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A NEW SENSOR FOR HYDROGEN PEROXIDE BASED ON CARBON PASTE ELECTRODES MODIFIED WITH COPPER MICROPARTICLES

SENSORI I RI PËR PEROKSID HIDROGJENI BAZUAR NË ELEKTRODAT PASTË KARBONI TË MODIFIKUARA ME MIKROGRIMCA BAKRI

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ABSTRACT
Copper microparticles (CuMP) were used as modifier of the carbon paste electrode for the detection of hydrogen peroxide. Due to biological importance of hydrogen peroxide, the objective of this work was investigating on detection of hydrogen peroxide in lower operating potential using CuMP as modifier compared to unmodified carbon paste electrodes. Cyclic voltammetry and hydrodynamic amperometry were used on the study of electrochemical reaction mechanisms of hydrogen peroxide, at modified sensor in phosphate buffer solution 0.1 M (pH 7.5). Further studies of its electrocatalytic activity for the amperometric detection of hydrogen peroxide were made in flow injection mode. The carrier solution in the FIA system was phosphate buffer 0.1 M (pH 7.5) and injection volume 200 µL. Optimal operation potential of sensor was -100 mV (v.s. Ag/AgCl) and a flow rate of a carrier 0.40 mL/min. Operating with the preferential working conditions, a quasi-linear relation between concentration and signal could be obtained for hydrogen peroxide concentrations up to 100 mg/L with a detection limit (3σ) of 1.8 mg/L. The sensor was tested as a response to the following compounds, uric acid, paracetamol and dopamine, also mixed in the same solution with hydrogen peroxide. The effect of investigated interferences was not fatal and could be eliminated by the use of the standard addition method. The new sensor was successfully applied to the determination of hydrogen peroxide in pharmaceutical products.

Key words: breed, crossbreeding, character, reproduction, acclimatization.
Fjalët çelës: raca, kryqezim, tipare, riprodhim, aklimatizim

1.INTRODUCTION

Hydrogen peroxide importance is not only in biochemical reactions as intermediate product but also in chemical processes such as disinfection. The development of electrochemical sensor for its determination in biological fluids is significant for the diagnosis and management of metabolic diseases and also environmental applications [1]. Hydrogen peroxide can be detected with chemically modified carbon paste electrodes in lower potential and are applicable for *in vivo* measurements, due to their bio-compatibility [2]. Furthermore carbon paste electrode has a lot of advantages including simple preparation and modification, also is easy to handle. Wide range potential applications makes them very useful in electrochemical analysis for different analytes [3].

Different modifiers were investigated on their ability to decrease over-potentials of hydrogen peroxide on carbon paste electrodes, such as iron and platinum metal, other transition metal oxides, or complexes or organic compounds [4]. Metal oxides such as MnO_2 [5], CuO [6], Fe_2O_3 [7], RhO_2 [8], RuO_2 [9], ReO_2 [10], SnO_2 [11], which were found useful for this purpose.

Our research in this study is focused on the possibilities of carbon-based heterogeneous electrodes modified with copper microparticles for the detection of

hydrogen peroxide. Cyclic voltammetry and hydrodynamic amperometry were used on study of electrochemical reaction mechanisms of hydrogen peroxide at modified sensor in phosphate buffer solution 0.1 M (pH 7.5). In flow injection mode (FIA) were estimated operating parameters of developed sensor and its application on determination of hydrogen peroxide in pharmaceutical products.

2. EXPERIMENTAL

2.1. Chemicals, Reagents and Solutions

All chemicals used were analytical reagent grade. Copper microparticles from Fluka. Phosphate buffer solution (PBS) was prepared by mixing aqueous solutions of sodium dihydrogen phosphate (0.1 mol/L) and disodium hydrogen phosphate (0.1 mol/L), until the required pH was achieved. Hydrogen peroxide stock solution of 1.000 $g{\cdot}L^{-1}$ were prepared and diluted appropriately. Solutions of uric acid, dopamine and paracetamol, were freshly prepared before use.

2.2. Apparatus

For cyclic voltammetry and hydrodynamic amperometry, a potentiostat Autolab PSTAT 10 with software GPES version 4.9 were used. The electrochemical cell consisted of a carbon paste electrode as the working electrode, an Ag/AgCl reference electrode (Metrohm 6.0733.100), and a platinum wire as the counter electrode. Nitrogen was used for degassing the solutions. A magnetic stirrer provided convection of the solution. The flow injection system was assembled from a potentiostat as a detector, high performance liquid chromatographic pump (510 Waters, Milford MA, USA in connection with a system controller, Waters 600E), a sample injection valve (5020 Rheodyne, Cotati, CA, USA), and a thin layer electro-chemical detector (LC 4C, BAS, West Lafayette, Indiana, USA) with a flow through the cell (spacer thickness 0.19 mm; CC-5, BAS). The working electrode was a carbon paste electrode (unmodified and modified with Cu microparticles), the reference electrode an Ag/AgCl (3 M KCl) electrode, and the counter electrode was the steel back plate of the cell. All potentials mentioned in this paper are referred to the Ag/AgCl reference electrode.

2.2.1. Preparation of Working Electrodes

Unmodified carbon paste was prepared by mixing 1.000 g graphite powder and 360 µL paraffin oil (Uvasol®, 0.84 - 0.89 kg/L,) in an agate mortar by gently stirring with a pestle until uniformity and proper compactness was obtained. Modified carbon paste was prepared by mixing 0.950 g graphite powder with 0.050 g Cu microparticles and 360 µL paraffin oil. The carbon pastes were transferred to glass vials and were kept overnight in a refrigerator.

2.2.2. Procedures

Cyclic voltammograms were scanned between +900 mV and −900 mV with a different scan rate. Hydrodynamic amperometric measurements were made at an operating potential of −100 mV, if not mentioned otherwise; 250 mg/L H2O2 were added per step. Amperograms in flow injection analysis were recorded at potentials at −100 mV. The flow rate of the carrier (PBS 0.1) was 0.4 mL/min and the injection volume of the analyte was 200 µL.

2.3. Analyses of Samples

Freshly sampled medicinal hydrogen peroxide was diluted with PBS (0.1 M). The determination of the hydrogen peroxide concentration was done in FIA mode using the standard addition method to exclude effects of the matrix and interferences. The reference determinations were made with potassium permanganate solution according to European Pharmacopeia assay method [12]

3. Results and Discussion

Carbon paste electrodes, unmodified and modified with Cu microparticles were studied to characterize the electrochemical behavior of the modifier and to evaluate its action on the analyte, hydrogen peroxide.

3.1. Cyclic voltammetry

Figure 1 compares the cyclic voltammograms of a plain carbon paste electrode and after addition of H_2O_2. At positive and negative potentials practically no difference can be noticed.

The cyclic voltammogram of hydrogen peroxide with an unmodified electrode shows negligible response in applied potential window. At more negative potentials a broad reduction signal can be observed with a peak maximum around −1 V (not shown); it represents the conversion of H_2O_2 to water.

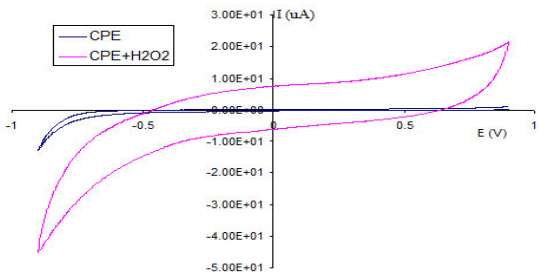

Figure 1. Cyclic voltammograms of a plain CPE (a), and after (b) the addition of 250 mg/L H_2O_2, scan rate 200 mV/s, $E_{init.}$ = -0.90 V, E_{final} = +0.90 V; phosphate buffer 0.1 M, pH 7.5.

The modified electrode shows a bit different picture (Figure 2), at positive potentials the oxidation of Cu can be noticed, probably to Cu (II) with a peak potential around 0 mV vs. Ag/AgCl. At negative potentials the reduction of Cu(II)/(I) can be noticed probably to Cu(I)/(0), with peak potential -0.30 V. At more negative potentials the reduction current of hydrogen peroxide increases and exceeds the reduction current of an unmodified electrode. The peak intensity is going to be increased with addition of hydrogen peroxide. Thus, it may be concluded that copper microparticles as a modifier has a significant effect on the analytical signal of the analyte, and is well suitable for its mediated detection. Cyclic voltammogram of modified electrode with scan rate 5 mV/s (Figure 3) shows two step reduction of copper (II). Peak (P1) shows oxidation peak of metallic copper, and first reduction peak around -0.20 V (P2) shows first step reduction of copper (II) to copper (I), and peak around -0.30 V (P3) reduction of copper (I) to copper(0).

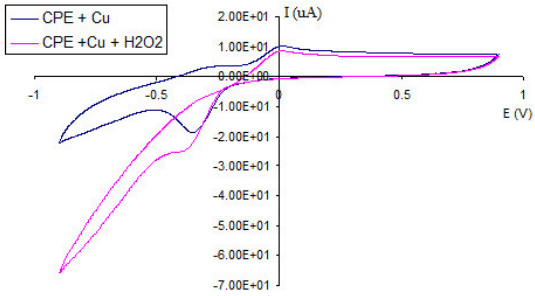

Figure 2. Cyclic voltammograms of modified CPE with Cu microparticles (a), and after (b) the addition of 250 mg/L H_2O_2, scan rate 200 mV/s, $E_{init.}$ = -0.90 V, E_{final} = +0.90 V; phosphate buffer 0.1 M, pH 7.5.

Based on the observations obtained from the cyclic vol tammograms a reaction mechanism is suggested which explains the electrocatalytic behavior of copper microparticles. Copper's oxidation potential is not high enough to further oxidize hydrogen peroxide to oxygen. Copper (II) is reduced already at slightly negative potentials, probably to copper (I) or (0). Metallic copper can be easily oxidized to copper (II) by H_2O_2. As reduction currents are measured the conversion of copper (II) to copper (I)/(0) is essential for the measurement. Thus, the action of the mediator on the reduction of hydrogen peroxide may be sketched as shown in Figure 4.

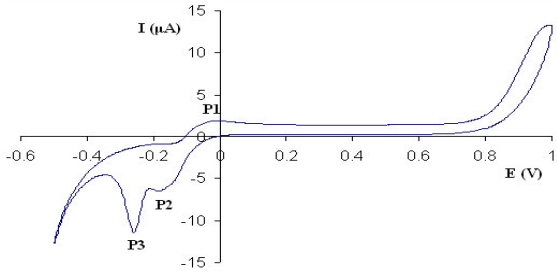

Figure 3. Cyclic voltammogram of modified CPE with Cu microparticles, scan rate 5 mV/s, $E_{init.}$ = -0.50 V, E_{final} = +1.00 V; phosphate buffer 0.1 M, pH 7.5.

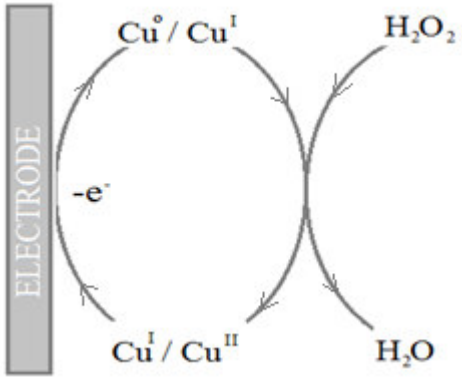

Figure 4. Suggested reaction mechanism of the electrocatalytic action of Cu microparticles on hydrogen peroxide.

Copper exists in high oxidation state because it is easily oxidized by oxygen to form copper (II) oxide. Primarily electrochemical reduction of Cu(II)/(I) → Cu(I)/(0) occurs, which in turn is oxidized chemically to copper (II) by hydrogen peroxide. Thus, the analyte is reduced in a mediated way generating the oxidized mediator, which is present at the surface of the electrode virtually in a higher concentration than in the absence of H_2O_2. Reduction currents at potentials more negative than −0.3 V reflect the

concentration of hydrogen peroxide already in the medium mg/L range.

3.2. Hydrodynamic amperometry

3.2.1. Operating potential

In hydrodynamic amperometry measurements, with a negative operating potential, 50 mg/L of hydrogen peroxide produce clearly distinct current steps. The electrode response time is rather quick; usually after two seconds the full signal has evolved including dispersion of the analyte in the measurement solution. The current steps decrease with increasing concentration of hydrogen peroxide, together with a very slightly increasing baseline.

In **Figure 5,** the hydrodynamic voltamperogram for different potentials are shown. Lowering the potential causes, an increase of the reduction current, but at the same time also the background current (current without hydrogen peroxide) increases. For practical application, more negative potentials also increase the possibility for co-education of other components in more complex samples.

Operation potential of −100 mV was chosen for further detailed studies. Compared to amperometrik signal of unmodified electrode, this potential provides still sufficient amperometric signal. The background current is reasonably small in combination with a good repeatability.

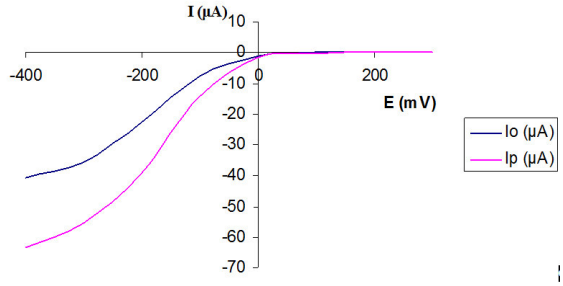

Figure 5. Hydrodynamic voltamperogram of hydrogen peroxide at a carbon paste electrode modified with Cu microparticles; H_2O_2 250 mg/L; (a)—background current, (b)—signal of the first step in the hydrodynamic amperogram.

3.2.2. Calibration curve and statistics

As we mentioned before, about significance of electrode operation potential, also linearity range is very important for its practical application. To find best operating potential for electrode on hydrogen peroxide detection, results are summarized in Figure 6 for different operating potentials in hydrodynamic studies. In order to give an idea about the size of the background current, the latter was not subtracted from the signal but is represented as the intercept of the graph with the current axis at c = 0 mg/L hydrogen peroxide.

At potentials of −100 mV and below there is a quasi- linear relation between current and concentration of H_2O_2 up to approximately 200 mg/L; at less negative potentials the electrode response is to low as can be seen from slope of calibration curve. Because −100 mV was chosen as a suitable operation potential, a practically linear relation between current and concentration at this potential exists up to a concentration of about 100 mg/L H_2O_2 with sensitivity of calibration curve 0.026 µA·L·mg^{-1} equation (1) and the correlation factor is R^2 = 0.9945

$$I\ (\mu A) = 0.026 \cdot C\ (mg\ L^{-1}) + 1.1862 \quad (1)$$

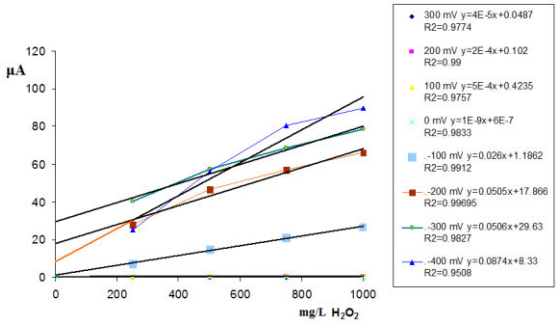

Figure 6. Hydrodynamic amperometric calibration curves for hydrogen peroxide of a CPE modified with Cu microparticles at various potentials 300, 200, 100, 0, -100, -200, -300 and -400 mV vs. Ag/AgCl

3.3.Studies with Carbon Paste Electrodes Modified with Cu microparticles using Flow Injection Analysis

Flow injection analysis (FIA) was used for further studies of modified carbon paste electrode. FIA allows simple operation and has a lot of advantages such as measurements repeatability, number of analysis in a comparably short time

etc. For the work presented here FIA was employed in one of its simplest forms. A pump was connected to an injection block for sample introduction and to a detector containing the electrochemical sensor (carbon paste electrode). The current flow was monitored in dependence on time.

Figure 7 shows a typical amperogram as obtained by flow injection mode. After injection the carries stream transport the analyte solution to the detector where it produces a current response. The signal is peak-shaped and of typical transient nature. When the sample plug reaches the detector it causes a sharp increase of the response. As the tailing part of the sample plug is more subject to dispersion in the carrier, the typical peak shapes from flow injection exhibit tailing with a slower relaxation of the signal to the background current value compared to the increase at the front end of the peak. Thus typically asymmetric peaks are obtained as a result. In fact the overall shape is dependent on the geometry of the practical setup and on some experimental parameters, such as injection volume and flow rate. For quantitative evaluation both, peak height and peak area may be exploited. Nevertheless for the sake of simplicity peak heights are evaluated in the current study.

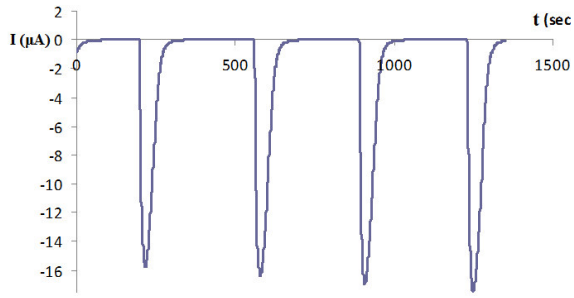

Figure 7. Amperogram obtained by FIA with a Cu-modifed CPE; operating potential −100 mV vs. Ag/AgCl, flow rate 0.4 mL/min, injection volume 200 µL, H_2O_2 concentration 100 mg/L

3.3.1. Operating potential

The dependence of the signal height in FIA on the operating potential is shows some difference in lower negative potentials (Figure 8) compared to hydrodynamic mode (Figure 5). Also the background current behaves different from hydrodynamic amperometry, because in FIA mode it is going to increase but not in the same tendency. The more negative the potential, the higher the signal and the background. For the same reasons as explained with hydrodynamic amperometry, an operating potential of −100 mV seems a favorable compromise still having a good signal-to-background ratio in combination with good repeatability.

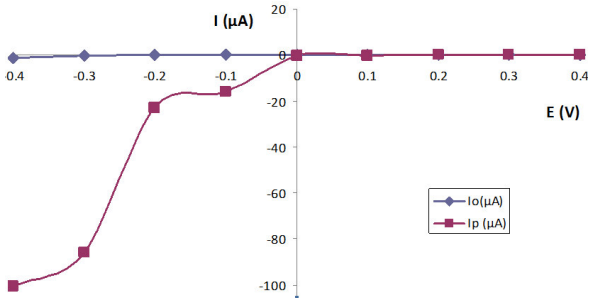

Figure 8. Dependence of the current in FIA mode on the operating potential; (a)—background current (b)—peak current; carrier phosphate buffer 0.1 M pH 7.5, hydrogen peroxide concentration 100 mg/L, flow rate 0.4 mL/s, injection volume 200 µL.

3.3.2. Flow rate

The peak shape, in particular the peak width and the peak height, are dependent on the flow rate of the carrier solution in FIA mode. The peak width is indirectly proportional to the flow rate, because with small velocities of the carrier the analyte resides longer over the electrode surface. The peak height of the current depends mainly on two effects: diffusion of the analyte to the reaction center and kinetics of the chemical reaction. The signal height didn't change in wide range of plow rate carrier. The choice of proper flow rate was guided by time for one injection.

From this point of view 0.1 mL/min or even slower would be the value of choice. At this rate the peaks are very broad and one injection lasts for about 8.5 min. A flow of 0.4 mL/min increases the speed of analysis considerably by more than the double, 3 min.

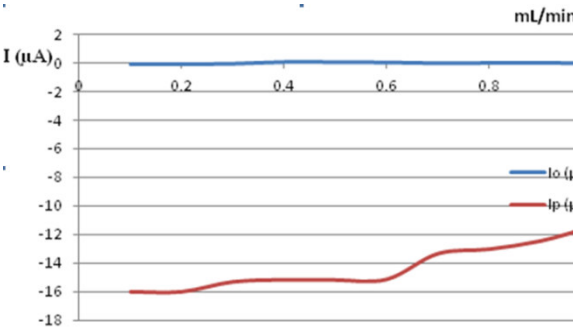

Figure 9. Dependence of the current in FIA mode on the flow rate operating potential -100 mV; (a)—background current (b)—peak current; carrier phosphate buffer 0.1 M pH 7.5, hydrogen peroxide concentration 100 mg/L, flow rate 0.4 mL/s, injection volume 200 µL.

3.3.3. Calibration curves and statistics

Calibration curves for the sensor at different operating potentials (−100 mV, −150 mV, −200 mV vs. Ag/AgCl) were made with hydrogen peroxide in the concentration range from 10 mg/L to 1000 mg/L. A quasi-linear relation between concentration and signal could be obtained for hydrogen peroxide concentrations up to 100 mg/L with a sensitivity 0.1222 µA $L \cdot mg^{-1}$ and a correlation factor R^2 = 0.9932 with operating potential -100 mV(Figure 9).

$$I(\mu A)=0.1222\ C(ppm)+0.1407 \quad (2)$$

The detection limit (3σ) estimated from the standard deviation of FIA-peaks for 10 mg/L hydrogen peroxide concentration is 1.8 mg/L. The relative standard deviation for the repeatability of measurements for 100 mg/L H_2O_2 was 2.0% (n = 10 measurements), and the corresponding reproducibility was 30% (n = 5 sensors).

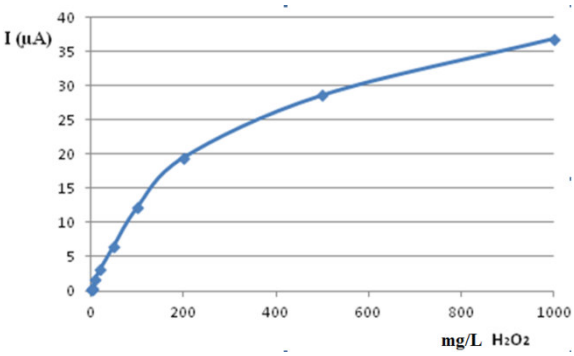

Figure 10. Calibration curve for hydrogen peroxide of a CPE modified with Cu microparticles at potential -100 mV vs. Ag/AgCl.

3.3.4. Interferences

The sensor was tested for its response to some recently possible interfering substances which might possibly occur in sample matrix. Tested compounds were uric acid, dopamine and paracetamol which may be present in the biological fluids.

Measurements were performed in two ways, signals from the substances alone (Figure 11a) and influence of interferents on the signal of hydrogen peroxide in the same solution (Figure 11 b).

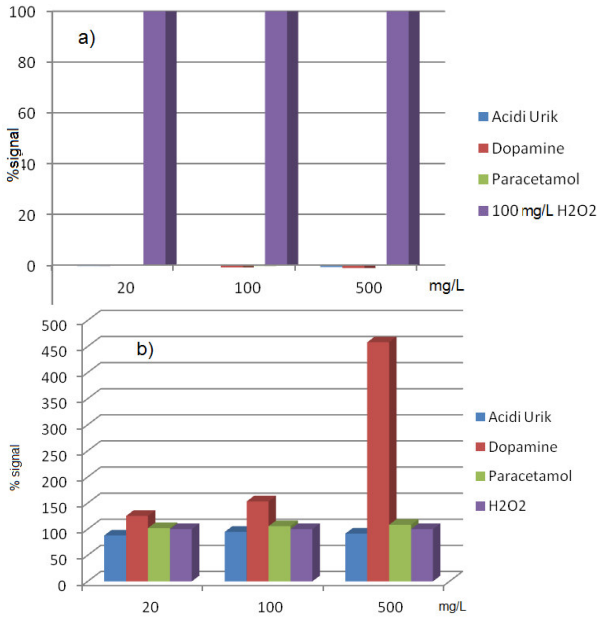

Figure 11. Interference of uric acid, paracetamol, and dopamine on the determination of hydrogen peroxide; (a) separate solution method with interferent signal expressed as relative signal of 100 mg/L H_2O_2; (b) mixed solution method showing the relative change of the signal of 100 mg/L H_2O_2 ; carrier phosphate buffer 0.1 M (pH 7.5), flow rate 0.4 mL/min, injection vol ume 200 µL, operation potential −100 mV.

Measured interferences such as uric acid and paracetamol did not cause a significant signal decrease or increase of hydrogen peroxide in all

tested concentrations, when they are in same solution. Sensor reponse to hydrogen peroxide increase significantly in presence of 500 mg/L dopamine, when they are mixed in the same solution with hydrogen peroxide. It is possible to occur reaction between analyte and measured interfernet dopamine.

3.3.5. Samples

Finally the sensor was tested for measuring hydrogen peroxide pharmaceutical preparations. 1.00 mL of the sample solution was diluted to 10 mL with phosphate buffer (0.1 M, pH 7.5) and was stored in the refrigerator. The determination of the hydrogen peroxide level was done with FIA mode using the standard addition method. The results for a hydrogen peroxide sample are presented in Table 1 compared to reference method by European Pharmacoepea. Two samples were measured, and the results between the method employing the new sensor and the references are in very good agreement.

Table 1. Determination of hydrogen peroxide in pharmaceutical preparations

Sample	CPE-Cu M	Reference method
S1 (3%)	2.23±0.07%	2.23±0.05%
S2 (3%)	1.74±0.08%	1.68±0.06%

4. Conclusions

The work presented here has clearly demonstrated that heterogeneous carbon sensors (carbon paste electrodes) with copper microparticles as a mediator exhibit improve the performance for the determination of hydrogen peroxide compared to unmodified electrodes because the modifier lowers the over-potential for the electrochemical reduction of the analyte. The suggested reaction mechanism assumes the reduction of copper (II) to copper (I)/(0), which in turn is oxidized by the hydrogen peroxide.

The modified electrodes have a long life time, good stability and high sensitivity which can be exploited for the determination of hydrogen peroxide up to 100 mg/L. The sensor may be used for two weeks or even longer with continuous operation.

