AMJ
ALBANIAN MEDICAL JOURNAL

ISSN 0255-6790

Number 3 - 2016

ALBANIAN MEDICAL JOURNAL

REPUBLIC OF ALBANIA
MINISTRY OF HEALTH

INSTITUTE OF PUBLIC HEALTH

ALBANIAN MEDICAL JOURNAL

3-2016

AMJ
ALBANIAN MEDICAL JOURNAL

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Design & Layout
Genc Musa

Technical Editors
Florida Beluli and Kleopatra Sava

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AMJ ALBANIAN MEDICAL JOURNAL
3 - 2016

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ORIGINAL RESEARCH

The relationship between spirometric parameters and hemodialysis adequacy in hemodialysis patients

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Abstract

Aim: In our study we aimed to investigate the relationship between spirometric parameters and Kt/V, which is an indicator of dialysis adequacy.
Methods: The study was conducted at four centers in Turkey using Kt/V values in evaluating hemodialysis adequacy. Subjects included 71 patients 18 years old and above who had been receiving hemodialysis treatment for at least six months. Patients' Kt/V values were calculated. Spirometric parameters of FEV_1, FVC, FEV_1/FVC, PE, and FEF_{25-75} were measured in all patients. Relationships between these parameters and URR, serum electrolytes, serum creatinine, blood urea nitrogen, and hemoglobin were assessed.
Results: Factors potentially affecting patients' spirometric parameters of FEV_1, FVC, FEV_1/FVC, PEF, and FEF_{25-75} were examined by using cascading multiple linear regression analysis method. The highest variance (R^2) model for FVC was determined as having two components, age and Kt/V. For the FEV_1, a three-factored model was formed, and age and Kt/V were determined to be independent predictor indicators. Regarding the PEF value, Kt/V and age were found to be independent predictors for the four-component model.
Conclusion: Our findings indicate that age and dialysis adequacy (Kt/V) constitute statistically significant independent predictors for spirometric parameters in hemodialysis patients.

Keywords: dialysis adequacy, hemodialysis, spirometric parameters.

Introduction

Chronic renal failure (CRF) is a chronic and progressive disease. A decrease in glomerular filtration rate (GFR) continues with deterioration of kidneys metabolic-endocrine functions and impairment of fluid-solid balance regulation.When creatinine clearance falls below 5-10 ml/minute/1.73 m^2, end stage renal failure is indicated and patients require renal replacement treatments, such as hemodialysis, peritoneal dialysis and renal transplantation. The most used renal replacement treatment is hemodialysis (HD). Studies have found that effective hemodialysis decreases morbidity and mortality. Pulmonary complications are frequently seen in these patients due to inadequate dialysis or because patients do not obey fluid restrictions. Pulmonary edema, pleural effusion, fibrosis, calcification, pulmonary hypertension, and/or hemosiderosis may occur in patients diagnosed with CRF (1). Also, bronchitis, interstitial fibrosis, and hyperemia are often detected in these patients in autopsy (postmortem) examinations (2). The most frequently seen complications are clinical or subclinical pulmonary edema and pleural effusion. When creatinine clearance falls below 50 ml/minute/ 1.73 m^2, fluid retention starts in the body, and due to excessive fluid uptake, pulmonary capillary pressure increases. Uremic toxins impair pulmonary capillary permeability and as a result pulmonary edema occurs. Also, cardiac disorders in uremic patients contribute to pulmonary edema and pleural effusion (2).

Getting efficient HD is dependent on several factors. Some of these factors include dialysis adequacy (Kt/V), patients' nutritional status, existing comorbid diseases, degree of anemia, social-economical state, patients' compliance, adequate bloodstream and the type of membrane used in hemodialysis. The most used indicators of dialysis adequacy are measurements of urea clearance in each HD session. For this purpose, urea reduction rate (URR) and Kt/V are calculated. It has been demonstrated that when Kt/V increases, morbidity and mortality rates significantly decrease (3).

Nowadays, after clinical inspection and lung graphics (X-ray), spirometric tests become one of the basic investigation methods in diagnosis, treatment and monitoring of diseases. With these tests, we can evaluate pulmonary functions in an objective and quantitative manner. There are few studies investigating the relationship between Kt/V adequacy and respiratory functions in hemodialysis patients. In our study we aimed to investigate the relationship between spirometric tests and Kt/V value, which is an indicator of dialysis adequacy.

Methods

The study was conducted at four centers in Turkey using Kt/V values in evaluating hemodialysis adequacy. Subjects included 71 patients who were 18 years old and above and who had been undergoing dialysis treatment for at least six months. Exclusion criteria included any pathological findings of respiratory or circulatory system in the anamnesis or physical examination, having a rheumatic disease, chronic lung disease or cerebrovascular disease, hemoglobin <7 gr/dlt, or ejection fraction <40%. Patients were undergoing dialysis twice or three times a week. Effective surface area changed between 1-2.2 m^2 according to the patient's body surface area (1 m^2/m^2 body surface area), FX class high flux hemodialyzer (FX 50, FX60, FX80) standard heparinization, and with Fresenius 4008-B trademark hemodialysis machines at a blood flow of 250-400 ml/minutes and a dialysate pump rate 300-700 ml/minutes, from arteriovenous fistula.

