maxilla and mandible 12 weeks after cyst enucleation is presented in Tables 5 and 6, indicating better bone density results for those treated with BCP/PLGA composite implantation for both maxilla and mandibula.

**Discussion**

After enucleation of cystic processes, bone resorption poses a huge problem (1), especially in the frontal region, where morphology and size of the alveolar bone are especially limited, and in the regions of premolars and molars, where the hi-
ghest burden to the alveolar bone is created during mastication. Therefore, cystic defects of the above regions were selected and analyzed. Restoration of normal trabecular bone structure 12 weeks after cystectomy and BCP/PLGA implantation is a significant result, indicating re-establishment of normal morphologic profile of the ridge in a relatively short period of time. Thus implanted, BCP/PLGA composite prevented bone resorption and invagination of the soft tissues into the defect, in addition to the replacement of bone tissue.

Positive results of this study are comparable with other positive results describing the efficacy of BCP/PLGA composite in the treatment of defects in an osteoporosis-weakened alveolar bone (12). The results justify the use of BCP/PLGA composite for successful regeneration of defects of the alveolar bone after cystectomy. Except in the regeneration of alveolar bone defects after cystectomy, some of the positive results have been stressed in other research papers, where BCP/PLGA has been used to replace alveolar bone damaged by osteoporosis, trauma, periapical processes, and after extraction of impacted teeth (12, 13).

After the implantation of a biomaterial into the alveolar defect, the first bone to be formed is trabecular bone, serving as a framework to support subsequent deposition of collagen and lamelar bone. The period of 6 weeks is too short for us to be able to spot these regeneration processes – the most optimal time is around 12 weeks (that was why we chose that period to monitor osteoregenerative processes) (17). In the treatment of enucleated cystic defects, some authors have used autologous platelet-rich gel concentrate combined with the granules of bovine derivative of hydroxyapatite xenograft, enabling bone regeneration at the cellular level and recuperation of soft tissues in the period of 4 months (16 weeks) (18), while the results of this study indicated that the period of 12 weeks was optimal for the healing of alveolar defects. From the clinical point of view, this period of time was

<table>
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<tr>
<th>Table 5. Results of radiologic criteria for the assessment of density of bone structure of maxillary defects 12 weeks after cyst enucleation</th>
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</thead>
<tbody>
<tr>
<td><strong>Experimental group (I)</strong></td>
</tr>
<tr>
<td>Maxilla</td>
</tr>
<tr>
<td>Number of defects</td>
</tr>
<tr>
<td>Frontal region (F)</td>
</tr>
<tr>
<td>Premolar region (P)</td>
</tr>
<tr>
<td>Molar region (M)</td>
</tr>
<tr>
<td>Density assessment in %</td>
</tr>
</tbody>
</table>

Note.

a – Density of frontal region of the maxilla increased by 10.57% in experimental group (I) compared to controls (II);
b – Density of premolar region of the maxilla increased by 20.11% in experimental group (I) compared to controls (II);
c – Density of molar region of the maxilla increased by 21.88% in experimental group (I) compared to controls (II);

<table>
<thead>
<tr>
<th>Table 6. Results of radiologic criteria for the assessment of density of bone structure of mandibular defects 12 weeks after cyst enucleation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental group (I)</strong></td>
</tr>
<tr>
<td>Maxilla</td>
</tr>
<tr>
<td>Number of defects</td>
</tr>
<tr>
<td>Frontal region (F)</td>
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<tr>
<td>Premolar region (P)</td>
</tr>
<tr>
<td>Molar region (M)</td>
</tr>
<tr>
<td>Density assessment in %</td>
</tr>
</tbody>
</table>

Note.

a – Density of frontal region of the mandible increased by 11.06% in experimental group (I) compared to controls (II);
b – Density of premolar region of the mandible increased by 28.67% in experimental group (I) compared to controls (II);
c – Density of molar region of the mandible increased by 28.97% in experimental group (I) compared to controls (II);
also sufficient to allow for soft tissue healing with the characteristics of an optimal trophism. The cited results corroborated other authors’ results, who, using hydroxyapatite gel and calcium sulphate in the treatment of bone defects in orthopedics, have confirmed biocompatibility of the composite and its capacity to occupy space, preventing defect filling-up with non-osteogenetic cells (19).

A significant contribution of BCP/PLGA composite is to induce the creation of new bone and improve healing of bone defects after the enucleation alveolar bone cysts, since it revascularize very quickly, and osteoprogenitor cells differentiate into osteogenetic ones more rapidly than with other biomaterials, reducing thus the number of additional interventions, providing for the creation of new trabecular bone similar to the natural bone tissue, and accelerating osteogenesis and preventing resorption.

BCP/PLGA composite implantation into the defects resulting from cystectomy reduces alveolar bone resorption and facilitates re-establishment of normal morphologic profile of the alveolar ridge, in contrast to other materials, which is very important in subsequent prothesesizing of the patient.

**Conclusion**

We may draw the conclusion from the presented clinical research that 12 weeks after implantation, BCP/PLGA composite produced complete soft tissue restoration, without invagination, in 21 treated cystic defects of the maxilla and mandible, out of 30 treated patients in total. This showed that BCP/PLGA composite induced complete wound closure, with well formed augmented tissue. Radiological studies after cystectomy and implantation of BCP/PLGA composite confirmed optimal clinical results, pointing out a visible presence of complete trabecular bone structure 12 weeks after implantation. Clinical and radiological results of the study demonstrated the capacity of BCP/PLGA composite implanted into the defects to support restitution ad integrum of both the soft tissue and alveolar bone after cystectomy.

The study demonstrated that this composite accelerates the rehabilitation of alveolar bone and creation of new bone tissue at the site of enucleated cystic process, and that its osteoconductive effects enable the composite to replace and renew completely the lost alveolar bone.

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**References**


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