The influence if possible intereferents (uric acid, paracetamol, and dopamine) on the determination of hydrogen peroxide has been estimated. The extent of all investigated interferences is not fatal and can be eliminated by the use of the standard addition method. The new sensor has been successfully applied to the determination of hydrogen peroxide in pharmaceutical preparations. The sensor seems also to promise to detect hydrogen peroxide as intermediate product of enzymatic reaction and to applicable as electrode material for new biosensor.

5. References

1. S. A. Kumar, P. H. Lo, S. M. Chen, Electrochemical analysis of H_2O_2 and Nitrite using copper nanoparticles/poly(o-phenylenediamine) film modified glassy carbon electrode. Journal of the Electrochemical Society 156 (7), (2009) pp. E118-E123

2. Ivan Svancara, Kurt Kalcher, Alain Walcarius, Karel Vytras. Electroanalysis with Carbon Paste Electrodes, CRC Press, USA 2012, pp.163

3. N. W. Beyene, P. Kotzian, K. Schachl, H. Alemuc, E. Turkušic, A. Copra, H. Moderegger, I. Švancara, K. Vytras and K. Kalcher, "(Bio)Sensors Based on Manganese Di- oxide-Modified Carbon Substrates: Retrospections, Fur- ther Improvements and Applications," *Talanta*, Vol. 64, No. 5, 2004, pp. 1151-1159.

4. P. Kotzian, P. Brazdilova, K. Kalcher, K. Handlir and K. Vytras, "Oxides of Platinum Metal Group as Potential Catalysts in Carbonaceous Amperometric Biosensors Ba- sed on Oxidases," *Sensors and Actuators B*, Vol. 124, No. 2, 2007, pp. 297-302.

5. K. Schachl, H. L. Alemu, K. Kalcher, J. Ježkova, I. Svan- cara and K. Vytřas, "Amperometric Determination of Hy- drogen Peroxide with a Manganese Dioxide-Modified Car- bon Paste Electrode Using Flow Injection Analysis," *Analyst*, Vol. 122, No. 9, 1997, pp. 985-989.

6. R. Garjonyte and A. Malinauskas, "Amperometric Sensor for Hydrogen Peroxide, Based on Cu2O or CuO Modified Carbon Paste Electrodes," *Fresenius' Journal of Analyti- cal Chemistry*, Vol. 360, No. 1, 1998, pp. 122-123.

7. J. Hrbac, V. Halouzka, R. Zboril, K. Papadopoulos and T. Triantis, “Carbon Electrodes Modified by Nanoscopic Iron(III) Oxides to Assemble Chemical Sensors for the Hydrogen Peroxide Amperometric Detection,” *Electro-analysis*, Vol. 19, No. 17, 2007, pp. 1850-1854.

8. P. Kotzian, P. Brázdilová, S. Řezková, K. Kalcher and K. Vytřas, “Amperometric Glucose Biosensor Based on Rhodium Dioxide-Modified Carbon Ink,” *Electroanalysis*, Vol. 18, No. 15, 2006, pp. 1499-1504.

9. P. Kotzian, P. Brázdilová, K. Kalcher and K. Vytřas, “Determination of *Hydrogen Peroxi*de, Glucose and Hypo- xanthine Using (Bio)Sensors Based on Ruthenium Diox- ide-Modified Screen-Printed Electrodes,” *Analytical Let- ters*, Vol. 38, No. 7, 2005, pp. 1099-1113.

10. A. Veseli, A. Hajrizi, T. Arbneshi and K. Kalcher, “A New Amperometric Glucose Biosensor Based on Screen Print- ed Carbon Electrodes with Rhenium(IV)—Oxide as a Mediator,” *Journal of Electrochemical Science and Engineering*, Vol. 2, No. 4, 2012, pp. 199-210.

11. L.Berisha, K. Kalcher, A. Hajrizi, T. Arbneshi, A New Biosensor for Glucose Based on Screen Printed Carbon Electrodes Modified with Tin (IV)-Oxide American Journal of Analytical Chemistry 4, 2013 pp 27-35.

12. European Pharmacopoeia 5 th edition, 15 June 2004

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STUDY ON PREHISTORIC IRON OBJECTS DISCOVERED ON THE OUTSKIRTS OF SHKODRA

STUDIM MBI OBJEKTE HEKURI PREHISTORIKE TË ZBULUARA NË RRETHINAT E SHKODRËS

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SUMMARY
In this study are studying three iron objects named machete, in order to study the technology of production in those periods. Objects belonging periods, cultures and different settlements on the outskirts of Shkodra. Analytical methods used in this study are: XRF-basic composition of the three objects as well as optical microscopy which is used only for machete 3, known as working leather machete. From the μ - X ray fluorescence results can be concluded that all the objects content iron over than 98%. The others present chemical elements are: Mn up to 0.38% (machete 3), and Co up to 0.99% (machete 1) . Co is thought to be as an impurity. At the machete 1 except Fe, Co, Mn, although it contained Cu in a very small content 0.1-0.01%. At machete 3 we can see clearly heterogeneous structure of widmanstatte.
Key words: Iron objects, machetes, XRF, MO

PËRMBLEDHJE
Në këtë studim janë studiuar tre objekte hekuri të emërtuara hanxhar, me qellim studimin e teknologjisë të prodhimit në këto periudha. Objektet i përkasin periudhave, kulturave si dhe vendbanimeve të ndryshme në rrethinat e Shkodrës. Metodat analitike që janë përdorur në këtë studim janë: XRF- për përbërjen elementare të të tre objekteve si edhe mikroskopia optike e cila është përdorur vetëm për objektin hanxhar 3 i cili njihet si hanxhar lëkurë punues. Nga rezultatet me μ - XRF mund të dalim në përfundimin që të gjithë objektet përmbajnë hekur mbi 98%. Elementë kimik të tjerë të pranishëm janë: Mn deri në 0.38% (hanxhar 3) dhe Co deri në 0.99% (hanxhar 1). Co mendohet të jetë si një papastërti. Tek hanxhar 1 përvec Fe, Co dhe Mn, përmban në masë shumë të vogël Cu 0.1-0.01%. Tek hanxhar 3 mund të shihet qartë struktura heterogjene e widmanstatte.
Fjalët kyce: Objekte hekuri, hanxharë, XRF , MO

INTRODUCTION

Interest on archaeological values of Albania launches in the XIX century, when historical geography researchers devoted to the localization and identification data from ancient sources. Shkodra is a city in northwest of Albania, established over centuries V - IV BC.The Shkodra district owns considerable potential in terms of archeology. In this city and its surroundings are revealed many iron objects of different periods through archaeological excavations and random findings [3].

Figure.1 Albanian map and the location of Shkodra, and Troshan.

There are discovered objects which show historical data for periods of special value. These objects are located in Historical Museum of Shkodra, that is situated in a characteristic house from Shkodra, buildet in eighteenth century called house of Oso Kuka. The part of archaeological objects in the museum contains about 500 objects representing all historical periods , from the Neolithic (6000 BC), until the late Middle Ages (XVI). In this work are studied three iron objects named machete, which are part of the Historical Museum of Shkodra, with the purpose of studying the technology of production in those periods. Iron objects studied in this work belong to periods, cultures, and different settlements on the outskirts of Shkodra, and border areas between Shkodra and Lezha . These objects could be used as a weapon , as an exchange tools, as well as a working tools. Iron metallurgy has come in the Balkans and in Albania from the Asia Minor. Iron as a raw material, has replaced gradualy bronze and copper in production of working tools and weapons. Although from 2000 years BC, some Illyrian tribes had learned how to glean iron from the mineral. It remained for a long time a rare metal, which were used to produce ornaments and arms especially [1].

Technological achievements in the iron period should be studied in order to understand their role in the overall development of society .

METHODS AND MATERIALS

Analytic methods which are used in this work are: XRF – for basic composition and optical microscopy. To study the basic composition it is used a portable μ - X Ray Fluorescence system, with 60 μm beam spot in two points, respectively on the handle and on edge of the objects (BRUKER μ - X Ray Fluorescence). Conditions of the used device are: 45 kV, 200 μA and 100 sec. To receive and analyze the different spectra are used software of: Spectra ARTAX version 7.2.5.0. and M-Quant-Calib (BRUKER). Objectes analyzed were painted with lacquer in order to be protected from corrosion, so it was necessary to remove the lacquer in the certain spot before analysed using diluent and abrasive paper. The objects are photographed and drawing before analysis with μ-XRF [4, 5].

In order to investigate the surface of micro structure, the preparation of the sample of machete 3 is done by polishing in different parts using silicon carbide paper from 320 up to 4000, and finally with cloth, diamante paste 6 , 3, 1 μm, and DP-Lubricant Blue. After survey of the sample surface with optical microscope, sample was etched with nital and picral mixed together in 1:1 proportion for different etching times from 3 to 20 seconds. It became possible taking micrography with the help of optical microscop (reflected and polarized light, Kozo XJP300), TCC-8.1 camera and soft TS View Version 1.0.0.1.

Stereo Microscop XTL6445 was used to enlarge small images.

The objects of study are three iron objects which are part of the Historical Museum of Shkodra. Acording to Zamir Tafilica an archaeologist, these objects belong to different periods and cultures, but they are made of iron and have the same processing technique. First object, "machete 1" was founded in archaeological excavations in Troshan village , which is located in Blinisht municipality between Lezha and Shkodra, the other two objects machete 2 and machete 3, are found on the outskirts of Shkodra . Following table presents the characteristics of each object, their images and sketchs.

Object	Period	Discovered	Object no	File no	Cultures	Tecniques	Mass
Machete 1	III century BC	Troshan	14639	112	Helenistic	Beating	337 gr
Machete 2	-	Shkodër	14717	130	Roman	Beating	400 gr
Machete 3	-	Shkodër	20233	87	Roman	Beating	300 gr

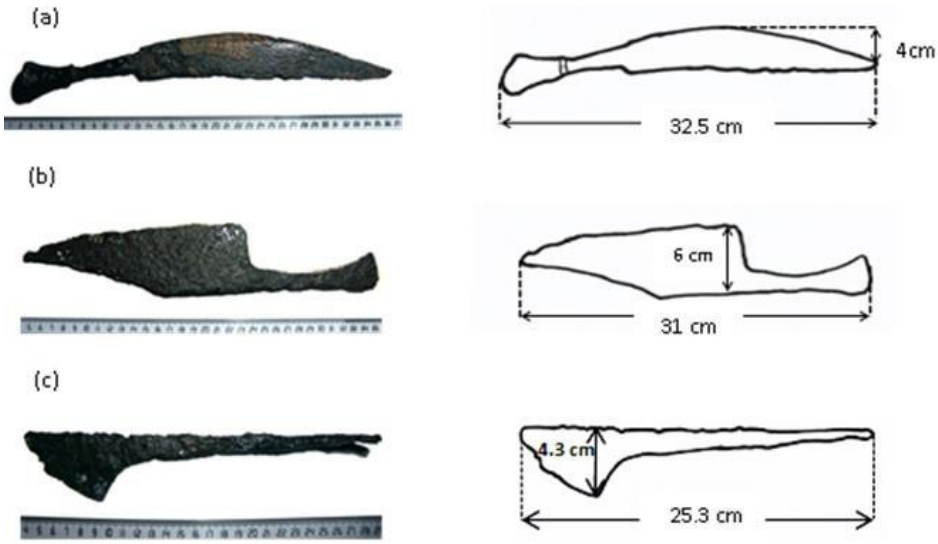

Figure.2 on the left photos of the objects, on the right sketches of the objects, (a) machete 1 , (b) machete 2 and (c) machete 3.

RESULTS AND DISCUSSION

µ - X Ray Fluorescence gains more and more importance for the analysis of objects of the cultural heritage. Non-destructive microanalysis of the chemical composition and the structure of ancient materials in the last decade has become an irreplaceable tool for the identification of archaeological material, the investigation of provenance or fabrication procedures.

The analyses were done in two certain spot, one on the edge, and one on the handle of each object. From results we saw that the percentage of iron is very high up to 98 %. We saw that on the analyses done on the handle of object, iron had a less percentage comparing to analyses done on the edge. The others chemical elements present are: Mn up to 0.38% on handel of machete 3, Co up to 0.99% on edge of machete 1 and Cu up to 0.1% on edge of machete 1 which is present only for this object.

After objects were analyzed with XRF, which was realized in the grounds of the Museum of Shkodra, in collaboration with archaeologist was possible to obtain a sample from object "working leather Machete" (known as “machete 3”) to study its microstructure. Below are present two photo from machete 3, photo on the left with reflected light and photo on the right with polarized light.

Name of object	Place of analysis	Fe (%)	Mn (%)	Co (%)	Cu (%)
Machete 1	edge	98.65±4.38	0.26±0.02	0.99± 0.26	0.1±0.01
	handle	99.37±4.79	0.25±0.02	0.38±0.25	-
Machete 2	edge	99.35±4.58	0.24±0.02	0.41±0.25	-
	handle	99.28±4.70	0.29±0.02	0.43±0.26	-
Machete 3	edge	99.44±4.51	0.29±0.02	0.27±0.21	-
	handle	99.42±4.04	0.38±0.02	0.20±0.18	-

Table.2. Quantitative analysis for each object on edge and on handle. For each chemical element are presented the percentage of the chemical elements and the standard deviation for each measure (% (m/m)±S.D.).

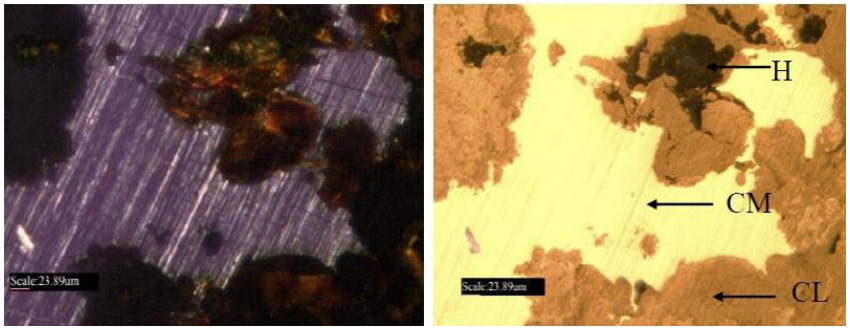

Figure.3 OM metallographic image of object machete 3, photo on the left with reflected light and photo on the right with polarized light. (Corrosion layer (light grey) CL, core metal CM (white), hole H).

Valuable information can be gained by examining samples in polarized light, as nonmetallic inclusions and corrosion products present on it. Examination under reflected polarized light yields a great deal of valuable information (Figure.3). Normally corrosion produces a buildup of insoluble products, both within and overlying the original metal volume. Corrosion products may be very informative; indeed, they may be all that is left of the original object [6]. Reddishorange powder rust and the presence of many cracks and cavities on the surface of the objects are indications of an ongoing active corrosion process, causing continuous loss of metal, as well as degradation of mechanical properties [7]. Corrosion products for the sample machete 3 were observed deep into the metal which means that these samples are authentic ancient objects and not fake replicas.

As a result of etching we can see clearly heterogeneous structure of widmanstatte. This heterogeneous microstructure contained ferrite, pearlite and Widmanstatten ferrite-pearlite. This kind of microstructure is typical of wrought iron which has been made by bloomer [2].

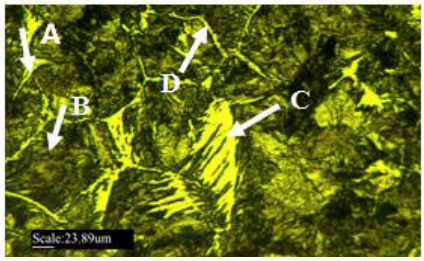

Figure.4 Metallographic image of machete 3 showing Widmanstatten microstructure: A-primary widmanstatten, B-pearlite, C-secondary widmanstatten, D-austenit grain.

The main factors affecting the formation of Widmanstatten structure in steels are the chemical composition of the steel, the cooling rate and the size of austenite grains. Widmanstatten structure is chiefly characterized by thickness of the lamellas and the amount of ferrite or cementite formations [2, 8]. Widmanstatten structure observed on machete 3 have a coarse austenitic grain, and was obtained under conditions of slow cooling from austenitic phase. Such structures are present in hypoeutectoid steels bearing 0.05-0.5% C [8]. Acording to Scott, D.A most ancient steels were made from iron, containing about 0.1-0.5% C [6].

CONCLUSION

From the μ - X ray fluorescence results can be concluded that all the objects content iron over than 98%. The others present chemical elements are: Mn up to 0.38% (machete 3), and Co up to 0.99% (machete 1) . Co is thought to be as an impurity. At the machete 1 except Fe, Co, Mn, although it contained Cu in a very small content 0.1-0.01%. At “machete 3” we can say that this object is ancient and has been made by wrought iron but it still needs further studies.

REFERENCES

1. Anamali.S. (1979): Arkeologjia dhe bujqësia, p13-39, Tiranë, Albania.
2. Ashkenaz. D, Mentovich.E, Cvikel.D, Barkai.O, Aronson.A and Kahanov.Y, Archaeometallurgical Investigation of Iron Artifacts from Shipwrecks – A Review
3. Ceka. N, (2002), Ilirët, Tiranë,Albania.
4. Einarsdóttir Sif.S. (2012), Mass-conservation of Archaeological Iron Artefacts, A Case Study at the National Museum of Iceland.
5. MIT. (2003), The Metallographic Examination of Archeological Artifacts, Laboratory Manual. Summer Institute in Materials Science and Material Culture. Massachusetts Institute of Technology USA. p4-6.
6. Scott,D.A.(1991), Metallography and microstructure of ancient and historic metals. The J. Paul Getty Trust, p16.
7. Selwyn, L. (2004). Overview of archaeological iron: the corrosion problem, key factors affecting treatment, and gaps in current knowledge. Proceedings of Metal 2004 National Museum of Australia Canberra ACT (4–8 October), 294–306.
8. Todorov, R.P. & Khristov K.G. (2004). Structural transformations: Widmanstatten structure of carbon steels. Metal Science and Heat Treatment 46 (1–2), 49–53..

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EFFECTS OF GAMMA AND ALPHA IRRADIATION ON POTASSIUM CURRENTS IN HUMAN GLIOBLASTOMA MULTIFORM CELL LINE

EFEKTET E RREZATIMEVE GAMA E ALFA MBI RRYMAT ELEKTRIKE TË KALIUMIT TË QELIZAVE NJERËZORE TË LINJËS GLIOBLASTOMA SHUMËFORMËSHE

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PËRMBLEDHJE
Në terapinë e tumoreve përdoren disa tipe rrezatimesh jonizuese, por kjo sjell edhe efekte anësore si induktimi i tumoreve dytësore apo vdekja e qelizave normale rreth atyre të rrezatuara. Mekanizmat që shpjegojnë këto efekte në lidhje me tipin dhe dozën e ulët të rrezatimit ende nuk janë të qarta. Qëllimi i këtij studimi ishte vlerësimi i efekteve të rrezeve gama dhe alfa mbi rrymën elektrike të kaliumit (I_K) të membranës së qelizave njerëzore të linjës glioblastoma shumëformëshe T98G. Qelizat janë ekspozuar në doza nga 0 në 2 Gy dhe I_K është matur duke përdorur teknikën e sofistikuar patch clamp. Rrezet gama nuk kanë shkaktuar asnjë efekt në I_K. Amplituda e I_K, e regjistruar nga qelizat e rrezatuara nga grimcat alfa, pësoi një rënie statistikisht domethënëse për dozën 0.25 Gy dhe jo për dozë më të lartë. Këto rezultate tregojnë se I_K varet fuqishëm nga doza dhe tipi i rrezatimit.
Fjalët çelës: rrezatimi jonizues, dozë e ulët, membranë qelizore

SUMMARY
Many types of ionizing radiation are commonly used to treat cancer, but there are side effects such as radiation-induced second cancer or normal cell death around the cancer cells. The mechanisms underlying these effects together with their inter-relationship and the correlation with type and low dose radiation are not yet clear.
The aim of this study was to estimate the effects of gamma and alpha irradiation on membrane potassium currents (I_K) in human glioblastoma multiform cell line T98G. The cells were exposed at different doses, from 0 to 2 Gy and the amplitude of I_K was measured using sophisticated patch-clamp technique. The gamma rays did not have significant effects on I_K amplitude. The I_K recorded from cells irradiated by alpha particles showed a significant decrease at dose 0.25 Gy and not for higher dose. The results show that the I_K is strongly dose and radiation type dependent.
Key words: ionizing radiation, low dose, cell membrane

INTRODUCTION

Medical use of ionizing radiation (IR) represents the principal contribution to the irradiation of the human population from artificial sources. Many types of high energy radiation, such as gamma rays and charged particles, can be used to treat a wide range of tumors but the risk to damage normal cells surrounding treated region is very

high. The risks of cancer after moderate and high doses of radiation are relatively well understood from the linear no-threshold concept derived from detailed epidemiological studies of the Japanese atomic bombing survivors and others [1]. It follows the notion that increases in the physical energy deposition of IR linearly increases the carcinogenic risk with increasing dose. The linear no-threshold concept has been widely used to establish international rules and standards of radiation protection. However, recent findings have strongly put into question this concept and its scientific validity for very low doses and dose rates [2]. Several lines of evidence from molecular, biochemical, and biological studies have outlined that low dose IR involve formerly unexpected cellular phenomena such as hyper-radiosensitivity, induced radioresistance, non-targeted (bystander effect) and delayed radiation effects in terms of various biological endpoints, contradicting the linear no-threshold concept and the classical paradigms of radiation biology considering DNA as primary target of interaction [3,4,5,6,7]. The mechanisms underlying these effects together with their inter-relationship and the correlation with type and low dose radiation are not yet well elucidated [8].

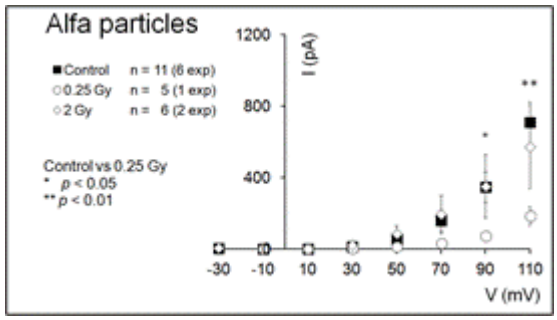
In this work it is evaluated the effects of acute low dose (from 0 to 2 Gy) gama and alpha irradiation on outward membrane potassium currents (I_K) recorded from glioblastoma multiforme cell line T98G, derived from a human tumor.

Material and Method

Cell preparation

The cells were maintained in RPMI medium, supplemented with 10% fetal calf serum, L-glutamine (1%), sodium pyruvate (10%) and antibiotics (penicillin, streptomycin). Cell cultures conditions were kept in incubator at 5% CO_2 humidified atmosphere at 37°C. Eighteens hours before irradiation the cells were plated as a monolayer on specially designed stainless-steel Petri dishes for irradiation [9]

Cell exposure



Gamma ray irradiations have been performed at 662 keV using a blood irradiator equipped with a 137 Cs source, available at the University Hospital "S. Orsola – Malpighi" (Bologna, Italy). Alpha irradiations have been performed at the Radiobiology facility of the INFN -LNL 7MV Van de Graaff CN accelerator (Legnaro – Padova, Italy). Irradiation facility, beam dosimetry and irradiation modalities have been described in detail elsewhere [9]. Alpha particles of 12 MeV (8.4 MeV energy at the cell entrance surface, corresponding to LET value of 62.3 keV/µm) have been used. Sham irradiated cells were used in all the experiments as control (un-irradiated) cells. The T98G cells were detached 2 hour after irradiation using trypsin-EDTA (0.02%) and re-suspended in culture medium at room temperature. Within 6 hours cells were transferred to a Petri dish and allowed to attach to the bottom for 30 min.

Chemical solutions

Culture medium was substituted with the bath solution (extracellular electrolyte solution, pH 7.4) contained (in mM): 133 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES and 10 glucose. The electrode solution (capillary electrode solution, pH 7.2) contained (in mM): 10 NaCl, 120 Kasp, 2 MgCl2, 4 CaCl2, 10 HEPES, 10 EGTA, 3 Mg-ATP, 0,2 GTP tris (Sigma Chemical Company, St. Louis, MO).

Potassium current recordings

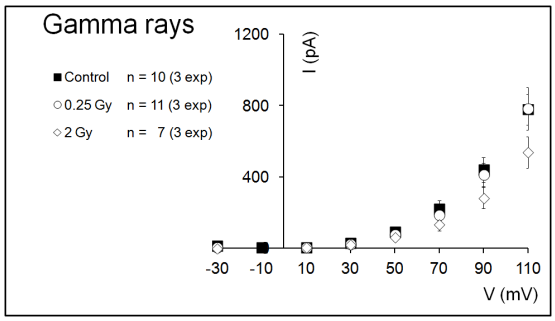
In all the experiments membrane I_K from T98G cells were recorded in the whole-cell configuration of the patch clamp technique [10]

at room temperature (22-24°C) with a EPC-10 amplifier (HEKA Instruments, Darmstadt, Germany). When the seal was confirmed by the absence of leakage current, a pulsed protocol was applied through the recording electrode. Voltage steps of 20 mV amplitude and of 100 ms duration increasing from -30mV to +110 mV, were delivered at intervals of 1s, with an holding potential (V_h) set at 0 mV. Fast capacitance transients were minimized on-line by the patch amplifier software which also performed, by global parameter setting (macro) a tracked leak compensation. This software setting allowed the automatic measurement of cell capacitance and resting membrane potential. The patch micropipette tip resistances ranged between 4 and 10 MΩ, when filled with electrode solution.

Data analysis

The statistical analysis consist in applying T-test ($P < 0.05$) between each of the exposed group and the control group. Data are expressed as mean ± standard error.

Results and discussion



The amplitude of I_K, recorded from T98G cells, irradiated by gamma rays and alpha particle, showed, as a function of the applied voltages, a general decrease compared to un-irradiated cells (Fig.1).

Fig.1. Mean current amplitudes recorded from un-irradiated (control) and irradiated (gamma and alpha) T98G cells, at different doses as a function of voltage (I-V graphs). Data are expressed as mean ± S.E.M. Student's t test was used to compute p values.