According to the NFK-DOQ criteria (4), blood samples were taken via arterial needles before starting dialysis procedure and applying heparin or serum in order to establish pre-dialysis urea values. To establish post-dialysis urea values, we decreased the pump rate of hemodialysis machine to 50 ml/ minute for a period of 15 seconds. At the end of this period, we took blood from the nearest blood collection port on the hemodialysis set and finished

the dialysis procedure. Blood samples were taken in the middle session of the week from patients undergoing dialysis three times a week, and at the beginning session of the week from patients undergoing dialysis twice a week. We calculated the Kt/V values of patients who were included to the study twice a month for a total of 12 times. Kt/V value was calculated with Daugirdas-2 formula [Kt/V= -1n (R-0.008 t) + (4-3.5×R) × UF/KVA] (3). The arithmetic average of these Kt/V values was decided as patients Kt/V value. Normal Kt/V value was decided as $\geq$1.2 according to National Kidney Foundation - Dialysis Outcomes Quality Initiative criteria because there is a consensus on $\geq$1.2. URR, which shows the reduction rate of urea in one dialysis session, was calculated as URR=100-(1-BUN post-dialysis/BUN pre-dialysis). It is suggested that minimum target URR value should be 0.65 (4).

Creatinine, total calcium (Ca^{+2}), phosphate (P), albumin, uric acid, hemoglobin (Hgb), iron (Fe^{+3}) and total iron binding capacity (TIBC), ferritin, and cholesterol were also assessed from the serum samples. Patients underwent spirometric tests after hemodialysis, after becoming clinically stabile. The spirometry test was performed using a spirometric V plus spirometric device, with the patients sitting at a 90º sitting-up position. Patients were all previously informed about the tests. Spirometric tests were performed with at least three technically acceptable methods approved by the American Thoracic Society criteria (5). The highest values obtained from three different curves were taken into the study. Forced vital capacity (FVC), forced expiratory volume at first second (FEV_1), FEV_1/FVC, forced expiratory flow rate (FEF_{25-75}), and peak flow rate were evaluated among spirometric parameters.

Statistical analysis

The basic and definitive data were defined as mean and standard deviation, in those showing a normal distribution and, median and a low-high interval, in those not showing a normal distribution. For group comparisons, Student's t-test and the non-parametric Mann-Whitney U-test were used. Multiple cascading regression analysis was used while searching for factors that affected spirometric parameters. Predictors having the highest variance (R^2) were introduced into the regression analysis. All P-values were calculated as double-sided. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

There were 39 males (54.9%) and 32 females (45.1%), for a total of 71 hemodialysis patients in this study. The mean age was 54.9±13.4 years (range: 20-82 years) and the disease follow-up period was rather skewed with a median of 57 months (range: 6-216 months). The demographic characteristics, laboratory parameters and primary diseases of the patients are shown in Table 1.

Because Kt/V values were inadequate (Kt/V<1.2) for seven patients, we could not make a group comparison. There were 15 patients whom URR were not enough (<65%). Factors potentially affecting FEV_1, FVC, FEV_1/FVC, PEF and FEF_{25-75} as spirometric parameters, were searched by forming multiple cascading linear regression analysis models. In regression analysis, predictor factors supplying the highest R^2 were, respectively, Kt/V, age and hemoglobin for FEV_1; Kt/V and age for FVC; and Kt/V, age, hemoglobin and calcium levels for PEF. A regression model containing these factors was formed. For FVC, the two component model including age and Kt/V had the highest variance (R^2). Regression equation was as follows: FVC = 5.79 – (0.039*age) – (0.864*Kt/V). Remarkably, age and Kt/V were found to be statistically significant independent predictors (Table 2). The parameters showed normal distribution. A normal probability graphic of FVC values is shown in Figure 1.