The amplitude of I_K recorded from cells irradiated by gamma rays did not showed a significant variation, compared to un-irradiated cells at none of the dose tested while I_K recorded from cells irradiated by alpha particles showed a significant decrease when irradiated by 0.25 Gy and not for higher dose 2Gy (Fig.1,2), contradicting the linear no-threshold concept.

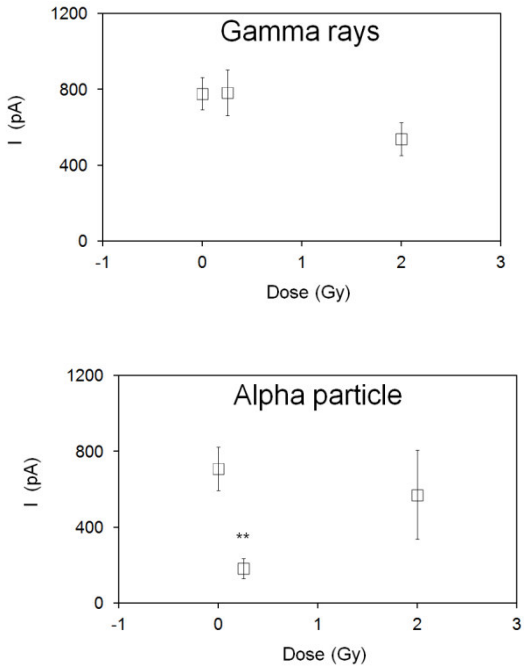


Fig.2. Mean current amplitudes recorded from T98G cells at 110 mV as a function of radiation doses (gamma and alpha). 0 dose point correspond to un-irradiated cells. Data are expressed as mean ± S.E.M. Student's t test was used to compute p values ** $p < 0.01$.

These 'non-linear' effects, observed at low doses of IR, might not be completely attributed to direct transfer of energy to the nuclear DNA macromolecule and indicate that the cellular response to low levels dose, at least in vitro, is influenced in a complex manner by several factors, including the type of radiation and the dose. Since I_K channels are involved in volume regulation, hormonal secretion, cell proliferation and apoptosis [11], including cells migration and proliferation, understanding their interaction with IR may be helpful in radio-therapy planning and radiation protection.

Conclusion

The results show that the I_K is strongly dose and radiation type dependent, but not according to the linear no-threshold concept.

References

1. Wakeford R, (2004), The cancer epidemiology of radiation. Oncogene 23, 6404–6428

2. Averbeck D (2009), Does scientific evidence support a change from the LNT model for low-dose radiation risk extrapolation? Health Phys. 97(5):493-504.

3. Cherubini R, De Nadal V, Gerardi S, Guryev D (2010), Lack of hyper-radiosensitivity and induced radioresistance and of bystander effect in V79 cells after proton irradiation of different energies. Radiat Prot Dosimetry 143(2-4): 315-319.

4. Zironi I, Gavoçi E., Virelli A., Lanzarini C., Capri M., Remondini D., Cherubini R. , De Nadal V., Gerardi S., Castellani G (2012), Effects of Proton Irradiation on Ionic Currents and Gene Expression in Human and Rodent Cell Lines. INFN- LNL ANNUAL REPORT 2011, 113 – 114.

5. Song H, Hedayati M, Hobbs RF, Shao C, Bruchertseifer F, Morgenstern A, Deweese TL, Sgouros G (2013), Targeting Aberrant DNA Double-Strand Break Repair in Triple-Negative Breast Cancer with Alpha-Particle Emitter Radiolabeled Anti-EGFR Antibody. Mol Cancer Ther.12(10):2043-2054.

6. Marples B, Collis SJ. (2008), Low-dose hyper-radiosensitivity: past, present, and future. Int J Radiat Oncol Biol Phys. 70(5):1310-8.

7.Tsoulou E, Baggio L, Cherubini R, Kalfas CA (2002), Radiosensitivity of V79 cells after alpha particle radiation at low doses. Radiat Prot Dosimetry. 99: 237-40.

8. Averbeck D.(2010), Non-targeted effects as a paradigm breaking evidence. Mutat Res. 687: 7-12.

9. Belli M, Cherubini R, Galeazzi G, Mazzucato S, Moschini G, Sapora O, Simone G, Tabocchini Ma, Nuclear instruments and methods in physics research, A256, 576-580, North-Holland, Amsterdam.

10. B. Sakman and E. Neher (1995),Single Channel Recording,Plenum Press, New York.

11. Nilius B., Droogmans G(2001), Ion channels and their functional role in vascular endothelium. Physiol. Rev. 81:415–459.

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IDENTIFICATION OF ASPIRIN TABLET COMPONENTS BY FTIR SPECTROSCOPY

VLERËSIMI I PËRBËRËSVE NË TABLETAT E ASPIRINËS ME SPEKTROSKOPINË FTIR

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PËRMBLEDHJE
Në këtë studim paraqitet një analize e detajuar e aspirinës së prodhuar nga kompania Profarma sh.a. ne Tiranë. Me anë të sistemit FTIR, Nicolet 6700, me rrezatim IR te mesëm (4000 – 400 cm^{-1}) u përftuan spektrat e absorbimit të tabletës të aspirinës dhe të komponimeve përbërëse të saj, te cilët janë: acidi acetilsalicilik (AAS), celuloza , kroscarmelosa e natriumit , acidi stearik , talk etj, të cilët shoqërohen nga lidhjet te tyre kovalente karakteristike. Ne spektrin FTIR të AAS u identifikuan disa prej lidhjeve kryesore kovalente: C=C, C=O, C-H etj. Identifikimi i komponimeve të përftuara nëpërmjet spektroskopisë FTIR shërben për hartimin e një metodike për vlerësimin cilësor dhe sasiorë te përbërësve të medikamenteve të ndryshme. Ajo gjithashtu shërben në përcaktimin e një raporti ndërmjet mbushësve kryesorë dhe komponimit bazë.
Fjalët kyçe: Spektroskopia molekulare, FTIR, grupet funksionalë, Aspirina, Acidi acetilsalicilik.

SUMMARY
In this study is determinated a detailed analysis of aspirin produced by Profarma company, Tirana. The absorption spectra of aspirin and its fillers, which are: acetylsalicylic acid (ASA), cellulose sodium croscarmellose , stearic acid , talc etc., are obtained by using FTIR spectroscopy, Nicolet 6700 with mid infra-red radiation (4000 – 400 cm^{-1}). Each of these components is characterized by its characteristic covalent bonds. In FTIR spectrum of ASA are identified some of the major covalent bonds: C = C, C = O, C - H etc. Identification of compounds obtained by FTIR spectroscopy serves to develop a methodology for a qualitative and quantitative assessing of various drugs components. Through this method it is possible to determine the ratio of fillers to the main active component.
Key words: Molecular spectroscopy, FTIR, functional groups, aspirin, Acetylsalicylic acid.

INTRODUCTION

Drugs consist of the base composition which is related to the therapeutic effect and other components as additions and fillers. One of the drugs that we use very often is the Aspirin. Aspirin is a drug in the family of salicylates (carboxylic acid), often used as an analgesic, antipyretic, and anti-inflammatory. It has also an anticoagulant effect and is used in long-term low-doses to prevent heart attacks. Aspirin was the first discovered member of the class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs), but not all of them are salicylates, Acetylsalicylic acid (ASA) is the main active component of aspirin tablet.

The study of these components can be carry out by IR molecular spectroscopy which is a preferred method for investigation of covalent bonds. The interactions of infrared radiation with matter could be described in terms of

changes in molecular dipoles associated with vibrations and rotations.

Vibrations can involve either a change in bond length (*stretching*) or bond angle (*bending*) and some bonds could be stretch in-phase (*symmetrical* stretching) or out-of-phase (*asymmetric* stretching) [8].

Infrared spectroscopy is one of the most important analytical techniques used in research and laboratory control. One of the greatest advantages of the IR spectroscopy is that any sample in any state may be analyzed. Fourier transform infrared spectroscopy (FTIR) has facilitated many different IR sampling techniques and improved the quality of infrared spectra and minimized the time required to obtain data. The increased speed and higher ratio of signal-to-noise of FTIR relative to dispersion infrared has lead to a substantially greater number of applications of infrared spectroscopy.

The complexity of an infrared spectrum arises from the coupling of vibrations over a large part of or over the complete molecule. Such vibrations are called *skeletal* vibrations. Bands associated with skeletal vibrations are likely to conform to a pattern or *fingerprint* of the molecule as a whole, rather than a specific group within the molecule.

In this study is presented a detailed analysis of the aspirin tablet produced by PROFARMA Company in Tirana, using the mid-IR radiation (4000 — 400 cm^{-1}), focused on the analysis of FTIR spectra of aspirin tablet and its components.

MATERIALS AND METHODS

Infrared spectroscopy is one of the most important analytical techniques used in research and laboratory control. One of the greatest advantages of the IR spectroscopy is that any sample in any state (solid, liquid) may be analyzed. Fourier transform infrared spectroscopy (FTIR) has facilitated many different IR sampling techniques and improved the quality of infrared spectra and minimized the time required to obtain data. The increased speed and higher ratio of signal-to-noise of FTIR relative to dispersion infrared has lead to a substantially greater number of applications of infrared spectroscopy.

FTIR spectra were obtained by Nicolet 6700 spectrometer, manufactured by Thermo Electron. This allows us to obtained spectra in NIR (12000 - 4000 cm^{-1}) and MIR (4000 - 400 cm-1) region.

This system works in two geometries, the geometry of the transmission and reflectance (Attenuated Total Reflection - ATR). The basis of this method is Michelson's interferometer, which consists of two mirrors, one of which moves with constant speed and the other is stationary, the beam-splitter and the laser that controls the speed of the moving mirror. The radiation emerged from the IR source passing through the interferometer and the sample reaches a detector. All IR wavelengths, passing through the interferometer, they generate the interferogram, which is a complex pattern. The absorption spectrum as a function of wavenumber (cm^{-1}) is obtained from the Fourier transform of the interferogram, which is a function of mirror movement (cm).

In this study is used the transmission geometry using mid Infra-Red (4000 – 400 cm^{-1}) range and the spectra are obtained and analyzed using OMNIC program. Aspirin tablet, produced by PROFARMA Company, consists of the following components (Table 1).

The KBr pellet technique is used for the components analysis. 20 mg of each component is mixed with 400mg spectroscopic KBr using a mortar. Before mixing, it is necessary to dry KBr powder at 120°C for 24h in an oven. Otherwise the broad spectral peak due to free O-H will seriously affect the interpretation on the bound hydroxyls associated with any of the aspirin components. [3]

Table 1 Aspirin components and their amount

Component	ASA	Cellulose	Sodium croscarmellose	Sodium glycolat	Stearic acid	Talc	Starch	Tartaric acid
Amount (mg)	500	28	7.5	7.5	6	6	5	2.5

RESULTS AND DISCUSSIONS

The base composition of aspirin tablet is acetylsalicylic acid (ASA) (Fig. 1) which is related to the therapeutic effect and other components as additions and fillers.

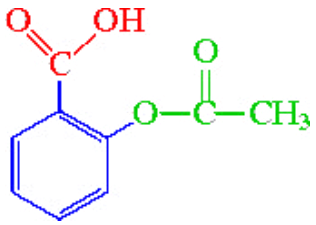

Figure 1: ASA chemical structure

At first were obtained FTIR spectra of aspirin Profarma, acetylsalicylic acid and fillers. Based in large information on functional groups that belong to ASA and different filler, through the OMNIC program were identified the peaks and we evaluated the chemical bounds and functional groups belong to them. **ASA** characteristic lines are 1605 cm^{-1} (asymmetric stretching) for the functional group C=C and 2847 cm^{-1} stretch for C-O functional group [3]. Tablet fillers are croscarmellose sodium, starch, tartaric acid, cellulose, stearic acid, talc and sodium starch glycolate. The characteristic lines of **croscarmellose sodium** are 3441 cm^{-1} (stretching) and 1417 cm^{-1} (bending) of the functional group O-H, 2916 cm^{-1} (stretching) and 2850cm^{-1} (stretching) of the functional group CH, 1328 cm^{-1} (bending) of the CH_2 functional group and 1605 cm^{-1} (stretching) of covalent bonding C = O [4, 7]. For **starch** the lines are 3382.34 cm^{-1} (stretching) to the functional group OH, 2916 cm^{-1} (stretching) for CH, 1158 cm^{-1} to C-C and 1134 cm^{-1} for C-O [1]. For **tartaric acid** the lines are at 1308 cm^{-1} of the O-H functional group, 1754 cm^{-1} (stretching) of the functional group C=O and 1471 cm^{-1} of C-O functional group [5]. For **cellulose** the lines are at 3347 cm^{-1} (stretching) of the functional group O-H, 2900 cm^{-1} (stretching) 1429 cm^{-1} (bending) 1373 cm^{-1} (bending) for functional group C-H, 1164 cm^{-1} (stretching) to C-C, 1677 cm^{-1} (stretching) and 1189 cm^{-1} (bending) for C-O and 1637 cm^{-1} for adsorbed water [1]. For **stearic acid** are identified at 2916 cm^{-1} for CH_2 functional group and 1701cm^{-1} (stretching) for C = O [6]. The characteristic line of **talc** is at 3676 cm^{-1} for O-H functional group [2]. The characteristic line of **sodium starch glycolate** are as follow O-H 3407.96 cm^{-1} (stretching), C-H 2931.60 cm^{-1} (stretching) and C=O 1598 cm^{-1} (stretching) [7] (Table 2). For a better evaluation of filler components in aspirin tablet it is obtained the subtract spectrum of aspirin Profarma 20mg and ASA 20mg and identification of fillers is analyzed through its visible lines. After the separately analyses of fillers FTIR spectra, where we evaluate their characteristic lines, we carefully identify the characteristics predominant lines of fillers in the subtracting spectrum between aspirin tablet and ASA (Fig. 5). Using FTIR spectra of aspirin tablet and their component, we could also evaluate the percentage of ASA related to aspirin tablet. In our case we evaluate that the percentage of ASA is 0.88 +/- 0.02, which fit well with the value from the tablet composition (Table 1).

Table2: Table of aspirin tablet fillers – the main functional groups and their wave number.

Functional groups	Wave number (cm^{-1})						
	Croscarmellose sodium	**Starch**	**Tartaric acid**	**Cellulose**	**Stearic acid**	**Talc**	**Sodium starch glycolate**
	$NaC_6H_7O_6$	**$(C_6H_{10}O_5)_n$**	**$C_4H_6O_6$**	**$(C_6H_{10}O_5)_n$**	**$CH_3(CH_2)_{16}CO_2H$**	**$H_2Mg_3(SiO_3)_4$**	**$C_2H_4O_3{\cdot}xNa{\cdot}x$**
O-H	3441.8 (stretching) 1417.44 (bending)	3382.34 (stretching)	1308 def. vibration	3347.34 (stretching)		3676.29- (stretching)	3407.96 (stretching)
C-H	2916.78 (stretching) 2850.07 (stretching)	2916.78 (stretching) 1401.66 (bending) 1384.00 (bending)		2900.56 (stretching) 1429.37 (bending) 1373.14 (bending)		empty	2931.60 (stretching)
CH_2	1328.27 (bending)	empty		1337 (bending-wagging) 1281 (bending-twisting)	2916.78 2850.08	empty	empty
C=O	1605.12 (stretching)	empty	1754.71 (stretching)	empty	1701.01 (stretching)	empty	1598.00 (stretching)
C-C	empty	1158.28 (stretching -ring breathing, asymmetric)	empty	1164.54 (stretching -ring breathing, asymmetric)		empty	empty
C-O		1134.11 1095.21 1020 - (anhidroglukoze stretch)	1471.41 def. vibration 1095.21	1677.76 (stretching) 1189.87 (bending)		empty	1155.28 (stretching)

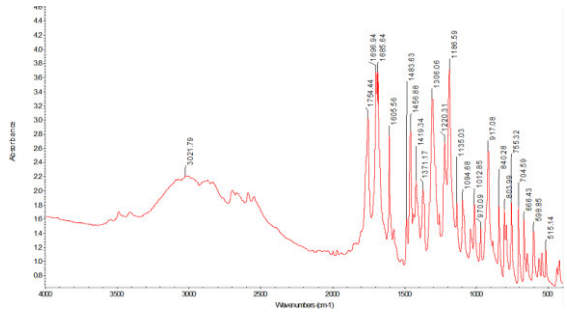

Figure 2. ASA + KBr FTIR spectrum

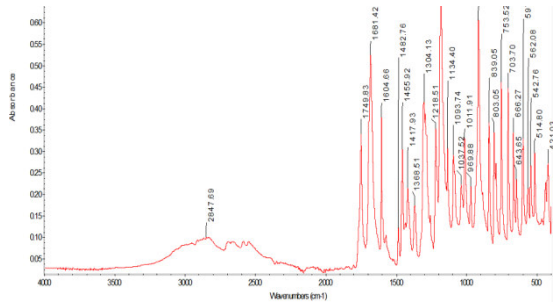

Figure 3. Aspirin Profarma + KBr FTIR spectrum

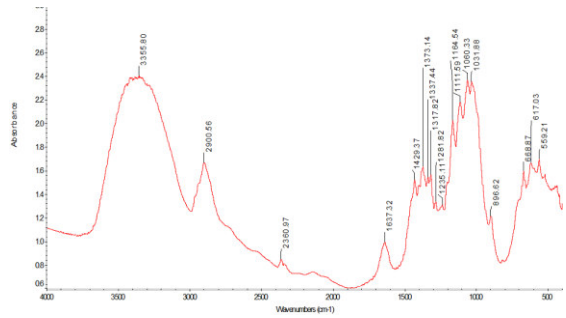

Figure 4. Cellulose + KBr FTIR spectrum

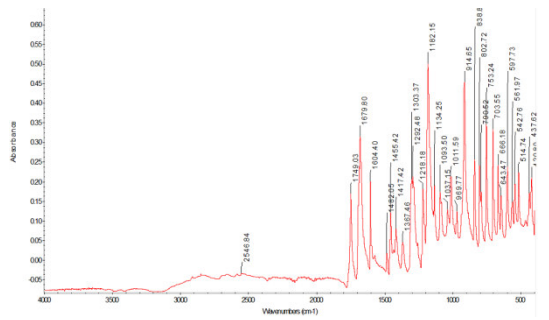

Figure5. Subtraction (Aspirin Profarma - ASA) FTIR absorption spectrum

CONCLUSIONS

Study of Albanian drugs by IR spectroscopy is important to introduce this technique in laboratory of pharmaceutical companies.

Through this method we could determine the functional characteristic groups of the main active component and the different fillers and we could enlarge this study to prepare of database of different pharmaceutical component.

Through this method it is possible to determine the ratio of the main active component to the aspirin tablet components.

BIBLIOGRAPHY

1. Chukwuemeka P Azubuike and Augustine O Okhamafe (2012), International Journal of Recycling of Organic Waste in Agriculture, Physicochemical, spectroscopic and thermal properties of microcrystalline cellulose derived from corn cobs, 1-9
2. Belgacem K, Lewellyn P, etc. (2008), Thermal behavior study of the talc. Optoelectronics and advanced materials – rapid communications, Vol.2, No.6, p.332-336.
3. Bunaciu A, etc. (2006), FT-IR Spectrophotometric analysis of acetylsalicylic acid and its pharmaceutical formulations. Canadian Journal of Analytical sciences and spectroscopy, Vol.51, No.5, p. 253-259.
4. Byrd, Houston; O'Donnell, Stephen E (2003), Spectroscopic Analysis of Aspirin", *J. Chem. Educ.*, *80*, 174
5. Fu B, Shen Q, etc. (2005), Interfacial interaction of tartaric acid with hydroxyapatite and enamel. Journal of Materials science: materials in medicine, No.16, p. 827-831.
6. Lian Y, Jiang Y, Tian Y (1991), FT-IR spectroscopic study on Langmuir-Blodgett films of stearic acid. Acta Physico-Chimica Simica, Vol.7, No.1, pp.72-76.
7. Rajeshree Panigrahl, etc. (2012), Formulation of fast dissolving tablets of Lisinopril using combination of synthetic superdisintegrants. Asian J. Pharm. Tech. Vol.2: Issue 3, pp. 94-98.

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EVALUATION OF ENTRANCE SURFACE DOSES FOR X-RAY DIAGNOSTIC RADIOGRAPHY BY TLD

VLERËSIMI I DOZËS HYRËSE SIPËRFAQËSORE NË RADIOGRAFINË DIAGNOSTIKUESE ME RREZE X ME ANË TË TLD

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PËRMBLEDHJE
Në radiografinë diagnostike me rreze X doza hyrëse sipërfaqësore (DHS) është një parametër i rëndësishëm i ekspozimit mjekësor. Ky studim ka për qëllim të vlerësojë dozën hyrëse sipërfaqësore gjatë disa ekzaminimeve radiografike (torakale PA, Pelvic-ut AP dhe shtyllës kurrizore AP/Lateral) në qendra të ndryshme mjekësore të rajonit të Prizrenit, të Republikës së Kosovës. Për të vlerësuar dozën hyrëse sipërfaqësore gjatë radiografisë me rreze X, përdorëm detektorët termolumineshentë (TLD) të tipit Harshaw dhe matja e dozave është bërë me anë të sistemit Harshaw Thermo Scientific 4500. Doza e përgjithshme është vleresuar në 10 pacientë, për çdo diagnostikim te veçantë. Për të patur një pamje të qartë të dozës hyrëse sipërfaqësore, përdorëm 3 detektorë TLD në të njëjtën kohë. Vlerat mesatare për dozën e pacientit janë përkatësisht: 0,34 mGy/ekspozim në radiografinë e toraksit dhe 5,39 mGy/ekspozim për radiografinë e shtyllës kurrizore.
Fjalët çelës: ekspozimi mjekësor, doza e hyrjes sipërfaqësore, TLD

SUMMARY
Entrance Surface Dose (ESD) from x-ray diagnostic radiography is an important parameter of medical exposure, and evaluates the patient dose during examinations. This study aims to evaluate the Surface Entrance Dose during some diagnostic radiographic examinations (Chest PA and Lumbar spine AP/Lateral) in different medical centers, and we are focused in Prizren region of Kosovo Republic. For the evaluation of ESD during x-ray diagnostic radiography we used Thermo Luminescent Detector (TLD) Harshaw made and dose measurement has been performed using Harshaw Thermo Scientific 4500 System. To have a good picture of the SED for each examination procedure we used three TLD in the same time. The average values for patient dose are respectively: 0.34 mGy/examination in Chest PA and 5.35 mGy/examination in Lumbar spine AP.
Key words: medical exposure, entrance surface dose, TLD

INTRODUCTION

According to the Basic Safety Standards for Protection against Radiation, three categories of exposure are defined: Occupational Exposure, Medical Exposure and Public Exposure. Dose limits for occupational exposures and public exposures are regulated by law. In existing legislation, the effective dose limit for occupational exposure is **20 mSv,** averaged to 5

years, while the limit for public exposure is **1 mSv** in a year.

For medical exposures, however, there are no dose limits; while for them **Dose Guidance Levels** are assigned. To put into practice these guidance levels, the basic principles are proposed by the International Commission on Radiological Protection (ICRP).

Based on recommendations of the ICRP, International Atomic Energy Agency (IAEA) gave guidance levels for medical exposures in Basic Safety Standards 1994. In case when the patient exposure level exceeds the guidance levels, should be reviewed relevantly, so that the dose can be reduced to appropriate levels. In this research, are taken into account all IAEA's guidance levels, so that measurements of the surface dose at the body of the patient, which are made in medical hospital centers in the Prizren`s region of the Republic of Kosovo, can be compared with guidance levels for all kinds of X-ray examinations

METHODS AND MATERIALS

The measurements and the evaluation of entrance surface radiation doses that patients receive, have been carry out using Thermolumniscent dosimeters (TLD) of HARSHAW type. Recording of doses using TLD has been carry out by Thermo Scientific 4500 HARSHAW system, located at the Institute of Applied Nuclear Physics in Tirana. The study and evaluation of entrance surface radiation doses on patients are conducted in these medical centers in the region of Prizren: Family Medicine Center (FMC) in Rahovec, Radiology Department of the Regional Hospital in Prizren, Radiology Clinic "Imazheria" in Rahovec.

Radiological equipments (X-ray devices) with which we have conducted experiments are: in Family Medicine Center (FMC) in Rahovec: SIEMENS OPTIPHOS, Tube Opti 135/30/55 R, nominal voltage 135 kV, operating voltage value by 30 kV - 150 kV, add filter - 1.5 mm Al, production – 2010; in Prizren's Regional Hospital: APELEM, operating voltage value by 20 kV - 150 kV, add filter - 1.2 mm Al +0.3 mm Cu, production – 2000; in Radiology Clinic "Imazheria" in Rahovec: SIEMENS - SIREGRAPH CF, operating voltage value by 20 kV - 100 kV, filter - 2 mm Al.

Before starting the measurements we take notes of all the technical characteristics, voltage, current, power, exposure time, filtering etc. Two thermoluminescent dosimeters (TLD) are put within the radiation beam before and after the patient, but without hindering the appearance of the organ, the image of which required (Figure 1). Also, another TLD was put on the side, outside the beam at 1 meter distance from the center of the flock, for scattering radiation evaluation. TLD dosimeters were held in those positions to 10 exposures in different patients for the same examination, and then we evaluated the average value of entrance dose at the surface of the body by HARSHAW Thermo Scientific System.

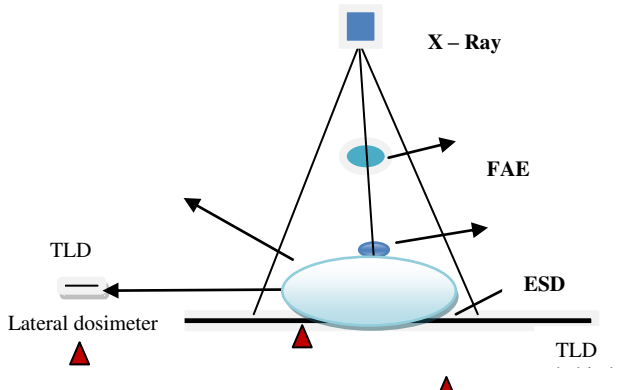

Figure 1. Method of measurement

RESULTS AND DISCUSSION

We have performed measurements by TLD detectors in three radiology centers in the region of Prizren, for three types of radiographic examination. Values obtained during the experimental procedures with the scope to evaluate the average entrance surface dose into patient's body, we could compare with theoretical calculations used by several methods.

The theoretical estimation of entrance surface dose could be called indirect assessment and evaluation, and the calculations will be carry out using the parameters of X – Ray equipments used during examination of patients, such as applied voltage and current in the X-ray tube, exposure values (mAs), Film Focus Distance (FFD), distance from the source to the patient's body surface - FSD, filtering, and other parameters.

In a first method, the evaluation of entrance surface dose (ESD) is done by this formula:

$$ESD = \text{BSF} \times \text{Tube Output}\left(\frac{mGy}{mAs}\right) \times \left(\frac{FFD}{FSD}\right)^2 \times \text{mAs}$$

where:

BSF – Backscatter Factor,

Tube Output – exposure that provides X-ray tube in the air at a certain distance,

FFD – Focus – Film Distance,

FSD – Distance from Focus to the surface of the patient's body,

mAs – Exposure.

Backscatter Factor (BSF) takes values from 1.1 to 1.5 for all kinds of examinations by X-ray radiography. To calculate the exposure to X-ray tube in a certain distance (mGy/mAs), we put a TLD dosimeter at the same distance from the source, which it is used to the relevant examination, without the presence of patient. In this case, the same voltage and current values, exposure and other settings were used, as in the case of the relevant examination. After reading the TLD on system HARSHAW 4500, in the case of X-rays of the lumbar spine, it appeared that the value of the dose received dosimeters is D = 3.58 mGy.

The parameters used with the first method are: exposure value 100 mAs, exposure time 355 ms, applied voltage in X – Ray tube 73 kV, Focus - Film Distance - FFD = 115 cm. Considering that the average body weight of patients examined was about 70 kg, the average patient width (chest level) is about 25 cm, then the distance from the source to the surface of the patient's body (Focus - Skin Distance) is 90 cm. The value of Backscatter Factor is 1.1. Using the appropriate formula for evaluating Entrance Surface Dose (ESD), the theoretically computed value is: **ESD = 6.39 mGy.**

Besides this method, an extensive use has also the method proposed by Tsai and Tong [ ]. This method suggests that incoming surface doses to be proportional to the production of air exposure with the relationship between the absorption coefficients of biological tissue and air and backscatter factor.

The formula proposed by Tsai and Tong has this form:

$$\text{ESD} = \text{FAE(mGy)} \times 0.00877\left(\frac{\text{mGy}}{\text{mR}}\right) \text{x} \left(\left(\frac{\mu}{\rho}\right)\text{tissue} \middle| \left(\frac{\mu}{\rho}\right)\text{air}\right) \text{x BSF}$$

where:

ESD – Entrance Surface Dose,

FAE – Free Air Exposure,

$0.00877\left(\frac{mGy}{mR}\right)$ is the factor that is used to convert the absorbed dose in mGy unit for 1 mR = 0.00877 mGy,

$\left(\left(\frac{\mu}{\rho}\right) tissue \middle| \left(\frac{\mu}{\rho}\right) air\right)$ is the ratio between the radiation absorption coefficients of tissue and air, and its value is approximately 1,06 for all the energies of X-rays used in radio diagnostics.

During the calculation we took into account the value of exposure to air at the examination of the Lumbar Spine according to the AP projection, which in our case is FAE = 3,58 mGy, and replacing this value and the values of other quantities in the above equation, we came up to this value of incoming surface dose:

ESD = 4.17 mG.

The following tables present direct measurements with TLD and their results.

Table 1. Results of TLD of chest radiography
Location of measurement: FMC - Rahovec

Position	TLD`s charge	Dose for 10 exposure	Dose for 1 exposure	ESD level by IAEA
Entry	129.2nC	3.44mGy	0.34mGy	0.4mGy
Exit	25.46nC	0.38mGy	0.038mGy	
Lateral	25.04nC	0.37mGy	0.037mGy	

Table 2. Results of TLD of chest radiography
Location of measurement: Regional Hospital of Prizren

Position	TLD`s charge	Dose for 10 exposure	Dose for 1 exposure	ESD level by IAEA
Entry	99.15nC	2.83mGy	0.28mGy	0.4mGy
Exit	13.20nC	0.3mGy	0.03mGy	
lateral	12.3nC	0.28mGy	0.028mGy	

Table 3. Results of TLD of the Lumbar Spine Radiography
Location of measurement: Radiology Clinic "Imazheria"

Position	TLD`s charge	Dose for 10 exposure	Dose for 1 exposure	ESD level by IAEA
Entry	1.843μC	53.91mGy	5.39mGy	10 mGy
Exit	34.1nC	0.705mGy	0.07mGy	
Lateral	20.04nC	0.29mGy	0.029mGy	

From the study in three radiological centers, TLD measurements and theoretical calculations that we carry out by implementing two methods that are used to evaluate the surface entrance dose, it results that theoretically calculated values are within the limits recommended by many international organizations. The organizations and institutions dealing with health care for exanimate patients by X-rays, recommend that the values calculated by theoretical methods have to be not outside ± 25 % of the values measured in practice using TLD.

In our case, doing the comparison between the values estimated by the methods and values of the surface entrance dose measured directly by TLD, appears that these values fit well within recommended limits. According to the first method, calculated value of the entrance surface dose (ESD) in the radiography of the spine (LS-AP) was 6.39 mGy, whereas with direct measurements with TLD measured values was 5.39 mGy. In this way the theoretically calculated value by method is not 25% higher than the experimentally measured value with TLD. According to the second method, to the same examination, the estimated value of the entrance surface dose was 17.4 mGy, which also is not 25 % less than the value measured by TLD.

CONCLUSIONS

We performed the evaluation of the Entrance Surface Dose In three radiological centers in the region of Prizren, Republic of Kosovo. From the experimental measurements performed during some diagnostic radiographic examinations, the recommended levels for guiding the surface entrance dose using X-rays are not exceeded.

This preliminary study and its results, produce a scientific base to continue in other medical centers and other diagnostic examinations.

The recommendations are given by the International Atomic Energy Agency (IAEA) have to presented as the guide for all diagnostic medical centers.

In view of TLD measurements, we can conclude that the levels of radiation doses of the three radio-diagnostic equipments and the patient exposures in these radiological equipments are within the recommended limits, recognized by the IAEA and other international agencies.

BIBLIOGRAPHY

1. Dosimetry in Diagnostic Radiology: An International Code of Practice. Technical Reports Series No. 457. IAEA, September 2007.
2. International Atomic Energy Agency, Operational Radiation Protection,A Guide to Optimization, Safety Series, No.101.IAEA, Vienna ,1990.
3. International Basic Safety Standards for Protection Against Ionizing Radiations and for the Safety of Radiation Sources. IAEA Safety Series, No.115 – I, IAEA, Vienna, 1994
4. International Commission on Radiation Units and Measurements, Quantities and Units in Radiation Protection Dosimetry, Rep. ICRU-51, Bethesda, MD (1993).
5. ICRP (1991). International Commission on Radiological Protection. Recommendations of the International Commission on Radiological Protection. ICRP Publication 60. Annals of the ICRP 21, No. 1-3 (Pergamon Press, Oxford),1990.
6. International Commission on Radiological Protection. Radiological Protection and 5.Safety in Medicine. ICRP Publication 73. Annals of the ICRP 26 (2). Oxford, UK: Pergamon Press, 1996.
7. Implementation of the International Code of Practice on Dosimetry in Diagnostic Radiology (TRS 457): Review of Test Results.
8. Recommendiations of the International Commission on Radiological Protection, Adopted by the Commission on November 1990, ICRP Publication 60, Annuals of the ICRP,21,Nos.1-3,1991
9. Tung CJ, Tsai HY: Evaluations of Gonad and Fetal Doses for Diagnostic Radiology, Proc. Natl. Sci. Counc. ROC(B), 1999 , 23(3) :107-113..

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FACTORS THAT DETERMINATE CLINICAL OUTCOME OF INTENSIVE CARE UNIT PATIENTS

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SUMMARY
Advances in critical care medicine have increased the chances of survival for patients of intensive care units. However, such patients consume a large proportion of medical resources. Aim: To identify factors that determinate the clinical outcome of patients treated in medical or surgical intensive care units (ICUs). **Results:** 432 patients aged 60.96 ± 16.2 years, 56.3 % male. The results of multiple regression analysis adjusted for confounders estimating the risk factors for mortality and complications were as follows: the age ≥ 60 years, APACHE II ≥15, presence of nosocomial infection, days on mechanical ventilation, presence of organ failure malnutrition and cumulated energy deficits during ICU stay. **Conclusion:** The risk factors for complications and mortality were age above 60 years, high APACHE II score, nosocomial infection, mechanical ventilation, malnutrition and cumulated energy deficit. Knowing the risk factors can be improved patient care, optimize resource planning and may decrease health care costs.
Key words: mortality, malnutrition, energy deficit, clinical outcome

Introduction

Advances in critical care medicine have increased the chances of survival for patients with acute exacerbation of underlying medical diseases or conditions, and/or extensive surgical intervention [1]. However, such patients are still at high risk for significant morbidity and death, and consume a large proportion of medical resources [2, 3]. There is a growing need to adjust clinical practice to improve the quality of care as well as conserve medical resources. Identifying modifiable risk factors that are associated with mortality is an important step in this direction.

This study sought risk factors for mortality, complications and longer ICU stays that have potential to be modified among patients treated in medical or surgical intensive care units (ICUs). The aim of the study was to determine the independent role of each risk factor associated with ICU mortality, complications and longer ICU stay in the hope that this information could provide a basis for the development of new policies to improve patient care and decrease the cost of ICU treatment.

Material and methods

In this prospective observational study, we evaluated the factors associated with ICU mortality among patients that stayed in medical or surgical ICUs for more than 4 days, who were

assumed to require prolonged physiologic support and were, therefore, theoretically at increased risk for nosocomial infection, complications and mortality. The study was designed as a prospective observational study, it was performed in the surgical and medical ICU of University Hospital Center of Tirana "Mother Teresa", Albania during January 2012-May 2013. Data were made anonymous for analysis.

Patient data: To control for the influence of severity of illness on ICU mortality and clinical outcome, we evaluated the severity of illness using the Acute Physiological and Chronic Health Evaluation (APACHE II) [4] upon admission to the ICU. Nutritional status on admission was assessed according to Nutritional Risk Screening 2002 [5]. The patients with a total score ≥ 3 was considered at nutritional risk. Undernutrition was evaluated by any of the 3 variables (BMI, recent weight loss, recent food intake).

As in our clinic is not available indirect calorimetry, energy target was set at 25 kcal/kg/day according to the ESPEN guidelines [6]. Energy delivery: total delivery includes energy from enteral and parenteral feeds, from non-nutritional sources (glucose and gluco-saline infusions used for drug dilution and fluid support). Energy balance was calculated as energy delivery – energy target, on daily basis. Data were collected on the nutritional risk screening, the time of start of feeding, energy delivery, and cumulated energy balance at the end of ICU stay. *Clinical follow-up:* Duration of ICU stay and length of ventilator stay, total complications, infectious complications and ICU mortality were recorded. Infectious complications were defined as sepsis or systemic inflammatory response syndrome [7], pneumonia, urinary tract infection, central venous catheter sepsis, and wound infection. Other complications were: post-operative and organ failure [8]. The duration of time in the ICU was defined as the time from admission of patients until they were ready for discharge. All patients were followed clinically until leaving the ICU or death and their outcome recorded.

Statistical analysis: A multiple logistic regression was applied to examine possible independent predictors of clinical outcomes and is calculated the odds ratio (OR) and its confidence interval. The relative risks (RR) between the risk factor variable and the mortality were calculated with their confidence intervals (CI). Statistical significance was considered at the level of $p \leq 0.05$. All tests were two tailed. SPSS 15.0 statistical package used to analyze the data.

Results

We studied 432 patients ≥ 18 years (aged 60.96 ± 16.2 years, 56.3 % male), that stayed more than 4 days in the ICU of University Hospital Center of Tirana "Mother Theresa" between 2010 and 2012. During ICU stay 143 patients (33.1%) have had nosocomial infections, 233 patients (53.9%) have had complications, ICU mortality rate was 28.7% (124 patients). The prevalence of malnutrition at the time of ICU admission was 63.6%. The results of multiple regression analysis adjusted for confounders estimating the risk factors for mortality ($p < 0.05$) were as follows: the age ≥ 60 years, APACHE II score ≥15, presence of nosocomial infections, days on mechanical ventilation, presence of organ failure, malnutrition at the time of ICU admission and cumulated energy deficits during ICU stay (table 1), it is also given by relative risk (table 2).

Table 1. Multiple logistic regression model for risk factors of mortality

Risk factors	OR	95% CI
Malnutrition	1.85	1.17 - 2.94
APACHE II ≥ 15	3.56	2.15-5.90
Age ≥ 60years	1.82	1.17-2.82
Presence of infections	1.64	1.06-2.52
Complications	2.24	1.44-3.47
Mechanical ventilation	1.90	1.38-2.62
Cumulated energy deficits during ICU stay	1.29	1.05 - 1.58

*$p < 0.05$

Table 2. Effects of sex, age, malnutrition, infections, complications, presence of malignity and the surgical treatment on mortality

	Dead (n)	Alive (n)	RR (95 % CI)
Male	60	128	1.21* (0.90-1.63)
Female	64	180	0.82* (0.61-1.10)
Age ≥ 60 years	84	165	1.54** (1.15-2.13)
Malnutrition	91	185	1.56** (1.10-2.21)
Nosocomial infection	51	92	1.41** (1.04-1.89)
Complication	84	149	1.79** (1.29-2.48)
Presence of malign disease	33	65	1.23* (0.88-1.71)
Surgical treatment	90	223	1.00* (0.72-1.40)

* P value > 0.05; ** P value < 0.05

Mortality was not related with the presence of malignant disease or urgent hospital admission.

Multivariate logistic regression analysis identified that risk factors for infectious complications and other complications were the age ≥ 60 year, APACHE II ≥ 15, mechanical ventilation, malnutrition, cumulated energy deficits during ICU stay, and the day when was started the nutritional support (table 3, table 4).

Table 3. Factors independently associated with complications

Risk factors	OR	95% CI
Age ≥ 60years	1.44	1.17 - 2.82
Malnutrition	5.37	3.50-8.26
APACHE II >15	2.31	3.15 - 5.90
Days on mechanical ventilation	1.14	1.07-1.21
Days on ICU	1.06	1.02-1.10
Urgent admission	1.62	1.08-2.43
Cumulated energy deficits during ICU stay	1.17	0.96 - 1.41
Day of start of nutritional support	1.27	1.08-1.49

*p < 0.05

Table 4. Factors independently associated with infectious complications

Risk factors	OR	95% CI
Age ≥ 60years	1.64	1.06-2.52
Malnutrition	2.25	1.44-3.52
APACHE II >15	1.03	1.00-1.07
Days on ICU	1.21	1.15-1.27
Days on mechanical ventilation	1.30	1.21-1.40
Urgent admission	2.79	1.83-4.24
Cumulated energy deficits during ICU stay	2.54	1.06-2.52
Day of start of nutritional support	1.36	1.19-1.56

* P value < 0.05

The results of multiple regression analysis adjusted for confounders estimating the risk factors for longer ventilatory support and ICU stay were as follows: urgent admission, surgical treatment, presence of nosocomial infections, presence of organ failure, malnutrition at the time of ICU admission and cumulated energy deficits during ICU stay.

Discussion

The ICU mortality of adult patients in the present study was 28.7%, which is higher than findings reported from the USA, Canada, and Japan (17–25%) [9]. This study sought to determine risk factors associated with ICU mortality that might be modified, to improve quality of care and patient outcomes. Multivariate analysis indicated that age above 60 years, high APACHE II score, nosocomial infection, mechanical ventilation, malnutrition and cumulated energy deficit were independently associated with ICU mortality.

The impact of ICU infections on hospital mortality is controversial. Prevalence and prospective cohort studies have reported various ICU infections to be independent risk factors for hospital mortality, including pneumonias or bloodstream infections [10, 11, 12], or ICU infections as a whole [13]. In the study of Vincent and colleagues [14], in the multivariate analysis of all patients, with hospital mortality as the

dependent variable and adjusting for possible confounders, infection was independently associated with a greater risk of hospital mortality (33.1% vs 14.8%; adjusted odds ratio, 1.51; 95% confidence interval, 1.36-1.68; $P < .001$). Infected patients had higher ICU and hospital mortality rates (25.3% vs 10.7% and 33.1% vs 14.8%, respectively; $P < .001$ for both) and longer ICU and hospital lengths of stay than those not infected. Our results support the findings of ICU-acquired infections increasing hospital mortality and the length of ICU stay.

The APACHE II score was initially developed for predicting the risk of death in an ICU population [4]. As in other studies high APACHE II score, in our study greater than 15 is associated with mortality [4].

The prevalence of 63.6% malnutrition in medical and surgical patients staying for more than four days in intensive care unit confirms the severity of this problem. A recent review of the world literature found that in 20 studies since 1990 the mean malnutrition rate in the hospital was 41.7% [15]. In another study the prevalence of malnutrition in the patients of the intensive care unit was as high as 47.6% on admission, using NRS 2002 method [16]. Previous studies have shown the impact of nutritional status on morbidity, mortality, LOS. ICU patients suffering from under-nutrition with a limited nutrition reserve have a poorer outcome [17].

Similarly to these studies, we were able to demonstrate that malnourished patients had significantly higher incidence of complications, increased mortality, longer ventilator and ICU stay. Giner showed that malnourished patients in an intensive care unit have a poorer prognosis and survival [17].

The present study as other studies [18], confirms that negative energy balance cumulated during inadequate nutrition support was associated with a higher rate of infectious complications, mortality, longer ventilator stay and longer ICU stay. Attempting to meet caloric targets may be associated with improved clinical outcomes in critically ill patients [19].

The mortality rate of critically ill patients under ventilatory support and patients with ARDS is elevated in both observational and interventional studies [20,21]. In the present study we found that ventilator support was correlated with infections, complications and mortality.

Conclusion: The risk factors for complications and mortality were age above 60 years, high APACHE II score, nosocomial infection, mechanical ventilation, malnutrition at ICU admission and cumulated energy deficit. Knowing the risk factors can be improved patient care, optimize resource planning and may decrease health care costs.

However, because the presence of severity of illness and age are not modifiable factors, increased emphasis on the control of a potentially modifiable factor such as nosocomial infection is particularly important.

ICU mortality was also significantly influenced by the nutritional status of the patients. Malnutrition was considered an independent risk factor, which significantly contributed to mortality, malnutrition was associated with a higher rate of infectious complications, overall complications, mortality, longer ventilator stay and ICU stay. Negative energy balance cumulated during inadequate nutrition support was associated with a higher rate of infectious complications, mortality, longer ventilator stay and ICU stay. Our findings suggest the need for implementation of Nutritional Risk Screening and Guidelines for nutrition support in critically ill patients in order to improve the clinical outcome of them.

References

1. Baue AE, Durham R, Faist E (1998), Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? Shock 10, 79–89.
2. Groeger JS, Strosberg MA, Halpern NA, et al (1992), Descriptive analysis of critical care units in the United States. Crit Care Med 20, 846–862.

3. Groeger JS, Guntupalli K, Strosberg MA, et al (1993), Descriptive analysis of critical care units in the United States: patient characteristics and intensive care unit utilization. Crit Care Med 21, 279–291.
4. Knaus WA, Draper EA, Wagner DP, Zimmerman JE (1985), APACHE II: a severity of disease classification system. Crit Care Med 13(10), 818-829.
5. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M (2003), ESPEN Guidelines for Nutrition Screening 2002. Clin Nutr 22, 415-421
6. Kreymann KG, Berger MM, Deutz NEP, Hiesmayr M, Jolliet P, Kazandjiev G, et al (2006), ESPEN guidelines on enteral nutrition: intensive care. Clin Nutr 25, 210-223
7. Bone RC, Balk RA, Cerra FB (1992), Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 101, 1644-1655.
8. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al (1998), Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on sepsis-related problems of the European Society of Intensive Care Medicine. Crit Care Med 26, 1793-1800.
9. Sirio CA, Tajimi K, Tase C, et al, (1992), An initial comparison of intensive care in Japan and the United States. Crit Care Med 20, 1207–1215.
10. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin M, Wolff M, Spencer RC, Hemmer M (1995), The prevalence of nosocomial infection in intensive care units in europe: results of the European Prevalence of Infection In Intensive Care (EPIC) Study. JAMA 274, 639–644.
11. Appelgren P, Hellstrom I, Weitzberg E, Soderlund V, Bindslev L, Ransjo U (2001), Risk factors for nosocomial intensive care infection: a long-term prospective analysis. Acta Anaesthesiol Scand 45, 710–719.
12. Craven DE, Kunches LM, Lichtenberg DA, Kollisch NR, Barry MA, Heeren TC, McCabe WR (1998), Nosocomial infection and fatality in medical and surgical intensive care unit patients. Arch Intern Med 148, 1161–1168.
13. Bueno-Cavanillas A, Delgado-Rodriguez M, Lopez-Luque A, Schaffino-Cano S, Galvez-Vargas R (1994), Influence of nosocomial infection on mortality rate in an intensive care unit. Crit Care Med 22, 55–60.
14. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin C.D, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K, for the EPIC II Group of Investigators (2009), International Study of the Prevalence and Outcomes of Infection in Intensive Care Units. JAMA 302(21), 2323-2329.
15. Norman K, Pichard C, Lochs H, Pirlich M: Prognostic impact of disease-related malnutrition (2008), Clinical nutrition 27(1), 5-15.
16. Nematy M, Mohajeri S, Moghadam Sh, et al (2012), Nutritional status in intensive care unit patients: a prospective clinical cohort pilot study. Mediterranean Journal of Nutrition and Metabolism 5 (2), 163-168.
17. Giner M , Laviano A, Meguid MM, et al (1996), In 1995 a correlation between malnutrition and poor outcome in critically ill patients still exists. Nutrition 12, 23–29.
18. Villet, S, Chiolero R, Bollmann M, Revelly J, Cayeux M, Delarue J, et al (2005), Negative impact of hypocaloric feeding and energy balance on clinical outcome in ICU patients. Clin Nutr 24(4), 502-509
19. Heyland DK, Naomi C, Andrew G (2011), Optimal amount of calories for critically ill patients: depends on how you slice the cake! Critical Care Med 39(12), 2619-2626.
20. Vincent JL, Akca S, De Mendonca A, Haji-Michael P, Sprung C, Moreno R, Antonelli M, Suter PM, SOFA Working Group (2002), The epidemiology of acute respiratory failure in critically ill patients. Chest 121**,** 1602-1609.

21. Behrendt CE (2000), Acute respiratory failure in the United States: incidence and 31-day survival. Chest 118, 1100-1105. .

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HELLP SYNDROME AS A COMPLICATION OF PRE-ECLAMPSIA IN PREGNANT ËOMEN ADMITTED TO THE UNIVERSITY CLINICAL CENTER OF KOSOVA

HELLP SINDROMA SI KOMPLIKIM I PRE-EKLAMPSISË NË SHTATZËNAT E HOSPITALIZUAR NË QENDRËN KLINIKE UNIVERSITARE TË KOSOVËS

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SUMMARY
Background - HELLP syndrome is thought to be a variant of preeclampsia, but it may be an entity all on its oën. The aim of investigation was to present the frequency of occurrence and its connection with other factors. Methods - Study included 430 subjects hospitalized in the University Clinical Center of Kosovo, in the period 2010-2012. Statistical analysis of data was conducted in SPSS. Results - The average age of patient's was 29.21 years. There is no significant difference between the sexes of babies. HELLP syndrome in our case is presented only in 4.2% of subjects. In subjects aged over 30 years HELLP syndrome is present in 14 cases and by those under 30 years old in only four cases. Conclusions - HELLP is a serious complication of preeclampsia which requires efficient management in order to prevent other more serious consequences for the health of pregnant women's.
Key words: pre-eclampsia, HELLP syndrome, pregnancy

PËRMBLEDHJE
Hyrje- HELLP sindroma është menduar të jetë variant i pre-eklampsisë por në shumë raste mund të zhvillohet edhe si entitet në vete. Qëllimi i kërkimit ishte që të paraqitet frekuenca e paraqitjes së kësaj sindrome dhe lidhshmëria e saj me faktorët tjerë. Metodologjia –Janë përfshirë 430 subjekte të hospitalizuar në Qendrën Klinike Universitare të Kosovës , në periudhën kohore 2010-2012. Analiza statistikore e te dhënave u krye në SPSS. Rezultatet - Mosha mesatare e pacienteve ka qenë 29.21 vjet. Nuk ka dallim sinjifikant në mes të gjinive të foshnjave të lindura. HELLP në rastet tona është paraqitur në 4.2 % të subjekteve. Në subjekte të moshës mbi 30 vjeç HELLP është paraqitur në 14 raste kurse nën 30 vjeç vetëm në 4 raste. Përfundimi– HELLP sindroma është komplikim serioz i cili kërkon menaxhim efikas në mënyrë që të parandalohen pasojat tjera më të rënda për shëndetin e shtatzënës.
Fjalët kyçe: pre-eklampsia, HELLP sindroma, shtatzënat

Background

Pre- eclampsai is pregnancy condition marked by high blood pressure and proteinuria after week 20 of gestation. This disorder appears and complicates 5-7% of pregnancies with an incidence of 23.6 cases per 100 000 thousand births in USA[1]. In recent years preeclampsia-eclampsia has been the second or third cause of the maternal deaths and is a frequent cause of fetal morbidity[2]. In overall, 10% -15% of maternal

deaths are directly linked to pre-eclampsia and eclampsia 3. Pre-eclampsia can cause serious complications to mother and fetus. One of the most sever complication is HELLP Syndrome (hemolysis, Elevated liver enzymes, low platelets). This is a serious complication of preeclampsia first described by Pritchard in 1954 while as the term was constituted by Weinstein in 1982. Among women with pre-eclampsia, 6% manifest abnormality associated with HELLP syndrome (typically increase of hepatic enzymes or decrease of the platelets), 12% manifest with two abnormalities and around 10% manifested with three abnormalities. HELLP syndrome can occur at any stage of pregnancy as well as in the puerperium but (the same as a pre-eclampsia) is very rare before the week 20th of gestation. A one third of all cases with HELLP syndrome occur after birth and only 80% of these patients have been diagnosed with pre-eclampsia before delivery [4, 5,6,7]. HELLP syndrome appears as a complication of 10-20% of the severe pre-eclampsia. In 20% of women occurs in late pregnancy, while in 30% occurs after birth. HELLP without hypertension and proteinuria appears to be in10-20% of cases [5,10]. The purpose of this study was to analyze cases of this syndrome in pregnant women hospitalized in UCCK and their links with other socio-demographic variables.