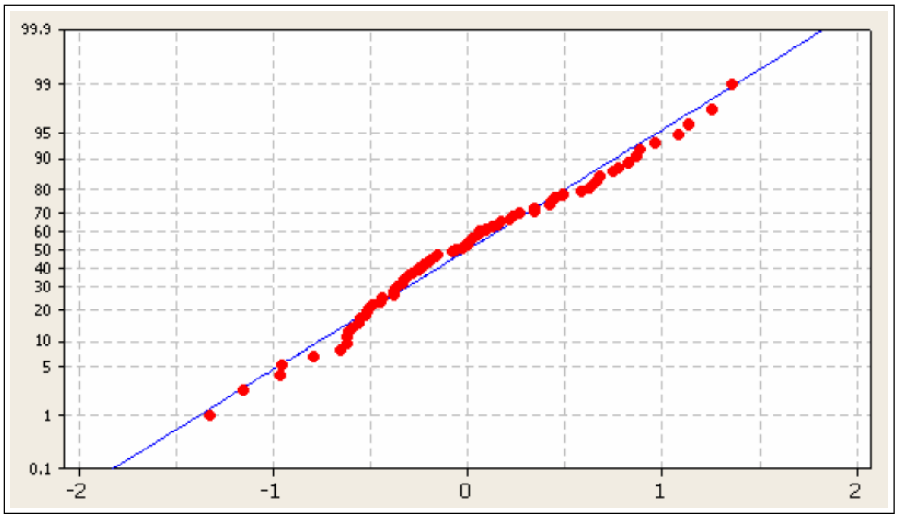
Table 1. Properties of hemodialysis patients

Demographic properties	Mean ± SD	Median
Patients' age (years)	54.86±13.4	54
Dialysis period (months)	57.14±51.6	57
Males (number)	39	
Females (number)	32	
BMI (kg/m^2)	26.74±4.3	26
Causes of chronic renal failure (numbers)		
Diabetic nephropathy	28	
Hypertensive nephropathy	14	
Glomerulonephritis	7	
Policystic kidney disease	6	
Urological diseases	5	
Pregnancy and complications	3	
FMF – Amyloidosis	2	
Other	6	
Laboratory Results		
Serum Albumin	3.87±0.5	3.8
Hemoglobin	11.2±1.4	11.2
Urea	146.9±38.7	146.9
Creatinine	6.96±2	6.9
Potassium	5.11±0.7	5.1
Calcium	8.4±0.7	8.4
Phosphate	4.9±1.4	4.9
Parathormone	284.6±356	284
Ferritin	1172.2±447	1172
Transferrin saturation (%)	35	
Total cholesterol	165±50	165
Working Parameters		
URR	67.57±10	67
Kt/V	1.4±0.2	1.4
FVC	2.35±0.9	2.3
FEV1	1.94±0.7	1.9
FEV_1/FEV	82.88±9.7	82.8
FEF_{25-75}	2.12±1	2.1
PEF	291.1±121	291

Table 2. Regression analysis: FVC and age, Kt/V

Predictors	Coefficient	T	P
Age	-0.039	8	<0.001
Kt/V	-0.864	-2.47	0.016
	S=0.764	R^2=32.3%	

Figure 1. Normal probability graph of FVC

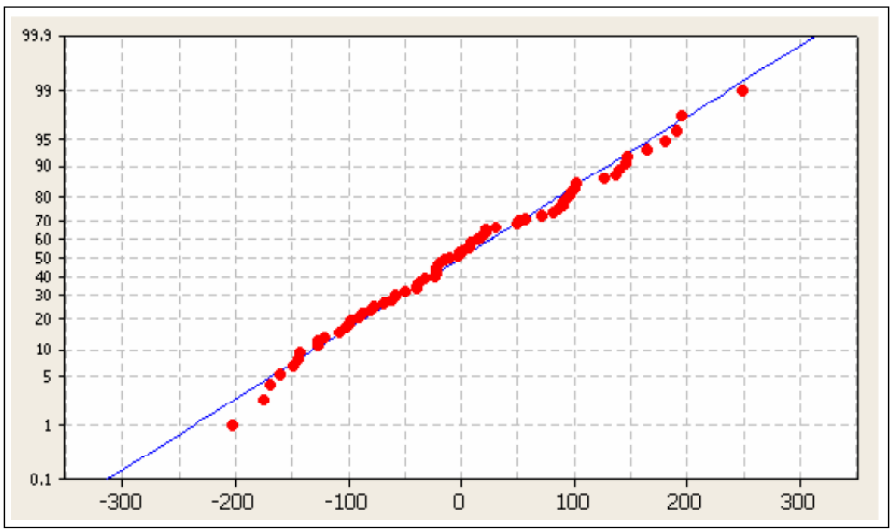


For the FEV_1, a triple model consisting of age, Kt/V and hemoglobin had the highest R^2 value. Regression equation was as follows: $FEV_1 = 4.13 – (0.031*age) – (0.627*Kt/V) + (0.042*Hgb)$. Age and Kt/V values were statistically significant among these three parameters as shown in Table 3. Parameters showed a normal distribution. The normal probability of FEV_1 value is shown in Figure 2.