Methodology

In transversal study type were included 430 subjects hospitalized in the Clinic of Gynecology at the University Clinical Center of Kosovo, in the period 2010-2012. Inclusion criteria: All pregnant women with arterial hypertension at or after the 20th week of pregnancy, patients who have swelling in or after week 20 of pregnancy, patients with proteinuria with excretion of 0.3g or more of the proteins in a sample within 24 hours . Exclusion criteria: all patients with prior history of essential hypertension, patients with a history of epilepsy, pregnancies with many children and patients with diabetes. Data collection has been retrospectively in 2010 and prospectively for another period of time. It consisted of a collection of schedule for each subject included in the study. Pearson Chi-square and Exact test was used to assess the relationship between variables and factors while T test was used to test independent samples, to determine the incidence of cases, their average and significance between variables. Statistical analysis of data was conducted in SPSS.

Results and Discussion

There are analyzed a total of 430 cases. The average age of patients was 29.21 years, DS 6.855. From them on the age of 30 or less were 135 and 295 patients have been older than 30 years. Nullipara were 56.5% while 43.7% have been nullipara belonging to the age group 18-35 years. There is no significant difference between the sexes of babies born from mothers with preeklampsia (X2 test> 0.005). Of the total number of cases based on the clinical picture and symptoms we found that 86.5% of patients have been with mild preeclampsia.

Table 1 General data and clinical characteristics of patients with preeclampsia (PE).

Clinical data		Nr of patients	p
Age (years)	≤ 30	295 (68.6%)	
	> 30	135 (31.4%)	0.004
Parity	nullipara	243(56.5%)	
	multiparity	187 (43.5%)	0.007
Nulliparas	< 18 year	107(24.9%)	
	18-35 year	188(43.7%)	
	>35 year	135(31.4%)	0.000
Baby sex	male	206(47%)	
	female	224(53%)	0.385
Type of PE	Mild	372(86.5%)	
	Severe	58(13.5%)	0.000

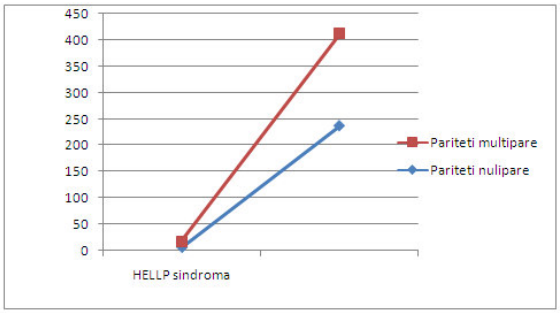
On the onset of HELLP syndrome as a frequent maternal complication in preeclampsia were found statistically significant differences between analyzed age groups. Of 135 subjects aged over 30 years HELLP syndrome is present in 14 cases and from 295 subjects aged less than 30 years only four cases have evolved in HELLP syndrome.

Table 2 Connectivity between age and HELLP syndrome

		HELLP Syndrome		
		Yes	No	Total
Age	≤ 30 year	4	291	295
	> 30 year	14	121	135
Total		18	412	430
Pearson Chi-Square=18.764		p= 0.000		

Among the subjects with HELLP syndrome as a maternal complication we found statistically significant difference between subjects with different parity. In the group of multiparity women are 12/135 subjects who developed HELLP whereas in nullipara HELLP was developed only in 6/295 subjects.

Figure 1 Connectivity between parity and HELLP syndrome



We attempt to find a distinction or preference of sex of the embryo on the presentation of HELLP syndrome but the differences are not significant, although here is a slight difference in favor of female fetus ratio 6.3% / 1.9 % of cases with HELLP syndrome.

Table 3 Connectivity between the gender of the embryo and HELLP syndrome in our subjects

		HELLP syndrome		
		Yes	No	Total
Baby sex	male	4 (1.9%	202 (98.1%)	206
	female	14 (6.3%	210 (93.8%)	224
Total		18 (4.2%)	412 (95.8%)	430
Pearson Chi-Square=4.966		p= 0.026		

There are a total of 430 cases analyzed patients. The average age of patients was 29.21 years. On the onset of HELLP syndrome we founded statistically significant differences between analyzed age groups by dominating in the onset of this syndrome at age greater than 30 years. Multiparity subjects in our study were presented with a significantly greater than nulliparities According to the paper from Rathore R, HELLP syndrome as a complication of preeclampsia appear in 4-12% of cases, which is identical to our findings, but there are other cases where it may appear in 25% of subjects[8]. Croatian authors have found that HELLP syndrome is present in 20% of cases which is very high number compared with our findings (4.2%). The same author has found that in these cases with HELLP and preeclampsia also was developed DIC in but much less than HELLP[9]. According to Lana K. Wagner, HELLP syndrome appears in 4-14% of cases, which is approximately the same result with our findings. In this paper was stated that HELLP syndrome may appear as a separate entity or as a variant of preeclampsia[1].

Conclusions

- Pre-eclampsia is the most common medical complication of pregnancy and is associated with substantial mortality and morbidity of mother and baby.
- They are found statistically significant differences between analyzed age groups .
- HELLP Syndrome is presented much often among the multiparity pregnancies.

- There are no differences in the onset of HELLP syndrome in women who have born children of different sexes.

References

1. Lana K. Wagner; Diagnosis and Management of Preeclampsia, American Family Physician, December 15, 2004, Volume 70, Nr. 12,
2. Longo, Sherri et al**;** Preeclampsia and Eclampsia Revisited, Southern Medical Journal: 2003 - Vol 96 - Issue 9 - p 891-899,
3. Jennifer Uzan et al; Pre-eclampsia: pathophysiology, diagnosis,and management, Vascular Health and Risk Management, 2011,
4. A.D.A.M. Medical Encyclopedia; HELLP syndrome , November 21, 2010,
5. B.H. Lim MRCOG et al; HELLP Syndrome: an Often Unrecognized Complication of Preeclampsia, Australian and New Zealand Journal of Obstetrics and Gynaecology, Volume 31, Issue 1, p 20–21, 1991,
6. Liliana S. Voto, Alicia M. Lapidus; Effects of preeclampsia on the mother, fetus and child, OBGYN.net, July 26, 2011,
7. Errol R. Norwitz et al.**;** Acute Complications of Preeclampsia, V. 45, Number 2, 2002,
8. Rathore R et al; Complications and Outcome of Patients of Pre-eclampsia and Eclampsia Presenting to Medical Wards of Mayo Hospital Lahore, ANNALS VOL 16. NO.1 JAN. - MAR. 2010,
9. Brezovec Cvetnić, Blanka; Bartolek, Dubravka; Patološka stanja u trudnoći (preeklampsija i eklampsija), Medicinska Naklada, Zagreb, 2005,
10. Eric A P Steegers; Pre-eclampsia,; 376: 631–44, Lancet 2010.

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A PROOF OF APPLICATION OF THE HEALTH BELIEFS MODEL: REFLECTIONS FOR THE HEALTH PSYCHOLOGY

NJË SPROVË E APLIKIMIT TË MODELIT TË BESIMEVE PËR SHËNDETIN : REFLEKTIM PËR PSIKOLOGJINË E SHËNDETIT

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PËRMBLEDHJE
Modeli i besimeve për shëndetin është shumë aktual në fushën e psikologjisë së shëndetit. Njohja e këtyre besimeve në mjedisin tonë nuk ka qenë objekt i hulumtimeve deri tani. Një pyetësor i përshtatur për matjen e këtyre besimeve është plotësuar nga pacientët, përcjellësit e tyre, studentët dhe punëtorët shëndetësorë. Analizat statistikore të përdorura na tregojnë se karakteristikat individuale si mosha, gjinia, vendbanimi, niveli i shkollimit, prezenca e sëmurjes dhe nervozizmi tregohen si variabla modifikuese të besimeve mbi shëndetin dhe deridiku parashikuese. Nuk gjenden dallime sinjifikative në grupet e respondentëve në nivelin e besimeve mbi shëndetin në konstruktet e matura. Në vendin tonë është shumë e dobishme të promovohet qasja e psikologjisë së shëndetit si një element shumë i rëndësishëm për efikasitetin e politikave shëndetësore në shëndetin publik.
Fjalë kyçe: besimet, shëndeti, pacientët, studentët, punëtorët shëndetësorë.

SUMMARY
Model of health beliefs is very actual in the field of health psychology. Knowledge of these beliefs in our country weren't subject of surveys. One questionnaire adopted for measuring of those beliefs has been filled out by patients, their companions, students and health workers. Used statistic analyses shows that individual characteristics such as age, gender, place of residence, education level, presence of illness and nervousness are shown as modified variables of beliefs on health and sometime are predictive. There are no significant differences found to the groups of respondents about the health and measured constructs. In our country is very useful to promote approach of health psychology as a very important element for health policy effectiveness in the public health.
Key words: beliefs, health, patients, students, health workers

INTRODUCTION

The health beliefs model is a most used theory in education and promotion of health[1]. This model has been developed on 1950 and its basic concept is that behaviours for health are determined from beliefs or personal perceptions about illness and possible strategies to avoid it [2, 5]. Basic constructs of this model are: perceptions about illness seriousness, perceptions for sensitivity or risk from illness, perceptions of the usefulness of an behaviour and perception about the obstacles for changing of something related to the health [4] . These perceptions as single or combined could be used for explaining of behaviours for about health. In this model are considered some individual characteristics which can change these constructs and they are named as modifying variables: culture, education level,

previous experiences, skills etc [4]. To this model were added later on: cues for action (events, people or events which are encouraging people for such action, ex. media, advertising, advices, etc...) [4] and self-effectiveness (personal beliefs in their abilities to do something) [6].

Main goal was measurement and estimation of health beliefs to the patients and their families, students and medical professionals as a pre-condition for increasing of positive behaviours about health. The importance of this paper is to promote health psychology approach in our country as a one important element for effectiveness of the health policy within the public health.

METHODOLOGY AND MATERIALS

It is a cross-sectional quantitative survey with a questionnaire which is based on the model of health beliefs (Health Beliefs Model Questionnaire) [3] which is adopted by authors and filled out by patients, their companions, students and medical professions. The questionnaire has measured perceptions on benefits from health protection behaviours, on the feeling overall threats from illness, for sensitivity to have an illness, the severity if the patient has an illness and obstacles for health protection. Questionnaire was provided directly to be filed out within the medical environment of the family medicine in Prizren, in Fama College, and also online through the posted link in Google Drive and mailed to the electronic mails of groups of students. Participants (N=116) were patients or their companions (37.9 %) respondents online (26.7 %), student (25 %) and health workers (10.3 %). Participant's age was 18-78 (the average 34, 19). The presence of female was biggest (66 %). Based on the residence – from urban places were (55 %). In regards the education, with the highest education were (64.3 %), high (21.6 %) and primary (13.8 %). Half of them were unemployed (52.2%). Majority of them didn't declare that are suffering from some illness. (82.8 %). All data has been analyzed by IBM SPSS statistics 19 and Microsoft Excel 2007.

RESULTS AND DISCUSSION

Respondents with the oldest age significantly have more beliefs that they are more sensitive for illness (r=.28, p<.00); and non- significantly have more beliefs to get ill severe, perceive many barriers, and in generally believe that are more threatened and the chances to benefit from their actions related to the health are small (non-significantly) (Tab 1.). Females does have beliefs significantly more that they get ill severely **(**r=.19, p<.04); non significantly they believe that are more sensitive, they shows more barriers for seeking help and perceive to have more opportunities to benefit from their actions related to the heath; in regards of the threats in general from the illnesses males shows themselves more threatened (Tab 1.). Respondents from the rural zone non - significantly believe that will benefit more from interventions, they feel are threatened, they are most sensitive; but they have less severity of illness and less barriers (Tab 1.). Persons with the low education non- significantly have beliefs that are most sensitive, most threatened, they get ill severely and they see many barriers and also they believe in fewer benefits from their interventions. Persons with illness significantly had beliefs that are most sensitive (r=.31, p<.00); non- significantly that they get ill severely and believe in more benefit from their interventions; they feel less threatened from the illness in general. (Tab.1.)

We have measured perception about how much respondent feel nervous and how much they are happy. 34.5 % of them are feeling nervous more than it expected and 40 % of them have expressed feeling happy. Persons with the high level of nervousness have declared that significantly are having more beliefs that are more sensitive for illness (r=.26, p<.00); and in generally feel threatened (r=.27, p<.00); non-significantly they believe in benefit from their interventions; also they believe less in severity of illness and perceive less barriers (Tab.2.). Persons with the highest levels of happiness have

declared non- significantly less beliefs that are sensitive; they believe in benefits from their interventions; they feel less threatened; and less illness severity, also they have perceived more barriers (Tab.2.).

Tab 1. Correlations of modifying variables with constructs of health belief model

Correlations						
Spearman's rho		Benefits	General threat	Sensitivity	Severity	Barriers
Age	Coeff.	-.090	.044	.289**	.097	.140
	Sig.	.340	.647	.002	.311	.159
Gender	Coeff.	.113	-.108	.007	.190*	-.028
	Sig.	.225	.252	.939	.044	.782
Residence	Coeff.	.138	.156	.071	-.059	-.100
	Sig.	.148	.103	.467	.545	.326
Education	Coeff.	.033	-.055	-.179	-.159	-.121
	Sig.	.725	.563	.061	.095	.222
Illness presence	Coeff.	.159	-.095	.313**	.073	.024
	Sig.	.087	.315	.001	.440	.811
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

Tab 2. Correlations of nervousness and happiness with constructs of health belief model

Correlations						
Spearman's rho		Benefits	General threat	Sensitivity	Severity	Barriers
Nervousness	Coeff.	.108	.270**	.265**	-.009	.008
	Sig.	.249	.003	.005	.925	.938
Happiness	Coeff.	.000	-.088	-.122	.017	-.036
	Sig.	.997	.354	.201	.856	.715
**. Correlation is significant at the 0.01 level (2-tailed).						

We found significant difference in between the groups without illness and those with illness in the level of nervousness where first group (md=1, n=96) and second group (md=2, n=20) U=691.500; z=-2.155; p=.031; r=.20 (effect size small). We found significant difference between the groups without illness and those with illness in the line with the happiness where first group is (md=2, n=95) and second (md=1, n=20) females are having highest level of those U=595.500; z=-2.721; p=.007; r=.25 (effect size small). It is

obvious that presence of illness is influencing level of nervousness and feelings of happiness.

The analysis of Kruskal Wallis Test doesn't shows significant differences in the level of perceptions between respondent's patients/companions, students, health workers and those filled online.

We found significant differences from the analyses of Mann-Whitney U between females and males in level of perception of seriousness if they get ill from the heart diseases, cancer and paralyze and.

Medical staff is seeing more probability for nerves diseases, blood pressure, cancer but non-significantly; fear to be ill is seeing as a main barrier for not receiving medical help and they are showing high levels of believes about sensitivity.

Regression analyses and multiple standard is used to see the model of variable (gender, age, residence and presence of illness) and model of variables (nervous and happiness). First model have achieved statistical significance only for prediction of variables for benefits from their behaviours related to the health and the significant unique contribution were given by gender and residence, education and presence of illness but not age (Tab.3) ; and to variable for sensitivity on illness the unique significant contribution gave only presence of illness (Tab.3). Beta coefficient of model is presented in table (Tab.3).

Second model have achieved statistical significance on for prediction of variables of threatening in general by illness and unique contribution significant is given only by nervousness (Tab.4). Beta coefficient of the model is presented in the table (Tab.4).

Tab 3. Analysis of multiple standard regressions for age, gender, residence and presence of illness predicting benefits and sensitivity

Model variables	Benefits		Sensitivity	
	R square (.128) Sig (.013)		R square (.188) Sig (.001)	
	Beta	Sig	Beta	Sig
Age	-.053	.632	.205	.060
Gender	.221	.029*	.059	.545
Residence	.232	.026*	.008	.937
Education	.244	.035*	-.138	.216
Illness presence	.230	.025*	.222	.026*

Tab 4. Analysis of multiple standard regressions for nervousness and happiness predicting general threat and sensitivity

Model variables	General threat		Sensitivity	
	R square (.011) Sig (.002)		R square (.103) Sig (.003)	
	Beta	Sig	Beta	Sig
Nervousness	.305	.001*	.280	.003*
Happiness	-.079	.388	-.108	.250

CONCLUSIONS

Beliefs on health should be investigated and to be target of preventive programs aiming to increase positive behaviours for improvement of the heath of population.

Individual characteristics like, age, gender, residence, education level, and especially being ill are like predictor for beliefs on benefits from behaviours for protection of health and also for perception of sensitivity, and as such they have to be addressed. Level of nervousness is predictable of perception of general threats and sensitivity.

There were no differences found between patients/ companions; online respondents; students and health professionals in beliefs on health.

BIBLIOGRAFIA

1. Glanz K, Rimer BK, (1997), Theory at a Glance: A Guide for Health Promotion Practice. Bethesda, MD: National Cancer Institute.
2. Hochbaum GM, (1958). Public Participation in Medical Screening Programs: A Sociopsychological Study.PHS Publication No. 572. Washington, DC: U.S. Government Printing Office.
3. Norman P, Fitter M, (1989) Intentions to attend a health screening appointment: some implications for general practice, Counselling Psychology Quarterly ,2: 261–72.
4. Stretcher V, Rosenstock IM (1997), The Health Belief Model. In Glanz K, Lewis FM, Rimer BK, (Eds.). Health Behavior and Health Education: Theory, Research and Practice. San Francisco: Jossey-Bass.
5. Rosenstock IM (1974), Historical origins of the Health Belief Model. Health Education Monographs, 2,328-335.
6. Rosenstock IM, Strecher VJ, Becker MH (1988), Social learning theory and the Health Belief Model. Health Education Quarterly, 15(2), 175–183.

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FEATURES OF HER2/neu BREAST CANCER IN ALBANIAN WOMEN

TIPARET E KANCERIT TË GJIRIT ME HER 2/neu NË GRATË SHQIPËTARE

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SUMMARY
Overexpression of glycoprotein HER2/neu, that act as a growth factor receptor on cell surface, is a marker for poor prognosis. We studied a case-control study of 25 patients with breast cancer, diagnosed between January 2006 and December 2007 at "Nene Tereza" Oncologic Hospital in Tirana to compare the clinical features and outcomes for women with HER2/neu breast cancer. **Results:** In this study, we found association between size of the tumor, nodal status, Ca 15-3 and relapse with HER-2/neu overexpression ($p<0.05$), while presence of estrogen receptor (ER; 57%/78%) and progestron receptor (PR; 57%/67%) correlates inversely with HER-2/neu overexpression. **Conclusion:** Patients with excessive expression of HER 2/neu have worst prognosis and should be taken into consideration when selecting appropriate schemes of treatment.
Key-words: Breast cancer, HER 2/neu, receptor status, relapse.

PËRMBLEDHJE
Mbishprehja e glikoproteinës HER 2/neu, që vepron si receptor i faktorit të rritjes në sipërfaqe të qelizës, është tregues i një prognoze të varfër. Në këtë studim rast-kontroll të 25 pacienteve me kancer gjiri, të diagnostifikuara gjatë Janarit 2006 deri në Dhjetor 2007 në spitalin Onkologjik "Nënë Tereza" në Tiranë për të krahasuar tiparet klinike dhe rezultatet për gratë me kancer gjiri me HER2/neu. **REZULTATET. N**ë këtë studim duket shoqërimi i përmasave të tumorit, statusit të nodusit, CA 15-3 dhe rishfaqjes së tumorit me mbishprehjen e HER2/neu ($p<0.05$), ndërsa prania e receptorëve të estrogjenit (ER; 57%/78%) dhe progesteronit (PR; 575/67%) shoqërohet në mënyrë inverse me mbishprehjen e HER2/neu. **KONKLUZIONI.** Pacientet me mbishprehje të HER2/neu kanë prognozën më të rëndë dhe duhet të merren në konsiderate në zgjedhjen e duhur të mënyrës së trajtimit.
Fjalë-çelës: Kanceri i gjirit, HER 2/neu, statusi i receptorit, rishfaqja

INTRODUCTION

The HER2/neu (HER2) proto-oncogene, located on chromosome 17, encodes a 185-kDa glycoprotein that acts as a growth factor receptor on the cell surface (Roskoski R, Jr 2004). In the breast, oncogenic overexpression of the HER2 protein is both a marker for poor prognosis and a target for trastuzumab (Herceptin). Cells

transfected with HER-2/neu acquire a more malignant phenotype with stimulation of cell proliferation¸ invasion and metastasis. This has been confirmed in the clinic: women with Her-2/neu positive breast cancer have a worse prognosis than those with Her-2/neu negative cancer (4, 6, 7, 9, 11, 19).

Although Her-2/neu overexpression has been correlated with poor prognostic tumor characteristics such as higher histological grade¸ increased tumor size¸ increased number of involved lymph nodes and negative or lower estrogen receptor (ER) expression.

The HER2 oncoprotein has emerged as an essential biomarker in the treatment of breast cancer patients. Once the primary breast cancer is removed, there is an increasing need to detect breast cancer recurrence. Many studies show that the HER2 status of the primary tumor may reflect recurrent cancer. Currently, in Albania tumors from breast cancer patients are tested quantitatively for HER2 positivity using immunohistochemistry (IHC). Determination of the correct HER2 tumor status is critically important for guiding the therapy of patients with HER2 positive breast cancer treated with Trastuzumab (Herceptin) (16).

Although the biological and clinical implications of cytoplasmic HER2 proteins are largely unknown, recent studies indicate that some variant truncated forms of HER2 proteins play a significant role in the growth of human breast cancer (2, 13, 14). Shedding of the extracellular domain of HER2 is known to affect the binding of HER ligands and Her receptors and to affect the relevant signaling in the cells. In addition, a recent study indicated that the expression of p95 HER2, which is an NH2-terminally truncated fragment, was correlated with the nodal involvement and poor prognosis of patients with primary breast cancer. Therefore, it is important to study the role of HER2 proteins in more depth.

In the present paper we have evaluated overexpression of HER2 in 25 breast carcinomas to evaluate its potential prognostic value in relation to conventional clinicopathological parameters and ER/PR status.

MATERIALS AND METHODS

The association between breast tumor characteristics and expression of ER, PR¸ Her-2/neu overexpression, patient's age, tumor size, tumor nodus, tumor grade and tumor marker were retrospectively evaluated in 25 women with primary breast cancer diagnosed between January 2006 and December 2007 at "Nene Tereza" Oncologic Hospital in Tirana, treated till January 2010- December 2011.

HER-2/neu Evaluation. HER-2/neu expression was assessed in formalin-fixed, paraffin-embedded tumor specimens. For immunohistochemical staining, 4 µm-thick sections were cut from a representative paraffin block of breast carcinoma. The sections were mounted on charged slides, deparaffinized in xylene, and rehydrated in descending grades of ethanol. Sections were then subjected to heat-induced epitope retrieval by immersion in a 0.001 M concentration of citrate buffer preheated to >90°C and heated in an electric steamer. Endogenous peroxidase activity was blocked by a 5-min treatment with 3% hydrogen peroxide. The slides were incubated with anti-HER-2/neu monoclonal antibody (1:100 dilution) on a Dako Autostainer. The staining was performed by using peroxidase kit (Dako). 3-3-diaminobenzedine was used as chromogen.

The slides were counterstained with Mayer hematoxylin. The intensity of HER-2/neu overexpression was graded by 0, 1+, 2+, or 3+. Overexpression of HER-2/neu was defined as positive 3+ membranous staining in >10% of the invasive carcinoma cells.

ER/PR Status. The information regarding ER and PR status of the primary tumors was retrieved from patients medical records. Tumor specimens were considered negative for ER and PR if staining for both receptors was negative; specimens were considered positive for ER/PR if

staining for either or both receptors was positive.

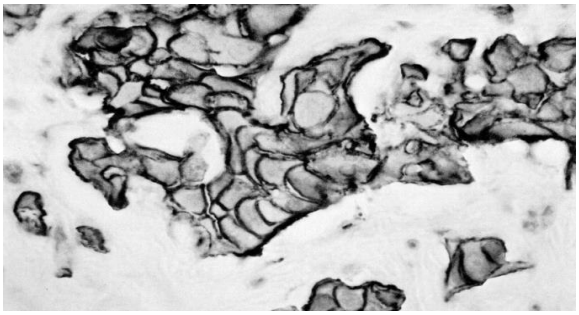

Fig 1. Immunohistochemical analysis for Her-2/neu. Positive staining of tumor cell membranes (400×).

Statistical Analyses. The χ2 test was used to examine the categorical variables and to assess possible associations between clinicopathological and molecular factors and HER-2/neu expression. The results were considered statistically significant if the P value was < 0.05. All analysis were performed with SPSS version 15 for windows.