Table 3. Regression analysis: FEV_1 and age, Kt/V, Hemoglobine

Predictors	Coefficient	T	P
Age	-0.031	0.0056	<0.001
Kt/V	-0.627	0.276	0.027
Hgb	0.042	0.048	0.388
	S=10.5	R^2=34.7%	

Figure 2. Normal distribution of FEV_1

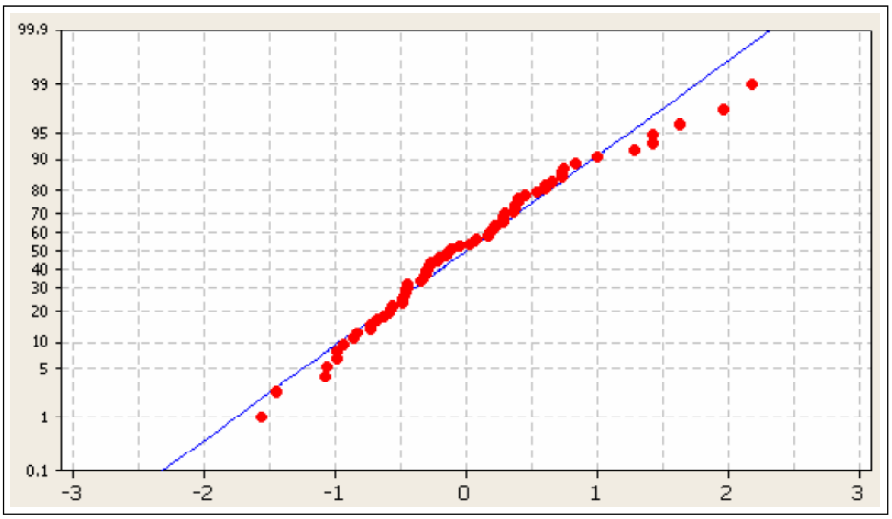


For the PEF, a tetra model consisting of age, Kt/V, Hgb and Ca^{+2} had the highest R^2. Regression equation was as follows: PEF = 430 – (4.6*age) – (130.5*Kt/V) + (9.3*Hgb) + (23.8*Ca^{+2}). Among these, only age and Kt/V values were statistically significant as presented in Table 4. Parameters showed normal distribution. A normal probability graph of PEF value is shown in Figure 3.

Table 4. Regression analysis: PEF and age, Kt/V, Hgb, Ca^{+2}

Predictors	Coefficient	T	P
Age	-4.6	-4.74	0.000
Kt/V	-130.5	-2.69	0.009
Hgb	9.3	1.09	0.28
Ca^{+2}	23.8	1.46	0.15
	S=10.5	R^2=22.9%	

Figure 3. Normal distribution of PEF



The highest R^2 value of the regression analysis, which was formed by calculating FEV_1/FVC values, was 34.2%. Age, Kt/V and ferritin levels were accepted as the best indicators.

Discussion

We observed 71 patients regularly undergoing hemodialysis for chronic renal failure, and found that age and Kt/V values are independent predictors for FVC, FEV_1 and PEF, ferritin and Kt/V values are independent predictors for FEV_1/FVC percentages. The significance values are in the order of PEF> FVC> FEV_1.

There may be pathological changes without respiratory symptoms or findings in the lungs of uremic CRF patients undergoing hemodialysis. Respiratory system complications in chronic renal failure patients, having regular hemodialysis treatment, are well studied. However, the affect of hemodialysis on pulmonary function is less known (6).

Reduction in lung diffusion capacity, decrease in tidal volume with restrictive type pulmonary disease, and interstitial edema are the most frequent of these pathologies (7). Research involving PEF measurements has showed that 75% of hemodialysis patients have a restrictive type of pulmonary diseases. Also, it has been reported that restrictive type pulmonary diseases are most seen in hemodialysis, peritoneal

dialysis and renal transplant patients (8). In the results of our study, the highest relationship with Kt/V was detected as the values of PEF.

There are two ways of respiratory function loss in CRF patients who do not have primary pulmonary diseases. Firstly, long-term interstitial and alveolar edema causes fibrosis in CRF patients. The basic property of dialysis is that it removes body's volume overload and excess fluid from the lungs. Kovelis et al. showed in their study that there was a statistically significant increase in FVC values and statistically insignificant increase in FEV1 values at the first week of dialysis, in patients undergoing dialysis for the first time (9).

In addition, we showed in our study that there is a significant relationship between dialysis adequacy indicators and respiratory parameters also in patients undergoing dialysis for a long time. Kovelis et al. showed that the most important factor playing a part in the increase of FVC is the reduction of body weight by removing excess fluid through dialysis (9). Despite this fact, in another study no relationship was detected between improvement in spirometric parameters and weight loss and laboratory indexes after dialysis (10). The second factor related to impairment in respiratory functions is when patients' blood has an allergic reaction with the dialysate membrane and an inflammatory response occurs. This condition may be eliminated with an increase in respiratory distress after dialysis session. None of the patients in our study reported an increase in respiratory distress.

It has been shown in previous studies that hemodialysis corrects spirometric parameters (11), improves exercise toleration and increases the quality of life (12). Compared with healthy people, CRF patients in our study had significantly lower spirometric values. Furthermore, patients' symptoms significantly decreased after dialysis. However, in some other studies no effects of dialysis on spirometric parameters could be found (13). Also, no significant difference between spirometric parameters, as measured at the beginning, middle, and end of the dialysis, could be detected (14). This condition can be explained as the minimal alveolar edema at the pulmonary tissue of the patients undergoing hemodialysis may correct symptoms but make no change in spirometric parameters. In our study, there is a relationship between Kt/V and spirometric parameters and the significance degree is PEF> FVC > FEV_1.

In another study, it was found that patients who were on a long-term hemodialysis program showed a significant decrease of the FVC following five years' treatment and none of the recorded spirometric parameters improved significantly one-hour after hemodialysis compared to the pre-hemodialysis period. They also found out that, although changes in spirometry observed in the population getting hemodialysis treatment have a reversible character during the first years of renal replacement therapy, after five years these changes become irreversible (6). In our study, we searched dialysis adequacy and found out that long-term efficient dialysis may be the cause of the significant relationship between respiratory parameters and dialysis adequacy. The dialysis adequacy rate was 90% in our patients and the inadequate dialysis rate was 10%. In those studies, because dialysis adequacy and spirometric parameters were not compared, inadequate dialysis may have been the cause of the ineffectiveness of hemodialysis on respiratory function improvement or inadequate dialysis itself may be a major cause of respiratory function deterioration. Most studies have shown significant improvements in respiratory symptoms at the end of the dialysis (12-14).

One study compared patients undergoing hemodialysis with bicarbonate and acetate dialysate and found that improvement in spirometric parameters was only significant in patients undergoing hemodialysis with bicarbonate. All spirometric parameters showed significant increases in the bicarbonate group, except FEV_1/FVC ratio (10). In our study, we used bicarbonate dialysate in all patients and found that the relationship between dialysis adequacy and increase

in spirometric parameters such as PEF, FVC, FEV_1 was statistically significant. However, similar to a previous study (10), no increases in FEV_1/FVC and FEF_{25-75} were observed in our study and predictor factors could not be detected.

In conclusion, in our study, age and dialysis adequacy (Kt/V) were found to be the most significant indicators among the respiratory functions' parameters in hemodialysis patients. It is known that Kt/V affects morbidity and mortality of hemodialysis patients to a high degree. Respiratory distress in hemodialysis patients or deterioration of spirometric parameters can show that clearance with dialysis is inadequate. We expect that dialysis adequacy will reduce the loss of pulmonary capacity and thus increase the survival rate of patients.

Conflicts of interest: None declared.

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Hepatitis and biochemical markers in correlation with alpha-fetoprotein as a diagnostic indicator for the HBV and HCV differentiation

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Abstract

Aim: The number of HBV and HBC patients is growing every day, with the dominance of HBV patients. The aim of this research work was to analyze biochemical and liver tumor parameters in HBV and HCV patients, including AFP correlation with other blood parameters in order to determine the diagnostic parameters important for differentiation of HCV and HBV.

Methods: The research included 48 patients with HBV and HCV during the period 2010-2013 at the University Clinical Center of Sarajevo. The analysis includes biochemical parameters and liver markers, CT, ECHO, MRI, cytology and pH findings.

Results: Most of the affected patients were middle-aged. There were no significant differences between HBV and HCV patients. AST, ALT, γGT, AFP and bilirubin were high in all patients. There was a correlation between the AFP and AST ($r=0.383$, $p<0.05$) and ALT ($r=0.501$, $p<0.01$). Test normality showed significant differences for most parameters ($p<0.05$). In all HBV patients there was anti-HBc (100%) and HBsAg (89.28%), while in HCV patients the anti-HBc (57.14%) dominated. Sex-specific analysis showed almost significant differences for γGT ($P=0.059$).

Conclusion: Increased serum bilirubin, AST, ALT and γGT are present in hepatitis. AFP and γGT are important biochemical markers of hepatitis. Anti-HBc and HbsAg are sufficient for HBV diagnosis, whereas for HCV there should be used biochemical and immune parameters (anti-HBc).

Keywords: alpha-fetoprotein, HbsAg, HBV, HCV, hepatic markers.