RESULTS AND DISCUSSIONS

Table 1. summarizes patient characteristics for the 11 patients with HER-2/neu-overexpressing tumors and for the remaining 14 patients with low HER-2/neu-overexpressing tumors. We found that 72% and 64% of cases were ER positive and PR positive, respectively. HER-2/neu-overexpressing tumors (3 of 4) were negative for ER, and 14 of 4 HER-2/neu-negative tumors were positive for ER. Presence of estrogen receptor (ER) and progestron receptor (PR) correlates inversely with HER-2/neu overexpression. Thus, 3 of 4 cases (43% / 22%) had correspondence between HER-2neu overexpression and absence of ER expression (p < 0.05).

Patients with HER-2/neu-overexpressing positive tumors tended to have worse prognostic features overall. Many others have found, as we did, that HER-2/neu overexpression correlated with absence of ER expression (10). The exact molecular mechanism of this phenomenon remains to be elucidated, but the finding of estrogen-mediated down-regulation of HER-2/neu in breast cancer cell lines implies the existence of reciprocal control at the level of gene regulation (5). In our study, 11 (39%) of the breast cancer women had HER2 overexpression. In general, 70–90% of all breast cancer patients are considered to be HER2-negative by standard tissue tests. This group, including triple-negative breast cancer (TNBC) patients, do not have access to approved HER2-targeted therapies, such as Trastuzumab.

It was reported (20) that approximately 17–20% of patients with breast cancer whose tumors initially tested negative for HER2 may develop a recurrent HER2 tissue-positive metastatic tumor. So, the HER2 status must be revaluated in the metastatic tumor to help identify patients with latent HER2-positive status as pointed out by Ardavanis and colleagues (1), as well as other groups (18).

No association were found between age of patient and HER-2/neu overexpression. We found association between size of the tumor, nodal status, Ca 15-3 and relapse with HER-2/neu overexpression (p<0.05).

Patients with HER-2/neu-overexpressing tumors were more likely to have been presented with metastatic disease. Many

Table 1. Summary of characteristics of HER-2/neu positive vs. negative

	HER2+		HER2-	
age	no	percent	nr	percent
>=45years	3	43	9	50
<45vjec	5	57	9	50
Tumor size				P<0.05
T1	0	0	6	61

T2	7	100	11	6
T3			1	6
Nodal status				P<0.05
N0	0	0	5	28
N1	7	100	11	61
N2			2	11
HG				P>0.05
I	1	14	2	11
II	6	86	15	83
III			1	6
ER receptor				P<0.05
pozitive	4	57	14	78
negative	3	43	4	22
PR receptor				P<0.05
pozitive	4	57	12	67
negative	3	43	6	33
Relapse				P<0.05
Jo	5	71	16	89
Po	2	29	2	11
Ca15-3				P=0.0059
≤ 35 U/ml	6	86	16	89
>35 U/ml	1	14	2	11
CEA				P>0.05
≤3.4 U/ml	6	86	14	78
>3.4 U/ml	1	14	4	22

publications that have compared elevated value of HER2 tissue status in the primary tumor with tumor size, nodal status, histological grade and hormonal receptor have clearly shown a strong association (4, 6, 7, 8, 9, 11, 19). There was an acknowledgement from the authors that there is an increasing body of evidence that shows HER2 levels are closely associated with adverse clinicopathological and molecular factors. These reports as well as several others showed that the HER2 status was a strong prognostic indicator and patients HER2+ had a worse clinical outcome than patients HER2- (3).

It has been shown over several years with numerous clinical studies and thousands of breast cancer patients that overexpression of HER2/neu reflects the activity of tumor cells and the clinical course of disease towards progression or regression. HER-2/neu plays an important role in cell proliferation and differentiation, and its overexpression seems to correlate with an increase in the proliferative activity of breast cancer cells (8).

Analysis of all breast cancer patients for elevated HER2 levels can provide valuable information for patient management in both the HER2-positive and HER2-negative groups. There is considerable preclinical evidence to support the existence of interaction between HER2 and estrogen-receptor (ER) signalling pathways in breast cancer and HER2 overexpression associated with resistance to hormonal therapy which would take in consideration alternative therapeutic strategies as the number of HER2 targeted drug choices continues to increase.

REFERENCES

1. Ardavanis A, Kountourakis P, Kyriakou F, et al (2008), Trastuzumab plus paclitaxel or docetaxel in HER-2-negative/HER-2 ECD-positive anthracycline- and taxane-refractory advanced breast cancer. Oncologist 13(4), 361–369.

2. Egeblad M, Mortensen OH, Jäättelä M (2001), Truncated ErbB2 receptor enhances ErbB1 signaling and induces reversible, ERK-independent loss of epithelial morphology. Int J Cancer 94(2, 185-91.

3. Finn RS, Gagnon R, Di Leo A, Press MF, Arbushites M, Koehler M (2009), Prognostic and predictive value of HER2 extracellular domain in metastatic breast cancer treated with lapatinib

and paclitaxel in a randomized phase III study. J Clin Oncol 27(33), 5552–5558.

4. Fornier MN, Seidman AD, Schwartz MK, et al (2005), Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate. Ann Oncol 16(2), 234–239.

5. Grunt T. W., Saceda M., Martin M. B., Lupu R., Dittrich E., Krupitza G., Harant H., Huber H., Dittrich C (1995). Bidirectional interactions between the estrogen receptor and the cerbB-2 signaling pathways: heregulin inhibits estrogenic effects in breast cancer cells. Int. J. Cancer 63, 560-567.

6. Köstler WJ, Schwab B, Singer CF, et a (2004), Monitoring of serum HER-2/neu predicts response and progression-free survival to trastuzumab-based treatment in patients with metastatic breast cancer. Clin Cancer Res 10(5), 1618–1624.

7. Kong SY, Nam BH, Lee KS, et a (2006), Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. Clin Chem 52(8), 1510–1515.

8. Molina MA, Sáez R, Ramsey EE, et al (2002), NH(2)-terminal truncated HER-2 protein but not full-length receptor is associated with nodal metastasis in human breast cancer. Clin Cancer Res 8(2), 347–353.

9. Molina R, Auge JM, Farrus B, et al (2010), Prospective evaluation of carcino embryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) in patients with primary locoregional breast cancer. Clin Chem 56(7),1148–1157.

10. Paik S., Bryant J., Park C., Fisher B., Tan-Chiu E., Hyams D., Fisher E. R., Lippman M. E., Wickerham D. L., Wolmark N (1998), erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. J. Natl. Cancer Inst. (Bethesda) 90, 1361-1370.

11. Sandri MT, Johansson H, Colleoni M, et al (2004), Serum levels of HER2 ECD can determine the response rate to low dose oral cyclophosphamide and methotrexate in patients with advanced stage breast carcinoma. Anticancer Res 24(2C), 1261–1266.

12. Roskoski R, Jr (2004), The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun 319, 1-11.

13. Sáez R, Molina MA, Ramsey EE, et al (2006), p95HER-2 predicts worse outcome in patients with HER-2-positive breast cancer. Clin Cancer Res, 12(2), 424-31.

14. Scott GK, Robles R, Park JW, et al (1993), A truncated intracellular HER2/neu receptor produced by alternative RNA processing affects growth of human carcinoma cells. Mol Cell Biol, 13(4), 2247-57.

15. Scaltriti M, Rojo F, Ocaña A, et al (2007). Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 99(8), 628–638.

16. Slamon DJ, Leyland-Jones B, Shak S, et al (2001), Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344(11), 783–792.

17. Sørensen PD, Jakobsen EH, Langkjer ST, et al (2009), Serum HER-2 concentrations for monitoring women with breast cancer in a routine oncology setting. Clin Chem Lab Med 47(9), 1117–1123.

18. Sørensen PD, Jakobsen EH, Madsen JS, et al (2013), Serum HER-2: sensitivity, specificity, and predictive values for detecting metastatic recurrence in breast cancer patients. J Cancer Res Clin Oncol 139(6), 1005–1013.

19. Tse C, Brault D, Gligorov J, et al (2005), Evaluation of the quantitative analytical methods real-time PCR for HER-2 gene quantification and ELISA of serum HER-2 protein and comparison with fluorescence in situ hybridization and immunohistochemistry for determining HER-2

status in breast cancer patients. Clin Chem 51(7), 1093–1101.
20. Xia W, Liu LH, Ho P, et al (2004), Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. Oncogene 23(3), 646-53..

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HTLV-1 AND HTLV-2 INFECTIONS IN ALBANIA

INFEKSIONET NGA VIRUSET HTLV-1 DHE HTLV-2 NË SHQIPËRI

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Address for correspondence: Shpëtim Qyra, HIV/AIDS Reference Laboratory, Institute of Public Health Tirana, Albania. shqyra@yahoo.com It is estimated that the number of infected people with HTLV-1 and HTLV-2 viruses around the world is about 20 million. The virus is endemic in African countries, but it has spread worldwide.

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Objectives: To see if HTLV virus is present in Albania.
Materials and methods: This study included 163 serum samples of which 81 (49.7%) were previously confirmed positive with HIV / AIDS and 82 (50.3%) samples of the contingent of female sex workers. Samples were stored at the Institute of Public Health at -20 ° C. The method used for sample testing was ELISA HTLV 1 & 2 Ab of ADALTIS Company with a specificity of 99.5% and a sensitivity of 100%. The calculation of the results was based on the cut-off, provided by the kit protocol. Interpretation of results was done by sample optical density ratio with cut-off. Samples with ratio greater than 1.1 were considered positive.
Results: Only 2 (1.23%) of 163 samples tested, proved positive for the presence of antibodies to HTLV 1 & 2 viruses. Only 1 (1.23%) of 81 HIV-1 positive samples was positive for the presence of antibodies to HTLV 1 & 2 viruses and only 1 (1.22%) of 82 samples of female sex workers was identified as positive.
Conclusion: This is the first study performed for HTLV virus infection. It shows the presence of the infection in Albania and the only route of transmission of it is through sexual intercourse. Albania and the only route of transmission of it is through sexual intercourse.
Keywords: HTLV, infection, HIV/AIDS, female sex workers, Albania

Introduction

HTLV type 1 and 2 viruses are two viruses that belong to the family of retroviruses, genus Deltaretrovirus. They carry their genetic material in the form of RNA. Besides these two types, in Africa recently are identified two other types, HTLV-3 and HTLV-4 similar to the first two types. For types 3 and 4, epidemiology and diseases that they cause are still unknown. Virus type 1 and type 2 have a similarity between them in 70% of the viral genome. These viruses have reverse transcriptase enzyme which enables the introduction of genetic material (RNA) into DNA lymphocytic cell type T. Most of the people infected with HTLV viruses remain asymptomatic, while those who exhibit clinical symptoms or signs of infection result to have taken it long time ago. HTLV-1 and HTLV-2 are associated with adult T cell leukemia (ATL) and myelopathy/tropical spastic paraparesis.

A) Leukemia / T-cell lymphoma in adults where virus affects type T lymphocytes which transform into malignant and carry provirus HTLV into their genome.

B) Myelopathy / spastic paraplegia which is a disorder of the nervous system characterized by progressive and permanent manifestations such as paralyzed legs, decreased sensitivity, urinary manifestations, etc.These signs are slow and progressively developed and they are caused by the loss of ability to walk [2, 3].

Leukemia / T-cell lymphoma pathology is associated with antigenic ability of viral genes that enable the changing of genes that control cell growth and their longevity. While Myelopathy / spastic paraplegia pathology is associated with the response of immune system after infection in order to control viral infection which is quite large and leads to nervous tissue damage. The source of HTLV - 1 & 2 viruses are infected persons who spread this infection through their biological fluids. The routes of transmission of HTLV are three: parenteral, vertical, and sexual. The possibility that these viruses are transmitted by sexual route is less compared to HIV because HTLV viruses are intracellular and they're rarely found in serum or plasma. HTLV -1 virus was first detected in CD4 T cells , but may be present in other leukocyte cell types such as CD8+ , B lymphocytes and monocytes. There is no current vaccine for these viruses. The use of corticosteroids, immunosuppressors, gamma-globulin and vitamin C has given moderate effects. Medications such as AZT (Zidovudine) and DDC (Zalcitabine) which are used to treat HIV infection inhibit the produce of proviral DNA of HTLV virus. The use of AZT has given improvements in neurological functions. Chemotherapy is used for the treatment of leukemia / lymphoma T cells but it has little effect on the acute form of the disease.

Epidemiology

It is estimated that the number of infected people with HTLV-1 and HTLV-2 viruses around the world is about 20 million. The countries with high endemicity are Japan, the Caribbean and sub - African Saharan countries respectively 10 %, 17 %, 3.8% and 6.6-8.5 %. In Europe the prevalence study of HTLV virus infection in blood donors in Scandinavia and Ireland appears to be 0-0.17/10.000 residents and 0.45-0.48/10.000 residents in France
and England, while in Romania appears to be 5.33/10.000 residents [7, 8, 9]

The prevalence in pregnant women in several Western European countries is about 4.4/10.000 residents. [2, 10, 11]. There is no data of HTLV - 1 and 2 viruses in Albania. The examination of these two types of viruses has not been performed until now, so it is unknown if they are present in our country.

Materials and methods

This study included 163 serum samples of which 81 (49.7%) were previously confirmed positive with HIV / AIDS and 82 (50.3%) samples of the female sex workers contingent. Samples were stored at the Institute of Public Health at -20 ° C. The method used for sample testing was ELISA HTLV I & II Ab of ADALTIS company with a specificity of 99.5% and a sensitivity of 100%. The calculation of the results was based on the cut-off (threshold of positivity) provided by the kit protocol. Interpretation of results was done by sample optical density ratio with cut-off. Samples with ratio greater than 1.1 were considered positive. Samples that resulted positive in ELISA are not confirmed with confirmatory test (Western Blot) for reasons of his absence.

Results and discussion

Only 2 (1.23%) of 163 samples tested, proved positive for the presence of antibodies to HTLV I & II viruses. Only 1 (1.23%) of 81 HIV-1 positive samples was positive for the presence of antibodies to HTLV 1 & 2viruses. This sample is also tested for HBsAg, HCV and Syphilis and was negative for these coinfections. The sample belongs to a male who has taken the infection abroad through homo-bisexual behavior. Coinfection by HTLV - 1 and HIV of the same cell is possible because both viruses have tropism for T - cells, but while HIV has highly cytopathic effect for CD4 + T - cells, HTLV - 1 and HTLV - 2 are non - cytopathic and they have the potential to cause clonal proliferation and transformation

of T - cells. [12,13]. According to a survey undertaken in Europe the prevalence of HTLV - 1 was 3.6 % in the HIV - 1 positive group and 0.9 % in the HIV - 1 negative group. All of the coinfected individuals HIV-1/HTLV-1 were males and females, transsexual sex workers in whom the overall prevalence of HTLV - 1 infection was 11.5 % while HTLV - 2 was only found in the HIV - 1 positive group with a prevalence of 1.2 % [14]. Only 1 (1.22%) of 82 samples of female sex workers was identified as positive. Even this sample was tested for other infections and it was found to be negative for HIV, positive for HBsAg, HCV and syphilis. FSWs are a high risk for transmitting STDs through contact with multiple partners. They may play an important role as a source of heterosexual transmission among clients who may, in turn, spread infection among the general population. [15, 16]

Conclusion

This is the first study performed for HTLV virus infection. The study was focused on these two groups of populations, diagnosed persons with HIV / AIDS and female sex workers as two of the most risked groups referring to the possibility of acquiring this infection. The amount of samples taken in the study (163 samples) was the reason for the limited amount of kits that were available. Despite of this, the study shows that infection by HTLV type 1 and 2 viruses is present in Albania and the only route of transmission of it is through sexual intercourse. Starting from this data, it is required that the study should be extended to other population risk groups, healthy population and blood donors in order to see the circulation of this virus, but also to intervene to prevent its spread. It is also important that this infection and pathologies caused by it should be considered by professionals of various medical specialties.

References

1. Wolfe ND, Heneine W, Carr JK, Garcia AD, Shanmugam V, Tamoufe et al. Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. Proc Natl Acad Sci USA, 2005 May 31;102 (22): 7994-9.
2. Goncalves DU, Proetti FA, Ribas JG, Araújo MG, Pinheiro SR, Guedes AC et al. Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. Clin Microbiol Rev. 2010 Jul; 23 (3):577-89.
3. Levin MC, Jacobson S. HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP): a chronic progressive neurologic disease associated with immunologically mediated damage to the central nervous system. J Neurovirol. 1997 Apr; 3 (2):126-40.
4. Manel N, Kim FJ, Kinet S, Taylor N, Sitbon M, Battini JL et al. The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. Cell. 2003 Nov 14:115 (4):449-59.
5. Manel N, Battini JL, Taylor N, Sitbon M, et al. HTLV-1 tropism and envelope receptor. Oncogene. 2005; 24 (39):6016-25.
6. Igakura T, Stinchcombe JC, Goon PKC, Taylor GP, Weber JN, Griffiths GM et al. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. Science. 2003 Mar 14; 299 (5613): 1713-6.
7. Sanzén L, Carlsson L. Transmission of human T-cell lymphotrophic virus type 1 by a deep-frozen bone allograft. Acta Orthop Scand. 1997 Feb; 68 (1):72-4.
8. Toro C, Benito R, Aguilera A, Bassani S, Rodriguez C, Calderón E, et al. Infection with human T lymphotropic virus type I in organ transplant donors and recipients in Spain. J Med Virol. 2005 Jun. 76 (2): 268-70.
9. WHO and IARC. Human Immunodeficiency Viruses and Human T-Cell Lymphotropic Viruses, in IARC Monogr Eval Carcinog Risks Hum. 1996; 67:1-424.
10. Kondo T, Kono H, Miyamoto N, Yoshida R, Toki H, Matsumoto I, et al. Age- and sex-specific cumulative rate and risk of ATLL for HTLV-I carriers. Int J Cancer. 1989 Jun 15: 43 (6): 1061-4.
11. Jain P, Manuel SL, Khan ZK, Ahuja J, Quann K, Wigdahl B, et al. DC-SIGN mediates cell-free infection and transmission of human T-cell

lymphotropic virus type 1 by dendritic cells. J Virol. 2009 Nov; 83 (21): 10908-21.
12. De Rossi A, Saggioro D, Calabro ML, Cenzato R, Chieco-Bianchi L. Reciprocal activation of human T-lymphotropic viruses in HTLV-I-transformed cells superinfected with HIV-1. *J Acquir Immune Defic Syndr* 1991; 4:380-385.
13. Gallo RC. Human retroviruses after 20 years: A perspective from the past and prospects for their future control. Immunol Rev. 2002; 185:236–265.
14. Zehender G, Colasante C, De Maddalena C, Bernini F, Savasi V, Persico T, Merli S, Ridolfo A, Santambrogio S, Moroni M, Galli M. High prevalence of human T-lymphotropic virus type 1 (HTLV-1) in immigrant male-to-female transsexual sex workers with HIV-1 infection. J Med Virol. 2004 Oct; 74 (2):207-15.
15. Rosenberg MJ, Weiner JM. Prostitutes and AIDS: a health department priority? Am J Public Health 1988; 78:418-423.
16. Day S, Ward H, Harris JR. Prostitute and public health. BMJ 1988; 297:158..

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PREVALENCE OF HERPES SIMPLEX TYPE-2 AMONG HIV SEROPOSITIVE PERSONS

PREVALENCA E HERPES SIMPLEX TIP-2 NË PERSONAT HIV SEROPOZITIVE

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PËRMBLEDHJE
Studime të shumta dëshmojnë se infeksioni prej HSV-2 shoqërohet me risk të rritur të transmetimit seksual si dhe marrjes së virusit të imunodeficenses së fituar në vendet në zhvillim. Qëllimi i studimit është vlerësimi i prevalencës së antitrupave HSV-2 në pacientët HIV seropozitive**.** Përcaktimi i antitrupave IgM dhe IgG ndaj HSV-2 u krye nëpërmjet metodës imunoenzimatike (ELISA). Në total u ekzaminuan 119 mostra të personave të diagnostikuar me HIV gjatë periudhës 1997-2010. Prej 119 mostrave të analizuara në total, 10.9% (13/119) e tyre rezultuan pozitive për antiHSV-2 IgM, dhe 68% e tyre (81/119) rezultuan pozitive për antiHSV-2 IgG. Rastet positive për antiHSV-2 IgM i përkasin grupmoshave 20 deri në 49 vjeç. HSV-2 është një infeksion bashkëshoqërues mjaft i rëndesishëm i HIV i cili duhet të merret në konsideratë. Nevojiten studime të mëtejshme me një numër me të madh rastet HIV pozitive si dhe në popullata me risk të lartë.
Fjalë kyçe: Herpex Simplex virus 2, bashkëshoqërim HSV-2/HIV

SUMMARY
There is increasing evidence that HSV-2 infection could significantly enhance the rates of sexual transmission and acquisition of human immunodeficiency virus (HIV) in developing countries. The aim of the study was to estimate the prevalence of HSV-2 antibodies in HIV seropositive patients. Antibodies to IgM HSV-2 and IgG HSV-2 were determined by enzyme-linked immunosorbent assays (ELISA). 119 samples of persons diagnosed with HIV during 1997-2010 were examined. Of 119 samples, 10.9 % (13/119) of them tested antiHSV-2 IgM positive, and 68% of them (81/119) antiHSV-2 IgG positive. The positive cases of antiHSV-2 IgM, belong to age groups of 20 years old to 49 years old. HSV-2 it's represents a HIV Co-infection that needs to be taken in consideration. Further studies are needed, with more HIV positive cases and based at high risk groups.
Key Words: Herpes simplex virus 2, co-infection HSV-2/HIV

Introduction

Herpes Simplex virus type -2 (HSV-2) is one of the most common sexually transmitted diseases in the world (3, 9). Transmission is facilitated by the frequent recurrence of infectious episodes of subclinical viral shedding (11). Undiagnosed and untreated genital herpesvirus infection in pregnant women can lead to vertical transmission from mother to newborn, causing infant morbidity and mortality (10). Although the prevalence of HSV-2 infection varies widely by country and population subgroup, generally higher rates are found in developing countries where the prevalence in adults can reach 50%. . In the recent decades, prevalence of herpes simplex infection is on the rise (6, 8) and herpes simplex virus 2 (HSV-2) infections is currently the most common cause of genital ulcerative disease. Hence, HSV-2 may act as a potential risk factor for the acquisition of HIV infection (10, 11).

Sexually transmitted infections are known to facilitate HIV transmission through a number of direct, biological mechanisms. Increasing evidence demonstrates a substantial link between the epidemics of sexually transmitted HIV and herpes simplex virus (HSV)-2 infections.

The seroprevalence of herpes simplex virus (HSV) antibodies among HIV patients in Albania is unknown. The objective of this study was to estimate the seroprevalence of HSV-2 infection in HIV positive patients.

Patients and method

A total of 119 sera samples of individuals diagnosed with HIV over the period 1997-2010 were examined. Sera were stored at laboratory of immunology, Institute of Public Health (IPH) in Tirana until examined for the presence of HSV 2 antibodies with commercial immunoenzymatic ELISA kit (Virion/Serion GmbH) The results were interpreted according to manufacturer instructions. Statistical analysis was performed using SPSS version 16 Software.

Comparison of quantitative variables was performed using Student's *t* test. Chi-square or Fisher's exact tests were used for comparison of the discrete data, and the odds ratio (OR) and 95% confidence interval (CI) were also calculated. A p value ≤ 0.05 was considered significant. Stepwise logistic regression analysis was used to identify the independent risk factors for HSv-2 infection. All tests were two tailed.

Results

Of the 119 individuals enrolled, 90 (75.6%) were males, 29 (24.4%) were females. The mean age of patients was 37.5 years (SD±11.8). The mean age of women was 34.2 years (SD±13.1) and the mean age of men was 38.7 years (SD±11.3), t= 2.4, p<0.01. Thirteen individuals tested positive for HSV-2 IgM. The seroprevalence of HSV-2 IgM antibodies at enrollment was 10.9% (95% CI 6.47 to 17.76) (Table 1). In a univariate analysis, none of variables were significantly associated with HSV-2 seropositivity.

Eighty one individuals tested positive for HSV-2 IgG. The HSV-2 IgG antibodies seroprevalence was 68.1% (95% CI 59.27 to 75.79) (table 2).

In a univariate analysis among the 81 subjects positive for HSV-2 I gG antibodies two variables were significantly associated with HSV-2 seropositivity, including no or primary education (OR=3.7 95%CI 0.29 - 10.46 p=0.01) and the place of contracting the infection which is in the country (OR=21.4 95%CI4.82 - 94.93 p<0.01). In a multivariate analysis, none of variables was identified as independent risk factor.