Introduction

Cirrhosis is the final stage of several types of chronic liver damage. The main pathological features are irreversible chronic damage of parenchyma accompanied by the formation of fibrosis and regenerative nodules (1). The formation of connective tissue deposits, nodular regeneration of parenchyma and distortion of vascular trough are microscopic features of this disease. In nodules, regenerating hepatocytes are arranged without any order, and various changes can be detected: focal atrophy, necrosis, dysplasia and hyperplasia and malignant alteration (1,2). The most common epidemiological causes of liver cirrhosis are excessive alcohol consumption (in 50-80% of cases) (3), and hepatitis B and C (in 10-30% of cases) (4). Important causes also are primary biliary cirrhosis (5-10%), hemochromatosis (2-5%) and Wilson's disease (about 1%) (5). Hepatitis B virus (HBV) infection causes acute and chronic liver disease and in chronic it carriers 100 times bigger risk of developing HCC (6). HBV contains a small partially double-stranded DNA and belong to the Hepadnaviridae family. Although still under discussion, it is considered that HBV can have direct and indirect effects on hepatocytes (7). The first direct effect is that the viral DNA can integrate into the genome of hepatocytes, causing disorder of chromosomal stability which leads to chromosomal rearrangements or deletions. Integration of HBV DNA can also lead to deregulation of the oncogenes or tumor suppressor genes expression involved in cell survival or cell death (7). Other direct effect of HBV can be attributed to 154 - amino acid (16.5 kDa) virus HBX protein, which transactivates genes involved in the control of cell proliferation, and deregulation of the cell cycle control, DNA repair and apoptosis (8). Hepatitis C virus (HCV) has recently been characterised as the leading cause of cirrhosis and HCC in developed countries (9). HCV belongs to the genus Hepacivirus, Flaviviridae family (6). Unlike HBV, HCV is an RNA virus and does not integrate into the host genome, but HCV infection can cause accumulation of abnormalities in the degeneration-regeneration process. HCV nuclear proteins and non-structural proteins NS3 and NS5A inhibit post-transcriptional expression of the cyclin-dependent kinase inhibitor, which plays an important role in cell-cycle control (10). HCV core protein has a particularly important role by modulating cellular proliferation, apoptosis and the immune response. This protein acts as transcriptional regulator of various cellular genes involved in the regulation of cell growth, including the c-myc proto-oncogene. HCV core protein can also induce carcinogenesis by other mechanisms, such as inhibition of several apoptosis activators, usually Fas and TNF-α (tumor necrosis factor-α) (10,11). Alcohol is an important cofactor in patients with HCV infection. Surprisingly high incidence of this infection (up to 55.5%) was found in alcoholics (12). Patients with HCV infection and simultaneous alcohol abuse develop severe fibrosis and have higher rates of cirrhosis than those who do not consume alcohol (12). In these patients have been reported higher rates of HCV replication and the inhibition of hepatic expression of Bcl-2 resulting in an enhancement of apoptosis and severe hepatic impairment (12). However, the dominant mechanism for the synergy between the alcohol and HCV infection seems to be an increase in oxidative stress. HCV core protein binds to the mitochondrial membrane, changes its permeability and promotes oxidative stress. Ethanol potentiates this mitochondrial injury by further increasing of reactive oxygen species (ROS) production and oxidation of hepatic glutathione. Moreover, the alcohol and the HCV core protein act synergistically in the induction of lipid peroxidation and enhancement of the liver expression of TGF-β and TNF-α (12). Complications of cirrhosis are portal hypertension, bleeding from esophageal varices (in 20-30% of patients with cirrhosis), ascites (most common complication), spontaneous bacterial peritonitis, hepatorenal syndrome, hepatopulmonary

syndrome, hepatic encephalopathy, cardiomyopathy and hepatocellular carcinoma (1,2,13). Treatment of patients with liver cirrhosis involves inhibition of etiological factors (e.g. abstinence from alcohol and treatment of chronic viral hepatitis), avoidance of additional risk factors and prevention of complications (AFP screening every six months, endoscopy, β-blockers, control of diuresis, treatment of infections, control of electrolyte and nutritional support). End stage of cirrhosis is treated by liver transplantation (1,2,5). For assessment of survival Child-Pugh score and MELD-score are the most commonly used (1).

The aim of this study was to analyze the biochemical parameters of liver and tumor markers with special emphasis on AFP in order to determine diagnostic parameters important for HCV and HBV as prognostic parameters.

Methods

Subjects

In this study we analyzed 49 patients with cirrhosis of viral B and C etiology (28 HBV and 21 HCV patients) who are under continuous AFP screening, HCC still unproved. This study was performed in the period January 2010 – March 2013 at the Clinic of Gastroenterohepatology, University Clinical Center, Sarajevo. There was selected a homogeneous sample of respondents in order to reduce multi-causality of the observed parameters. Approval for the study was given by the Ethics Committee of University Clinical Center Sarajevo. All patients were clinical, biochemical and histopathological treated.

Clinical, biochemical and pathohistological analysis

During hospitalization, all patients underwent liver function tests: bilirubin (total, direct and indirect, refferent values 6.8-20.5 mmol/L) and the value of enzymes: aspartate aminotransferase - AST (≤ 38 U/L), alanine aminotransferase - ALT GT (≤ 48 U/L) gamma-glutamyl transferase - gGT (11-55 U/L) and alkaline phosphatase - AP (60-142 U/L). Patients underwent immunological tests for hepatitis markers B and C (HBs Ag, anti-HBs, anti-HBc, Anti-HCV and anti-HBe), abdominal ultrasonography (US) and computed tomography (CT). All the patients were quantified for serum AFP (≤ 7 ng/ml) as a tumor marker. Bilirubin concentration and enzyme activity were determined by analyzer VITROS 5600 Integrated System (Ortho Clinical Diagnostics, USA). For hepatitis markers detection ELISA method was applied, while the concentration of AFP was determined using chemiluminescent microparticle immunoassay ARCHITECT AFP assay - (CMIA, Ireland).