Table 1. Data analysis in HIV positive patients according to HSV-2 status. N=13

Patient characteristics	**IgM n (%)**	**Crude OR, univariate (95%CI)**	**p**
Gender			
Male	7 (8.4)		
Female	6 (26.1)	3.09 (0.94 - 10.10)	0.06
Age (y)			
<25	1 (8.3)	0.65 (0.07 - 5.47)	0.6
25-35	3 (10.0)	1.02 (0.26 - 4.02)	0.9
36-45	5 (13.5)	0.95 (0.29 - 3.10)	0.9
>45	4 (14.8)	1.23 (0.35 - 4.34)	0.7
Educational level			
High school or university	9 (11.8)		
No or primary education	4 (13.3)	1.12 (032 - 3.93)	0.8
Mode of transmission			
Blood			
Heterosexual	12 (12.2)	0.97 (0.11 - 8.52)	0.9
Homosexual	1 (33.3)	2.86 (0.27 - 29.72)	0.3
Vertical			
Place			
Abroad	5 (12.2)		

Home	8 (12.3)	1.0 (0.30 - 3.29)	0.9

Table 2. Data analysis in HIV positive patients according to HSV-2 status. N=81

Patient characteristics	**IgG n (%)**	**Crude OR, univariate (95%CI)**	**p**
Gender			
Male	63 (70.0)	1.4 (0.59 – 3.42)	0.4
Female	18 (62.1)		
Age (y)			
<25	8 (61.5)	0.7 (0.21 - 2.37)	0.6
25-35	22 (66.7)	0.9 (0.38 - 2.15)	0.8
36-45	30 (71.4)	1.3 (0.56 - 2.89)	0.5
>45	21 (67.7)	0.9 (0.40 - 2.35)	0.9
Educational level			
High school or university	52 (61.2)		
No or primary education	29 (85.3)	3.7 (1.29 - 10.46	0.01*
Mode of transmission			
Blood	2 (66.7)	0.9 (0.08 - 10.66)	0.9
Heterosexual	75 (68.2)	1.1 (0.25 - 4.53)	0.9
Homosexual	3 (75.0)	1.4 (0.14 - 14.14)	0.7
Vertical	1 (50.0)	0.5 (0.02 - 7.59)	0.5
Place			
Abroad	37 (50.7)		
Home	44 (95.7)	21.4 (4.82 - 94.93)	<0.01*

* statistically significant

Discusion

HIV infected persons have high rates of HSV-2 infection, ranging from 50 to 90% in studies of HIV-infected populations from different parts of the world (12). In our study the IgG seroprevalence of HSV-G2 was 68.1%. Similar rates of seroprevalence had been previously reported in HIV infected population groups in different countries (6, 2, and 13). Previous studies had reported that the seroprevalence rates of HSV-2 infection were 48% and 75% in U.S. Brazil, France and German HIV-1-infected subpopulations, respectively (1,4,5,6,8,14).

In different parts of the world over the prevalence is higher in women compared with man, especially among the young (3). In our study the seroprevalence was higher among men 70.0 % than woman 62.1%. We think that the lower rate and the absence of significant difference in the present study may be due to a low number of female participants.HIV-HSV-2 coinfected patients may be a common source of horizontal or vertical HSV-2 transmission. HSV-2 antibody status is stated as a surrogate marker of sexual behaviour. This could explain the higher seroprevalence rate in older age. The trend of a higher prevalence of HSV-2 infection with increasing age, though not statistically significant, is reported by similar studies, which suggests that increasing age is a possible risk factor for HSV-2 infection and that HSV-2 infection is a lifelong infection with a high rate of transmission (17, 18). These findings are consistent with previous reports identifying the age and sexual behavior as a risk factor for HSV-2 seropositivity (13).

According to the transmission route 92.6% of cases (75/81) of those tested positive for antiHSV-2 IgG are infected with HIV through heterosexual route and 3.7% through homobisexual route, 2.5% through blood transfusion and 1.2% through vertical transmission. This establishes that heterosexual mode is the main route of transmission of HSV/HIV in this Albania. The observation of 68.1% seropositivity for HSV-2 in HIV-positive individuals is in correlation with previous studies (15, 16).

An important finding of our study was the significant association of educational level with seroprevalence. The HSV-2 IgG seroprevalence was higher among Individuals without education or primary education (85.3%) compared to individuals with high school or university (61.2%).

Unlike our findings, a review conducted in 2004 which showed that there is a common finding among sero-epidemiological surveys that socio-economic and educational status has no significant effect on the prevalence of HSV-2 infection (4).

In our study we did not have any information if the patients contracted initially HSV-2 or HIV but numerous epidemiological studies demonstrated that HSV-2 infection increases the risk for HIV-1 acquisition, even in the absence of clinical symptoms (19, 20).

There is epidemiological and biological evidence of an interaction between HSV-2 and HIV. The HIV infectiousness is increased in persons coinfected with HSV-2. HSV-2 reactivation and shedding are more frequent among those with higher HIV plasma viral load and lower CD4+ T-cells count by providing a portal of entry, at which more activated CD4+ T-cells as the target for HIV can be found (7,9). Recognition, diagnostics and treatment of genital herpes might prevent transmission of HIV.

Genital herpes is not similar to other genital ulcerative diseases. Individuals having genital herpes are often unaware of prior infection, thus serological evidence is an adequate predictor of prior genital herpes.

Symptomatic and asymptomatic HSV remissions are common among HIV-1-infected persons and can be a cause of significant morbidity. These asymptomatic seropositive patients continue to spread HSV-2 and HIV to uninfected partners due to unprotected sexual activity.

It is important to emphasize the role of education, with particular focus on the transmission of HSV, and the regular use of condoms, detection of the disease at initial phase and treatment as important measures to reduce the HSV dissemination among patients with less advanced educations and at high risk for STDs.

Limitations of our study should be noted. Small numbers of sera tested. Further studies, with more HIV positive cases and maybe at high risk groups based studies. It is recommended that positive cases with anti HSV-2 should be tested for HIV.

We studied 119 out of 665 HIV-infected patients registered in Albania from 1993 till 2013. There is no information regarding the clinical symptoms, marital status and the number of sexual partners of the patients enrolled in the study (samples were taken from the HIV positive serums already in IPH).

The results highlight the potential public health impact of HSV-2 in Albania where anti-HSV-2 testing is not generally performed in all populations, especially considering the risk of vertical transmission and the attendant complications at birth as well as the synergy between HIV and HSV-2 transmission (20)

REFERENCE

1. Da Rosa OL, Goncalves Da saliva A, Pereira AC Jr (1996).Herpes simplex virus type 2 in Brazil: seroepidemilogic survey. Int J Dermatol 35: 794-6.
2. Hook, E. W., III, R. O. Cannon, A. J. Nahmias, F. F. Lee, C. H. Campbell, Jr., D. Glasser, and T. C. Quinn (1992). Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. J. Infect. Dis. 165:251–255.
3. Low N, Broutet N, Adu-sarkodie Y,Barton P, Hossain M, Hawkes S (2006). Global control of sexually transmitted infections. Lancet: 368:2001.

4. Laurent Andreolleti, Emmanuel Piednoir, et al (2005).High seroprevalence of herpes Simplex Virus Type 2 infection in french

   Human immunodeficiency virus type-1 infected outpatients.Journal of Clinical Microbiology. P.4215-4217
5. Malkin, J. E., P. Morand, D. Malvy, T. D. Ly, B. Chanzy, C. De Labareyre, A. El Hasnaoui, and S. Hercberg (2002). Seroprevalence of HSV-1 and HSV-2 infection in the general French population. Sex. Transm. Infect. 78:201–203.
6. Malkin, J. E (2004). Epidemiology of genital herpes simplex virus infection in developed countries. Herpes 11 (Suppl. 1):2A–23A.
7. Posavad, C. M., A. Wald, S. Kuntz, M. L. Huang, S. Selke, E. Krantz, and L.Corey (2004).
   Frequent reactivation of herpes simplex virus among HIV-1-infected patients treated with highly active antiretroviral therapy. J. Infect.Dis. 190:693–696.
8. Pebody, R. G., N. Andrews, D. Brown, R. Gopal, H. De Melker, G. Francois, N. Gatcheva, W. Hellenbrand, S. Jokinen, I. Klavs, M. Kojouharova, T. Kortbeek, B. Kriz, K. Prosenc, K. Roubalova, P. Teocharov, W. Thierfelder, M. Valle, P. Van Damme, and R. Vranckx (2004). The seroepidemiology of herpes simplex virus types 1 and 2 in Europe. Sex. Transm. Infect. 80:185–191.
9. Palu G, Beneti L, Calistri et al (2001) Herpes,8 ,50
10. Sacks, S. L., P. D. Griffiths, L. Corey, C. Cohen, A. Cunningham, G. M. Dusheiko, S. Self, S. Spruance, L. R. Stanberry, A. Wald, and R. J. Whitley (2004). HSV shedding. Antiviral Res. 63(Suppl. 1):S19–S26
11. Sacks, S. L., P. D. Griffiths, L. Corey, C. Cohen, A. Cunningham, G. M. Dusheiko, S. Self, S. Spruance, L. R. Stanberry, A. Wald, and R. J. Whitley (2004). HSV-2 transmission. Antiviral Res. 63(Suppl. 1):S27–S35.
12. Strick LB, Wald A, Celum C (2006). Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. Clin Infect Dis; 43:347-56.
13. Wald, A (2004). Herpes simplex virus type 2 transmission: risk factors and virus shedding. Herpes 11(Suppl. 3):130A–137A.
14. Wutzler, P., H. W. Doerr, I. Farber, U. Eichhorn, B. Helbig, A. Sauerbrei, A. Brandstadt, and H. F. Rabenau (2000). Seroprevalence of herpes simplex virus type 1 and type 2 in selected German populations—relevance for the incidence of genital herpes. J. Med. Virol. 61:201–207.
15. Van Benthem BH, Spaargaren J, van Den Hoek JA, Merks J, Coutinho RA, Prins M, et al. (2001).Prevalence and risk factors of HSV-1 and HSV-2 antibodies in European HIV infected women. Sex Transm Infect; 77:120-4.
16. Bystricka M, Gasparovicova L, Stanekova D, Mokras M, Solarikova L, Russ G (2000). Prevalence of antibodies to herpes simplex virus 2 among homosexual men either positive or negative for human immunodeficiency viruses in Slovakia. Acta Virol; 44:163-7.
17. Weiss H (2Type-2 infections in the developing world. Herpes 11: 24A-35A.
18. Christenson B, Bottiger M, Swensson A Jeansson S (1992). A 15-year surveillance study of antibodies to herpes simplex virus 1 and 2 in a cohort of young girls. J Infect 25.
19. Royce, R. A., A. Sen˜a,W. Cates, Jr., and M. S. Cohen (1997). Sexual transmission of HIV. N. Engl. J. Med. 336: 1072–1078.
20. Fleming, D. T., and J. N. Wasserheit (1999). From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex. Transm. Infect.75:3–17.

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PREOPERATIVE VALUES OF CEA, A PROGNOSTIC FACTOR FOR POSTOPERATIVE RECURRENCE OF COLORECTAL CANCER

VLERAT PREOPERATORE TE CEA NJE FAKTOR PARASHIKUES PER REKURENCAT POSTOPERATORE TE KANCERIT KOLOREKTAL

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PERMBLEDHJE
Ne matem CEA ne 102 adulte me CA kolonorektal.Vlerat post-operatore te CEA u percaktuan me interval 3 muaj per dy vitet e para pas operacionit dhe cdo 6 muaj me vone.Mbijetesa e pacienteve pas operacionit ishte me e vogel te pacietet me nivel te CEA te larte para operacionit.61% e pacienteve me rekurenca te kancerit kishin CEA te larte gjata follow-up.Pacientet qe kane vlere preoperatore te larte te CEA parashikohen te kene rekurenca ne 80% te rasteve.Intervali midis rritjes postoperatore te CEA dhe diagnostikimit te rekurencave postoperatore ishte 3 muaj.Vlera e larte e CEA pre-operatore dhe ndjekja e vlerave te saj jane faktore prognostike per CA kolorektal.Survejanca e vlerave post-operatore te CEA eshte me efektive kur pacientet kane vlera te larta preoperatore te CEA.Ulja e vlerave postoperatore te CEA tregon per nje rezeksion rezultativ te kancerit dhe rritja e tyre vlen si faktor prognostik per rekurenca.
Fjalet çeles:kanceri kolorektal,CEA,rekurenca.

SUMMARY
We measured serum CEA levels in 102 adults with colorectal cancer. Follow-up was 6 months to 60 months with a median 2 years.The patients survival after resection was less in patients with higher preoperative CEA levels.Among the patients with a tumor recurrence 61% had high follow-up serum CEA levels.Higher preoperative and follow-up serum CEA levels were indipendent prognostic factors for tumor recurrence in 80% of cases.The mean interval between postoperative serum CEA elevation and the diagnosis of a tumor recurrence was 3 months. High serum CEA levels preoperatively and at follow-up are prognostic factors for colorectal cancer. Postoperative serum CEA surveillance is used most effectively when patients have high preoperative serum CEA levels.Normal value of postoperative serum CEA levels is an indicator of the optimal treatment and high value of CEA after resection is a predictive factor of recurrence after curative surgery.
Key words: Colorectal cancer,CEA ,recurrence.

Introduction

Colorectal cancer is the third cause of death in people diagnosed with cancer.Incidence and mortality is high both in man and women and effectiviness of treatment in advanced stage is poor. Therefore searching the prognostic factors of survival in colorectal cancer is nowadays a challenge.The most frequently used marker in

colorectal cancer is CEA. It was first described in 1965 by Gold and Freedman as an antigen that was present in both fetal colon and colon cancer .Because this glycoprotein was detected in cancer and embryonic tissue it was named carcinoembryonic antigen or CEA. Later subsequent work showed that CEA was also present in low
concetration in certain healthy tissues and it might have high values in some benign gastrointestinal diseases.CEA is the tumor marker most used in colorectal patients during follow up after surgery.High serum CEA level before surgery is associated with higher incidence of recurrence of tumor after curative surgery. The aim of this retrospective study in Albanian patients was to evaluate preoperative serum CEA levels by Duke`s stage and to predict postoperator recurrence during the follow up and to find possible relationship between high serum CEA level before surgery and its level after resection of tumor.

Patients and methods

Our study included 102 patients with histologically confirmed colorectal cancer.Median age was 60 years(range 25-80).These included 54 male (53%) and 48 female(47%).All included patients had operable cancer.We classified cancer by Duke`s stage(Duke`s A 6 patients, Duke`s B 54 patients,Duke`s C 42 patients).Patients with Duke`s D diseases were not included in our study. CEA was measured with ELISA method using Elisa kits from Diametra (Italy).Serum levels of CEA were measured preoperatively and than after curative surgery and during the follow up.The follow-up was 6 months to 60 months with a median 2 years. Reference value was 0 – 5 ng/ml. In this analysis five variables were employed: age, sex, tumour location, Dukes stage and preoperative CEA level.We calculated specifity, sensitivity,positive and negative prognostic value of CEA as an index of tumor recurrence. Significance was $p < 0.005$

Results

102 patients included in our study had 78 % or 72 patients CEA ≤ 5 ng/ml and 32 % or 33 patients CEA > 5 ng/ml.Higher incidence of CEA > 5 ng/ml was found in Duke`s C than in Duke`s A and B (Table 1).

Table 1 Number of patients with preoperative CEA ≤ 5 ng/ml or CEA > 5 ng/ml and their incidence

Duke`s stage	CEA ≤ 5 ng/ ml	CEA > 5 ng/ml	% per CEA> 5 ng/ml
A	5	1	17
B	44	10	18
C	30	12	28

The first month after curative resection serum CEA level was ≤ 5 ng/ml in 70% of patients Duke´s B and 50% of patients Duke´s C.During the follow up was observed recurrence in 36 patients . Before one year of tumor resection was observed recurrence in 15 patients and after one year of surgery was observed recurrence in 21 patients (Table 2).

Table 2 Number of patients with CEA ≤ 5 ng/ml or CEA > 5ng/ml and time of recurrence

Time of recurrence	CEA ≤ 5 ng/ ml	CEA > 5 ng/ml
< 1vit	5	10
> 1vit	9	12

Among 66 patients without recurrence during the follow up 87% of patients had CEA < 5 ng/ml and 61% of patient with recurrence had CEA > 5 ng/ml.(Table 3).

Table 3 Number of patients with or without recurrence and CEA ≤ 5 or > 5 ng/ml

	CEA ≤ 5 ng/ml	CEA > 5 ng/ml
With recurrence	14	22
Without recurrence	58	8

The sensitivity(S),specifity(E), the positive predictive value (PPV)and negative predictive value (NP) are :

$$S = \frac{\text{Number of pat.with recurrence and preoperative CEA>5}}{\text{Number of pat.with recurrence}}$$

$$S = \frac{27}{36} = 75\%$$

$$E = \frac{\text{Number of pat.without recurrence and preoperative CEA<5}}{\text{Number of pat.without recurrence}}$$

$$E = \frac{58}{66} = 87\ \%$$

$$PPV = \frac{\text{Number of pat.with recurrence and CEA>5}}{\text{Number of pat.with elevated CEA}}$$

$$PPV = \frac{22}{30} = 73\%$$

$$NPV = \frac{\text{Number of pat.without recurrence and CEA<5}}{\text{Number of pat.with normal CEA}}$$

$$NPV = \frac{58}{72} = 80\%$$

During the follow up was observed frequency of recurrence in Duke`s A 10 % , Duke`s B 70% and in Duke`s C 80% of patients. Recurrence of the disease was observed in 80 % of patients with preoperative CEA > 10 ng/ml and 70% of patients with preoperative CEA > 5ng/ml.Mean survival of patients with CEA <5 was about 60 months and the survival of patient with CEA >5 ng/ml was under three years.

Discussion

CEA is a glycoprotein released by about 90% of colorectal cells, that can be useful as a reliable tumour marker.Our study evaluated the prognostic value of preoperative CEA level in Albanian patients and showed that survival was higher for subjects with CEA ≤ 5 ng/ml compared to those with CEA > 5 ng/ml . As CEA levels reflect tumor volume and tumoral cell number we may say that patients with CEA > 5 ng/ml had larger tumor that results in higher risk of recurrence.Frequency of recurrence was 35 % and it was observed higher in Duke`s C than in Duke`s B or A. 61 % of patients with recurrence had CEA > 5 ng/ml . Patients with preoperative CEA value >10 ng/ml had decreased survival(they died within two years).We did not observed any differences of serum CEA levels when comparing age and sex.We observed CEA level higher for rectal cancer than for colon cancer because rectal cancer is more infiltrative and annular.The mean preoperative serum CEA levels were higher for patients in Duke`s C than for those in Duke`s B or A.

CONCLUSION

Preoperative serum CEA level is a reliable prognostic factor for recurrence in patients with colorectal cancer. The follow up of postoperative serum CEA level is more effective in high preoperative CEA.Normal value in postoperative CEA is an index of resultative resection and high levels after a few months is a predictive factor of recurrenceso we may say that value of CEA will be useful for therapeutic orientation in patients undergoing curative resection of colorectal cancer

References

1.Astler VA, Coller FA. The prognostic significance of direct extension of carcinoma of the colon and rectum. Ann Surg 1954;139:846–52.

2.FLETCHER, R.H. (1986). Carcinoembryonic antigen. Ann. Intern. Med., 104, 66-73.

3. Gold P, Freedman SO. Specific carcinoembryonic antigen of the human digestive system. J ExpMed 1965; 122:467–81.

4. National Institutes of Health Consensus Development Conference. Carcinoembryonic antigen: its role as a marker in the management of cancer. Cancer Res 1981;41:2017–8.

5.Fletcher RH. Carcinoembryonic antigen. Ann Intern Med 1986;104:66–73.

6.Dukes CE. The classification of cancer of the rectum. J Pathol 1932;35:323–32.

7.Wang JY, Tang R, Chiang JM. Value of carcinoembryonic antigen in the management of colorectal cancer. Dis Colon Rectum 1994;37:272–7.

8.Ladenson JH, McDonald JM. Colorectal carcinoma and carcinoembryonic antigen (CEA). ClinChem1980; 26:1213–20.

9. Wolmark N, Fisher B, Wieand HS, Henry RS, Lerner H, Legault-Poisson S, et al. The prognostic significance of preoperative carcinoembryonic antigen levels in colorectal cancer – results from NSABP clinical trials. Ann Surg 1984;199:375–81.

10.Chu DZ, Erickson CA, Russell MP, Thompson C, Lang NP, Broadwater RJ, et al. Prognostic significance of carcinoembryonic antigen in colorectal carcinoma. Arch Surg1991;126:3:314–6.

11.Goslin R, Steele G Jr, MacIntyre J, Mayer R, Sugarbaker P, Cleghorn K, et al. The use of preoperative plasma CEA levels for stratification of patients after curative resection of colorectal cancers. Ann Surg 1980;192:747–51.

12.Steel G Jr, Ellenberg S, Ramming K, O'Connell M, Moertel C, Lessner H, et al. Carcinoembryonic antigen monitoring among patients in multi-institutional adjuvant G.I. therapy protocols. Ann Surg 1982;196:162–9.

13. Moertal CG, O'Fallon JR, Go VLW, O'Connell MJ, Thynne GS. The preoperative carcinoembryonic antigen test in the diagnosis, staging and prognosis of colorectal cancer. Cancer 1986;58:603–10.

14. Scott NA, Wieand HS, Moertel CG, Cha SS, Beart RW, Lieber MM. Colorectal cancer: Dukes' stage, tumor site, preoperative plasma CEA level and patient prognosis related to tumor DNA ploidy pattern. Arch Surg 1987;122:1375–9.

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MACROSCOPIC AND MICROSCOPIC ASPECTS OF FRACTURE SURFACES

ASPEKTE MAKROSKOPIKE DHE MIKROSKOPIKE TË SIPËRFAQEVE TË THYERJES

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PËRMBLEDHJE
Në punim prezantohen disa aspekte vizuale dhe mikroskopike të sipërfaqeve të thyerjes të bashkësisë së salduar, përkatsisht tegelit (T), zonës së ndikimit termik (ZNT) dhe metalit bazë (MB), të tubave të salduar me tegel spiral dhe gjatësor pas realizimit të provave me shkatërrim. Provat me shkatërrim realizohen për vlerësimin e cilësisë së tubave dhe krahas rezultateve numerike, sipërfaqja e thyerjes së mostrave të provuara ofron informacion shtesë, mjaft të rëndësishëm për vlerësimin e cilësisë së tubave të salduar. Duke e marrë parasysh këtë, në punim trajtohet aspekti vizual dhe mikroskopik (LOM-Light Optical Microscopy dhe SEM-Scanning Electron Microscopy) i sipërfaqeve të thyerjes të mostrave të shkatërruara pas provave destruktive. Morfologjia e sipërfaqeve të thyerjes krahasohet me rezultatet numerike dhe konkludohet se ekziston korrelacion i drejtpërdrejt ndërmjet rezultateve të fituara dhe deformimit, përkatësisht sforcimit që e shkakton thyerjen duke siguruar kështu të dhëna shtesë për vlerësimin e cilësisë së tubave të salduar.
Fjalët çelës: kontroll vizual, mikrostrukturë, sipërfaqe të thyerjes, deformim.

SUMMARY
In this paper are presented some visual and microscopic aspects of welded joint fracture surfaces, respectively welded seam (W), heat affected zone (HAZ) and base metal (BM), of spiral and longitudinal welded pipes after conducting the destructive testing. Destructive testing are conducted to assess the quality of the welded pipes and besides the numerical results, the fracture surface of the tested samples offers additional information, very important for assessing the quality of welded pipes. Given this, in the paper are treated the visual and microscopic aspects (LOM-Light Optical Microscopy and Scanning Microscopy-SEM) of the fracture surfaces of samples which are fractured after destructive testing. The morphology of the fracture surfaces is compared with the numerical results and it concludes that there is a direct correlation between the obtained results and deformation, respectively the stress that causes fracture, providing thus additional information for assessing the quality of the welded pipes.
Key words: visual control, microstructure, fracture surface, deformation.

1. INTRODUCTION

A Fracture is the separation of an object or material into two, or more, pieces under the action of stresses and fractography can be defined as the science which deals with the description, analysis and interpretation of fracture surface morphologies (fracture topographies) and links them to the causative stresses, mechanisms and subsequent evolution of the fractures. Fractography can be classified according to the field (scale) of observation into [1]:

-macrofractography, dealing with features which can be studied visually using the naked eye or hand lens, and
-microfractography, dealing with features which can be studied at low magnification using Light Optical Microscopes (LOM), or at high magnification with Scanning or Transmission Electron microscopes (SEM or TEM). Energy-dispersive X-ray spectroscopy, used in conjunction with the SEM or TEM, is a very practical tool for elemental chemical analysis [1, 2, 3].
Fracture of materials depends upon many parameters like the state of stress, strain rate, the defect size, crystal structure, chemical homogeneity, grain size, grain boundary, formation of twins and slip plane, temperature and environmental conditions [4].
Based on the ability of a material to experience plastic deformation, for engineering materials, two fracture modes are possible: ductile and brittle [5]. Ductile fractures typically exhibit substantial plastic deformation with high energy absorption before fracture. On the other hand, brittle fractures display little or no macroscopically visible plastic deformation and require less energy to form.
Fractured surfaces display both macroscopic and microscopic features.
Ductile fractures exhibit certain characteristic macroscopic features:
-a relatively large amount of plastic deformation precedes the fracture,
-shear lips are usually observed at the fracture termination areas,
-the fracture surface may appear to be fibrous or may have a matte or silky texture, depending on the material,
-the cross section at the fracture is usually reduced by necking,
-crack growth is slow.
Macroscopically, brittle fractures are characterized by the following:
-little or no visible plastic deformation precedes the fracture,
-the fracture is generally flat and perpendicular to the surface of the component,
-the fracture may appear granular or crystalline and is often highly reflective to light,
-herringbone (chevron) patterns may be present
-cracks grow rapidly, often accompanied by a loud noise.
On a microscopic scale, ductile fracture in metals displays a dimpled surface appearance created by microvoid coalescence and transgranular separation.
Microscopically, brittle fractures have the following characteristics:
-transgranular cleavage or quasi-cleavage,
-intergranular separation,
-features on transgranular facets, such as river marks, herringbone patterns, or tongues.

2. MATERIALS AND METHODS

Spiral line pipes Ø813x12mm were fabricated using high strength steel coils X65 according to API 5L (American Petroleum Institute) standard, which chemical composition and mechanical properties are given in Table 1 and 2, according to the Certificate of Quality. Spiral line pipes Ø 813x12mm were welded in two-stage process by double sided Submerged Arc Welding (SAW) through an "X" groove configuration, according to the BLOHM+VOSS.