Statistical analysis

Data were analyzed by using SPSS Version 17 software to determine the mean values of different tumor markers and biochemical parameters and to assess the presence of significant difference in the above values with regard to factors supposed to cause variation. The 95% confidence interval and 5% absolute precision were employed for the analysis of variance test (ANOVA). Nonparametric Spearman's test was used for the analysis of correlation, while the normality distribution was determined by Kolmogorov-Smirnov and Shapiro-Wilk tests.

Results

Table 1 shows the percentage ratio of HBV and HCV patients by age. Patients were divided into four groups; young-aged group (age between 18 and 40; n=1), middle-aged group (age between 41 and 64; n=28), old-aged group (age between 65 and 75; n=13), and very old-aged group (≥76, n=7). The largest number of affected patients of both groups belonged to the category of middle-aged, followed by a group of old-aged patients.

In addition to the analysis of biochemical parameters, ultrasonography, CT and MRI of the liver (Imagining Methods), as well as cytology and pH findings were analyzed. The largest number of infected patients had liver cirrhosis.

Statistical testing of biochemical parameters HCV

Table 1. Percentage ratio of HBV and HCV patients

Type of patients	Young-aged (18-40 years)	Middle-aged (41-64 years)	Old-aged (65-74 years)	Very old-aged (≥75 years)
HCV	0	15 (71.42%)	4 (19.04%)	2 (9.52%)
HBV	1 (3.57%)	13 (46.42%)	9 (32.14%)	5 (17.85%)
Total	1	28	13	7

and HBV patients is presented tables 2-5. Table 2 presents the biochemical parameters of 21 patients with hepatitis C virus (males and females) including the mean and standard deviation. In addition, analysis of variance (ANOVA) between men and women was also performed and the resulting p-values are presented. All HCV patients had increased values of bilirubin, except for direct bilirubin in females. We detected very high values of AFP, especially in males. Analysis of enzyme activity showed that the AP value was in the reference interval, a very high value was characteristic for AST and γGT, while ALT was slightly increased, with higher values in males. All the analyzed parameters had higher values in males against females. The largest variations were typical for values of AFP, AST and γGT. Between males and females there was a borderline statistically significant difference (P=0.059) for values of γGT.

Table 2. Biochemical parameters of HCV patients (males and females)

Parameters	Total (n=21)	Males (n=8)	Females (n=13)	P-value
Total bilirubin	37.26±27.79	44.42±30.97	30.90±22.66	0.265
Direct bilirubin	23.88±13.79	27.66±15.07	19.15±8.66	0.182
Indirect bilirubin	37.39±16.67	37.86±14.33	36.80±19.95	0.476
AFP	59.62±135.03	78.85±175.27	42.62±83.35	0.409
AST	91.29±71.69	121.44±85.86	69.85±43.13	0.089
ALT	57.67±38.08	70.89±31.87	48.69±37.91	0.174
γGT	89.05±88.45	128.11±114.57	57.08±35.83	0.059
AP	117.90±43.28	119.89±57.13	113.08±26.11	0.73

Table 3 shows the results of biochemical parameters of 28 patients (including males and females) with hepatitis B virus diagnosis. The analysis included the mean, standard deviation and ANOVA. P values show statistically significant differences between males and females for HBV patients, and P values in the last column represent differences between HCV and HBV patients. For HBV patients we detected higher bilirubin concentration compared to HCV patients, and high variations were found for indirect bilirubin in females. These patients also had increased values of AFP, however they were much less compared to HCV patients. Also, AP values were within referent range. For AST, ALT and γGT, there were observed high values. HBV patients had higher levels of enzymatic activity in comparison to HCV patients. The values of all enzymes in females were higher compared to men, especially AST and γGT. The greatest variations in HBV patients were also present for the value of γGT. However, between HBV and HCV patients, as well as between males and females, there was no statistically significant difference.

Table 3. Biochemical parameters of HBV patients (males and females)

Parameters	Total (n=28)	Males (n=17)	Females (n=11)	P-value	
	Mean ± SD	Mean ± SD	Mean ± SD	Male-female (HBV)	HCV/HBV
Total bilirubin	48.24±37.95	46.06±41.75	51.33±33.35	0.723	0.229
Direct bilirubin	31.95±30.14	32.94±35.54	30.59±22.97	0.872	0.172
Indirect bilirubin	47.81±74.50	27.76±17.08	75.36±111.16	0.176	0.284
AFP	20.33±55.11	26.11±71.53	12.13±12.79	0.511	0.462
AST	122.2±180.8	90.18±63.06	167.67±271.3	0.263	0.452
ALT	88.5±114.95	84.00±105.33	94.92±131.99	0.806	0.236
γGT	126.8±201.6	101.71±122.8	162.50±281.4	0.434	0.389
AP	126.5±109.4	114.41±132	143.67±67.71	0.488	0.695

Correlation between AFP and other biochemical parameters as shown in Table 4 was mostly positive (except ãGT in HBV and total bilirubin and AST in HCV patients). A statistically significant correlation was observed in HBV patients with AST ($p < 0.05$) and ALT ($p < 0.01$).