Tab.1. Chemical composition of steel coils X65

Steel coils	Chemical composition wt-%					
	C	Mn	Si	P	S	V
X65	0.09	1.31	0.43	0.020	0.005	0.048

Tab.2. Mechanical properties of steel coils X65

Steel coils	Mechanical properties						
	Yield Strength	Tensile Strength	Elongation	Impact toughness			
				Individual values			Average values
	Re	Rm	A	Kv 1	Kv 2	Kv 3	Kv
				ISO-V-0°C			
X65	MPa		%	J			
	549	649	23.6	132	102	143	126

Charpy V-Notch (CVN) specimens were cut out from the double sided weld metal, perpendicular to the welding direction, as shown in figure 1.

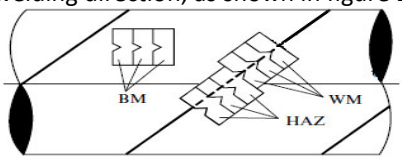


Fig.1. Schematic representation of the Charpy V-Notched specimens

The standard CVN specimens with V groove were cut from base metal (BM), heat affected zone (HAZ) and weld metal (WM) and tested at -20°C, 0°C and 20°C by use of the MOHR-FEDERHAFF LOSSENHAUSEN pendulum.

In a Charpy impact test, three key parameters are typically determined:

-impact toughness,

-percent shear fracture area and

-lateral extension.

A methodology for measurements of the percentage of shear (ductile) fracture and brittle fracture on the total fractured surface of a CVN tested specimens is shown in figure 2. The specimen cross-section after fracturing is divided into equal millimetric units and then by counting of the relevant squares into the entire cross-section, using Autocad software, determines percent of shear fracture (ductile) area and percent of brittle fracture area.

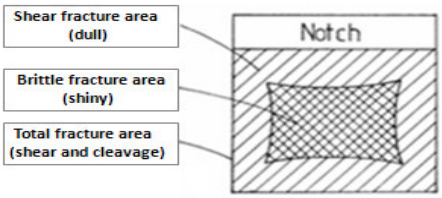


Fig.2. Determination of the percent of shear and brittle fracture

In order to obtain a more complete assessment of the fracture surfaces of Charpy V-Notched specimens, lateral extension were measured, in the height direction (h_1-h_0) and in the width directions (a_1-a_0), where:

-h_0, a_0 (mm)-initial specimen height and width, before fracture and

-h_1, a_1 (mm)-maximum specimen height and width after fracture.

According to the measured dimensions before and after fracture, relative strain in the height directions (ε_h) and relative strain in the width directions (ε_a) were calculated.

$$\varepsilon_h = \frac{h_1 - h_0}{h_0} x100\ [\%] \dots\dots\dots\dots\dots\dots\dots\dots (1)$$

$$\varepsilon_a = \frac{a_1 - a_0}{a_0} x100\ [\%] \dots\dots\dots\dots\dots\dots\dots\dots (2)$$

All fractured surfaces of CVN specimens after testing were examined visually with the unaided eye, at low magnification using Light Optical Microscope (LOM-NEOPOT 21) and at high magnification using Scanning Electron Microscope (SEM-Leo 1530).

3. RESULTS AND DISCUSSIONS

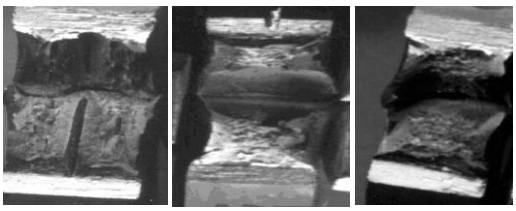
Impact toughness results of the Charpy-V Notch specimens are presented in table 3. These values represents average of three specimens measurements at different temperatures (-20°C, 0°C and 20°C). Table 3 shows decreasing tendency of BM, HAZ and WM impact toughness with decreasing of the temperature.

Table 3. Impact toughness (CVN)

Steel coils	Specimens	Average values of impact toughness-CVN [J/cm^2]								
		Base metal [BM]			Heat affected zone [HAZ]			Weld metal [WM]		
		Temperature [°C]								
		-20	**0**	**20**	**-20**	**0**	**20**	**-20**	**0**	**20**
X65	**C_0**	75.3	104	124	78.0	78.9	111	92.2	103	94.1
	C_1	78.7	102	115	73.0	99.2	111	84.2	99.0	113

	C_2	64.2	103	118	73.1	99.0	89.9	91.2	77.9	110
	C_3	80.1	100	120	74.5	80.1	91.1	80.6	90.1	108

Visual analysis of the Charpy V-notch fractured surfaces clearly shows that exist fracture morphology differences between BM, HAZ and WM, figure 3 [6].



BM HAZ WM

Figure 3. Typical fracture surfaces of BM, HAZ and WM

From the visual analysis, figure 3, there is notable evidence of plastic deformation in the fractured surfaces of all tested specimens of BM, HAZ and WM. Post-fracture visual analysis in all fractured surfaces shows presence of shear lips which are located at the opposite sides of the specimens width, figure 3. Presence of shear lips is also a characteristic of ductile fracture.

Macroscopic view of the fracture surface of the Charpy V-Notch specimen of BM, specimen marked as C_2, with lowest impact energy (Kv=64.2J/cm^2), tested at −20°C is presented in figure 4.

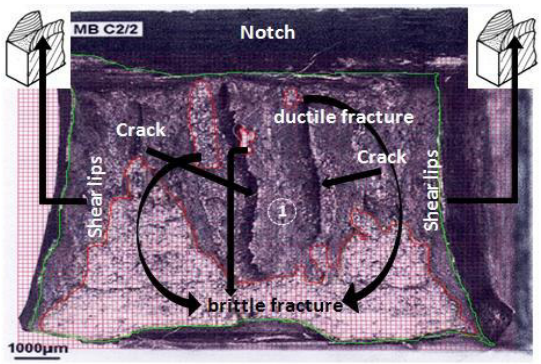


Figure 4. Macroscopic view of the fracture surface of BM

Table 4. Percentage of ductile and brittle fracture surfaces

Steel coils	Specimens	Percentage of ductile and brittle fracture %					
		[BM]		[HAZ]		[WM]	
		Temperature [-20°C]					
		Ductile	Brittle	Ductile	Brittle	Ductile	Brittle
X65	C_0	79.6	20.4	90.2	9.8	92.3	7.7
	C_1	82.7	17.3	88.7	11.3	94.7	5.3
	C_2	70.9	29.1	92.1	7.9	95.1	4.9
	C_3	89.4	10.6	90.5	9.5	93.3	6.7

Table 4 shows that shear or ductile fracture dominated in all cases. The largest percentage (29.1) of brittle fracture is in the sample marked with C_2, which is in accordance with the numerical value of the impact toughness, given in table 3.

In general, all fractured surfaces of the BM, HAZ and WM consisted mainly of a large ductile region and small amounts of brittle area. The fracture surface of BM is fibrous and its color is dull. Fracture surfaces of the WM and HAZ are also fibrous with a little presence of bright shiny color. In the case of BM with lowest impact energy (Kv=64.2J/cm^2), specimen marked with C_2 and tested at −20°C, brittle area appears flat and shiny, figure 4.

Fractured surfaces of some specimens of BM shows some cracks like separations perpendicular to the axis of the V-notch, figure 3 and 4. These cracks are referred to as delamination [6].

The percentage of shear or ductile and brittle fracture surfaces of CVN tested specimen in (-20°C) is shown in table 4. These values represent average of three specimens measurements.

Lateral extension in height (ε_h) and in width (ε_a) determined by formula (1) and (2), are presented in table 5.

Table 5. Relative strain of height (ε_h) and width (ε_a)

Steel coils	Specimens	Relative strain %					
		[BM]		[HAZ]		[WM]	
		ε_h	ε_a	ε_h	ε_a	ε_h	ε_a
X65	C_0	27.1	25.2	22.8	20.1	26.2	24.2
	C_1	26.3	24.1	21.9	18.1	24.5	20.2
	C_2	23.6	19.1	23.9	18.2	24.2	21.9
	C_3	24.3	21.3	24.3	20.6	23.7	20.7

From the table 5, a little difference of the lateral extension of height (ε_h) and width (ε_a) can be observed. Lateral extension in high (ε_h) almost in all specimens is slightly larger than lateral extension in width (ε_a).

The scanning electron microfractography (SEM) of the central portion of the base metal (BM) specimen, marked with circle 1, in figure 3, reveals numerous coarse and fine concave equiaxed dimples, characteristic of a ductile area. Partially in this electron microfractography (SEM) exist some cleavage facets, which are characteristic of brittle fracture, figure 5.

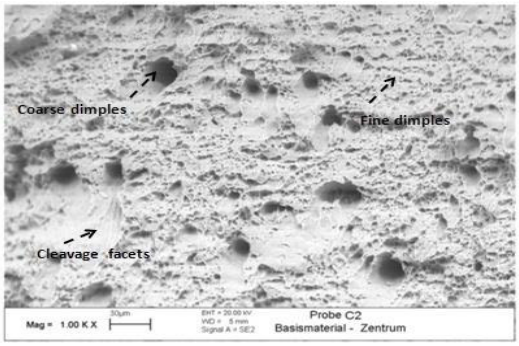


Figure 5. SEM microfractography of BM

4. CONCLUSIONS

On the basis of the results presented in this work, it is possible to draw the following conclusions:

-Impact toughness values accompanied with visual analysis of fractured surfaces of CVN specimens can provide additional information for quality evaluation of the welded joint, respectively BM, HAZ and WM.

-Lateral extension measurements with determination of the percentage of ductile and brittle fracture in the total fracture area are additional parameters and facilitate the assessment of the quality of the welded joint, respectively BM, HAZ and WM.

-Microscopic analysis with LOM and SEM can provide very important additional data for qualitative and quantitative assessment of fractured surfaces and mechanisms of fracture of welded joint, respectively BM, HAZ and WM.

BIBLIOGRAPHY

[1] Ronald J, Parrington PE, (2002), Practical Failure Analysis Volume 2(5) October, 33-38.

[2] Lynch P, Moutsos S, (2006), A brief history of fractography-Proceedings of the 16th European Conference of Fracture, Alexandroupolis, Greece, July 3-7.

[3] Mohammed SA, (2013), Facture topography as a tool in fracture mechanics and stress analysis. An Introduction. Special Publication No. 92, 1-10.

[4] Pradhan PK, Dash PR, (2012), Microvoid coalescence of ductile fracture in mild steel during tensile straining, Frattura ed Integrita Strutturale, 19, 51-60.

[5] George F, Vander V, (1987), Visual Examination and Light Microscopy, ASM Handbook, Volume 12, Fractography, 91-165.

[6] Maksuti Rr, Mehmeti H, Baum H, Rama M, Çerkini N, (2009), Correlation of microstructure and toughness of the welded joint of pipeline steel X65, Damage and Fracture Mechanics, Failure Analysis of Engineering Materials, Springer, 315-3.

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DATA FROM THE PELLET ANALYSIS OF THE BARN OWL (*TYTO ALBA*) IN THE SOUTHERN REGION OF ALBANIA

TE DHËNA NGA ANALIZA E VJELLJEVE TË KUKUVAJËS MJEKËROSHE (*TYTO ALBA*) NË RAJONIN JUGOR TE SHQIPËRISË

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PËRMBLEDHJE
Qëllimi i këtij studimi është të japë të dhëna të karakterit biologjik dhe statistik nga analiza e vjelljeve (pellets) të kukuvajës mjekëroshe (*Tyto alba*) mbi bazën e të cilave ne monitorojmë sjelljen ushqimore të saj si dhe praninë e gjitarëve të vegjël në një territor të caktuar. Gjatë periudhës 2010-2011 në territorin e qarkut të Gjirokastrës kemi grumbulluar 729 vjellje të kukuvajës mjekëroshe . 62.4% e vjelljeve të *T.alba* përmbajnë rodentin *Microtus thomasi*, ndërsa mbi 36% e vjelljeve përmbajnë përmbajnë respektivisht rodentin *Mus macedonicus* dhe insektivorin *Crocidura suaveolens.* Rreth 27% e vjelljeve të kukuvajës mjekëroshe përmbajnë vetëm mbetje kockore të *M.thomasi.* Tre gjitarët e mësipërm përbëjnë dhe prenë ushqimore më të preferuar për kukuvajën mjekëroshe.
Fjalët çelës: vjellje, Tyto alba, mbetje kockore, gjitarë të vegjël, Gjirokastër.

SUMMARY
The aim of this study is to provide biological and statistical data elaborated from the analysis of the pellets collected from the barn owl (*Tyto alba*), based on which we can monitor feeding behavior and small mammal community inside the hunting range of the owl. During 2010-2011 some 729 pellets of the barn owl (Tyto alba) have been collected and analysed in the territory of Gjirokastra region. In 62.4% of the analysed pellets the rodent species *Microtus thomasi* is contained, while some 36% of the pellets contains the rodent *Mus macedonicus* and the Soricomorph *Crocidura suaveolens.* About 27% of the barn owl pellets contains only remains of one single species, that's of *M. thomasi.* All three above mentioned small mammal species compose the most preferable food prey of the barn owl.
Key words: pellets, *Tyto alba*, skeleton remains, small mammals, Gjirokastra region.

INTRODUCTION

The barn owl (*Tyto alba* Scopoli, 1769) is the strigiform with the broadest worldwide distribution (5), and its diet has been studied more extensively than that of any other bird of prey (8). Small mammals are the main components of its diet throughout its range, along with variable proportions of birds, reptiles,

amphibians, fishes and arthropods (6,9). Ruprecht (1979) concluded that the relatively small individual territory of the barn owl and low degree of digestion of skeletal elements of prey make it an ideal species for use in investigations on the distribution and age structure of populations of small vertebrates. The analysis of the T.alba pellets by Albanian biologists is used as an extremely effective tool to identify a significant number of small mammals and particularly some species that it appears it is more difficult to capture by traps such as *Suncus etruscus, Micromys minutus*.etc. In Albania, at least 18 species of small mammals have been identified through the analysis of T.alba pellets (1,2,3,14,15). The aim of our study through the analysis of T.alba pellets is to provide biological and statistical data on the basis of which we monitor their feeding behavior and the presence of small mammals in the southern region of Albania.

MATERIAL AND METHODS

During the period September 2010-September 2011 in the district of Gjirokastra pellets of T.alba have been collected on a regular basis for each month and in all seasons. Preliminary in the study area were made careful observations to identify the places in which barn owls sheltered as well as the removal of old pellets from surrounding territory so that the accurate monitoring of their seasonal diet could take place. The pellets were collected in abandoned buildings and in old churches. Only entire pellets were collected which were sent to the laboratory where they were placed in the open area for them to dry. After that pellets were weighted one by one and measurements made by means of a caliber pellets were carefully opened one by one, in dry conditions, with help of a forceps, to avoid the damage of biological material included inside them. Bone remaining was preserved in separate plastic boxes, and each plastic box was accompanied with specific information on the prey items, time and location. Prey was identified at genius and species level, using reference books and articles (7,10,11,12,13,17).

RESULTS AND DISCUSSION

In the district of Gjirokastra during the period 2010-2011, 729 pellets have been collected from which 2083 individuals are selected. The material was collected in the Drinos valley and in Permet area (Table 1). Pellets were gray to black with average size 3.97 cm in width and 2.97 cm in length. The average weight of pellets was 4.03 gr.(min.0.6gr, max.9gr). In 62.4% of the analysed pellets the rodent species *Microtus thomasi* is contained, while some 36% of the pellets contains the rodent *Mus macedonicus* and the Soricomorph *Crocidura suaveolens.* About 27% of the barn owl pellets contains only remains of one single species, that's of *M. thomasi.* The number of individuals in pellets range from 1 to 10 with an average of 2.90 individuals for each pellet. The maximum number of individuals was found in autumn in the greenhouses of Mashkullora (Drinos valley). After the treatment of the material 14 species of small terrestrial mammals have been found. Mammals with greater frequency and distribution which also constitute the T.alba's most preferable food are *Microtus thomasi* (frequency, F = 32.9%) followed by *Crocidura suaveolens* (F = 23,5%) and *Mus macedonicus* (F = 17.8%). (Figure 1). The highest values in the trophic niche of the Barn owl is registered in Autumn (3.16) ,followed by Spring (3.11), while the lowest value are registered in summer (1.66) and winter (2.60). By statistically processing the data through the incresing number individuals observed in pellets, we have noticed and a nearly linear increase in the number of insectivores (mainly Crocidura sp.) and the contrary, a declining for rodents. (Figure 2). In the pellets with a number of individuals

	Bodrishtë Bularat	Sofratikë	Libohovë	Lazarat	Gjirokastër	Arshi Lengo	Serat e Mashkullorës	Ura e Kardhiqit	Ballaban-Suk.
Number of pellets	36	8	45	72	5	225	143	128	67
Number of individuals	101	23	143	180	9	637	432	426	132
Averag.nr.of ind./pellets	2.80	2.88	3.17	2.50	1.80	2.80	3.02	3.32	1.97
Number of species	7	7	9	9	3	12	13	12	9

Table 1. Stations where the pellets were collected with respective data for each station. In the right,(with black) shows the area of our study.

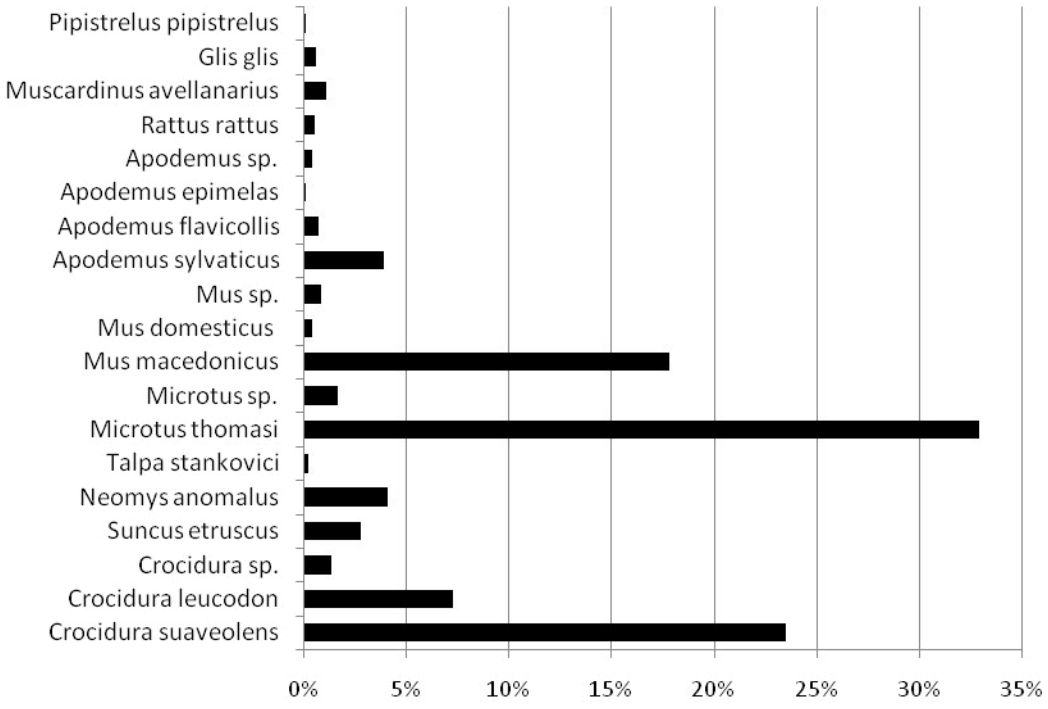

Figure 1. Average frequencies of mammal species found in the barn owl pellets.

greater than 5, insectivores predominate over the rodents (F insektiv = 76%, F rodent. = 24%), while in pellets with a number of individuals less than 6, rodents prevail over insectivores (F insectiv. = 35%, F rodent. = 65%).
The obvious increase of insectivores (mainly Crocidura sp.) in relation to rodents, in the pellets with a large number of individuals is probably related to the fact that insectivores, since they have a body weight several times smaller than the rodents, compensate the T.alba's demand for biomass by increasing the number of insectivores being consumed prey. Of course this phenomenon depends on a number of ecological factors such as spawning period, season,vegetation, fluctuations of climatic factors that may favor or prevent the development of such a phenomenon. Although shrews are generally not a preferable food item to most birds of prey, barn owls catch them in abundance(from our study it comes out that they constitute about 40% of individuals being consumed prey) which is perhaps due to their local availability (4;9). The relatively high number of individuals (from 1-10) and prey species (from 1-6) met in the barn owl pellets reflects the feeding behavior of the barn owl that tries to preserve food balance, both in quantity by consuming the necessary biomass regardless of the prey type, and in quality by consuming different species of mammals which makes its daily meals more "delicious".

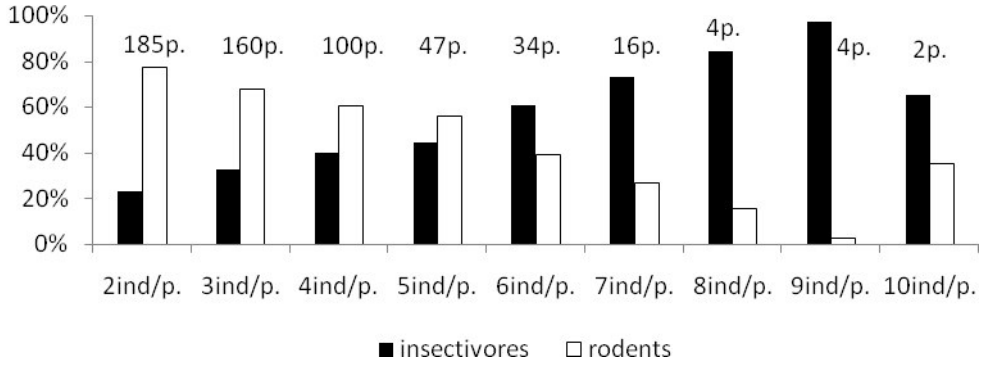

Figure 2. Average frequency of rodents and insectivores in the barn owl pellets (p) according the number of individuals found in them.

CONCLUSIONS

Albania's southern region is a highly preferred habitat by small terrestrial mammals and, therefore for the T.alba that eats them. This is confirmed by the significant number of shelters we have found as well as by the large number of pellets we have collected. Owl pellets contain mainly rodents (60%) and insectivores (40%). The highest percentage in this type of prey caught *M.thomasi, C. suaveolens* and *M.macedonicus* that cover about 75% of the T.alba mammal diet.

REFERENCES

1.Bego F (2003), Të dhëna mbi komunitetet e gjitarëve të vegjël (Mammalia: Rodentia dhe Insectivora) të fushës së Vurgut(Mesopotam) dhe Myzeqesë (Apolloni) [Data on the small mammals (Mammalia: Rodentia and Insectivora) of Vurgu field (Mesopotam) and Myzeqe field (Apolloni)]. Akademia e Shkencave: Studime Biologjike. No. 7, pp. 123–131 (in Albanian with English summary).

2.Bego F, Kryštufek B, Paspali G, Rogozi E (2008) Small terrestrial mammals of Albania: annotated list and distribution. Hystrix Ital. J. Mamm. 19(2): 83–101.

3.Bego F, Kadiasi N (2008), Përberja dhe dinamika e komuniteteve te gjitarëve të vegjël (Mammalia: Insectivora dhe Rodentia) të ekosistemit ligatinor Divjake–Karavasta [Structure and dynamics of the communities of small mammals (Mammalia:Insectivora and Rodentia) in the ecosystem of the Divjaka–Karavasta area.]. In: Proceedings of the InternationalConference on Biological and Environmental Sciences. UT,FSHN, pp. 280–286 (in Albanian with English summary).

4.Bunn DS, Warburton AB, Wilson RDS (1982), The Barn Owl.T. and A.D. Poyser, Carlton, UK.

5.Burton JA (1984), Owls of the World. Tanager Books, Dover, New Hampshire, USA.

6.Cramp S. (ed.) (1985), The birds of the Western Palearctic, Vol 4. Oxford University Press, Oxford.

7.Erfurt J (2003), Bestimmung von Saugetierschadeln in Frasresten und Gewollen [Determination of mammalian skulls in nutrition remnants and pellets].Methoden feldokol. Saugetierforsch 2:471–535 (in German).

8.Everett M, Prestt I, Wgagestaffe R (1992), Barn and bay owls. In: Owls of the World: Their Evolution, Structure and Ecology (Ed. J.A. Burton). Peter Lowe, London, pp. 35–60.

9.Mikkola H (1983), Owls of Europe. T. and A.D. Poyser, Carlton, UK.

10.Niethammer J, Krapp F (1977), Handbuch der Säugetiere Europas– Band 1, Nagetiere 1 [Manual of the European Mammals,Volume 1, No 1]. AULA–Verlag, Wiesbaden (in German).

11.Niethammer J, Krapp F (1982), Handbuch der Säugetiere Europas– Band 2/1, Nagetiere 2 [Manual of the European Mammals,Volume 2/1, No 2]. AULA–Verlag, Wiesbaden (in German).

12.Niethammer J, Krapp F (1983), Handbuch der Säugetiere Europas– Band 3/1, Insektenfresser – Herrentiere [Manual of EuropeanMammals, Volume 3/1, Insectivora]. AULA–Verlag, Wiesbaden (in German).

13.Niethammer J (1989), Gewöllinhalte der Schleiereule (Tyto alba) von Kos und aus Südwestanatolien. Bonn. Zool. Beitr. 40: 1–9.

14.Paspali G, Bego F (2008), On the small mammals (Mammalia:Rodentia and Insectivora) of the Drinos valley (Gjirokastër) and Vurgu field (Delvine). In: Proceedings of the International Conference on Biological and Environmental Sciences. UT,FSHN, pp. 265–270 (in Albanian with English summary).

15.Paspali G, Oruci S, Koni M, Wilson FI, Krystufek B, Bego F (2013), Seasonal variation of small mammals in the diet of the barn owl (Tyto alba) in the Drinos river valley, southern Albania. Turkish Journal of Zology, 37, 97-105.

16.Ruprecht AL (1979), Food of the Barn owl, Tyto alba guttata (C.L.Br.). Acta ornithologica, Warsaw, 16(19): 493-511.

17.Yalden DW (1977), The identification of remains in owl pellets. Occas.Publ. Mammal. Soc., London..