Table 4. Nonparametric Spearman's correlation test between AFP and biochemical parameters

Parameters		Total bilirubin	Direct bilirubin	Indirect bilirubin	AST	ALT	γGT	AP
HBV	**r**	0.139	0.049	0.351	0.383	0.501	-0.065	0.104
	P	0.480	0.842	0.141	0.04*	0.07**	0.744	0.599
HCV	**R**	-0.42	0.619	0.643	-0.002	0.112	0.107	0.253
	P	0.864	0.102	0.086	0.993	0.628	0.644	0.283

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 5 presents the percentage ratio of patients with positive and negative findings for hepatitis markers, as well as their reference range. All HCV patients did not have a positive test for HBs Ag and anti-HBs. Most important diagnostic immunological liver marker was anti-HBc (57.14%). However, HBV patients usually have a present and the high percentage of almost all markers (except for anti-HCV). Anti-HBc was a marker present in all HBV patients (100%). Kolmogorov-Smirnov normality test (for samples of >50 individuals) and Shaphiro-Wilk normality test (for samples of ≤50 individuals) for HBV and HCV patients including males and females were performed. Most biochemical parameters in HBV patients did not have normal distribution.

Table 5. The link between hepatitis markers for HBV and HCV patients

	Parameters	HBs Ag (0.00-0.99 S/CO)	anti HBs (0.00-9.99 mLU/mL)	anti HBc (0.01-0.99 S/CO)	Anti HCV (0.00-0.99 S/CO)	Anti Hbe -
HBV	**Reactive**	89.28%	68%	100%	45.55%	84.21%
	Nonreactive	10.71%	32%	-	55.55%	15.78%
	Range	0.18-5204.5	0.01->1000	0.14-14.47	0.05-13.65	0.02-1.35
HCV	**Reactive**	30%	38.09%	57.14%		
	Nonreactive	70%	61.90%	42.85%	-	-
	Range	0.25-0.44	0.98-871.84	0.13-12.26		

Discussion

HBV and HCV viral infections are the dominant infectious disease among the world's population. In developed countries, HCV infection is more prevalent compared to HBV infection. Both diseases make important candidates for public health in terms of prevention, early diagnosis and treatment (14). Individuals with HBV and HCV have a high risk for development of cirrhosis and hepatocellular carcinoma in the end. In this study, we analyzed the biochemical and immunological parameters of patients with HBV and HCV viral etiology with the aim of identification of the most important liver markers for differentiation the hepatitis type, the prognosis and course of the disease. Serum bilirubin is one of the earliest known markers of liver. But as its activity increased in other liver diseases too, bilirubin is not sufficient for diagnosis. The concentration of bilirubin in our study is very high as referred in other studies (16). Damage of liver cells cause the release of large amounts of bilirubin which increase levels of serum bilirubin. The ratio of direct and indirect bilirubin depends on the degree of hepatocytes damage. In most patients there were observed necrosis of hepatic tissue, and thus much increased serum bilirubin. However, the bilirubin is not sufficient for diagnosis, but has great diagnostic significance.

In clinical practice, the level of AFP is elevated in a variety of clinical situations, including hepatocellular carcinoma, acute or chronic viral hepatitis, chronic liver disease and gonadal tumors (17). Elevated levels of AFP are used as a marker of liver regeneration after destruction of hepatocytes in viral hepatitis and as a dependent predictor for the patients with HBV and HCV (18). AFP is typically produced during fetal neonatal development of liver, and its concentration decreases after birth when it is present only in trace amounts (less than 10 ng/mL) (19). There is a much more records that AFP levels are significantly raised in anti-HCV-positive patients in comparison with HBsAg-positive patients (17). Serum AFP levels were higer in HCV (59.62 ± 135.03 ng/mL) and HBV (20.33 ± 55.11ng/mL) patients in comparison with referent values ($\leq$7 ng/mL) and there were a non significant difference in HBV patients compared to HCV ($p>0.05$). Increased values of AFP in HBV and HCV patients were reported in the earlier studies (18,21).

We detected a very high value of enzymes AST (91-122 U/L), ALT (57-88 U/L), AP (117-126 U/L) and γGT (89-126 U/L) in HCV and HBV patients. Compared with previous studies (18) our values are significantly higher. Increase in enzyme activity may be a result of the destruction of liver cells and the release of enzymes and increase in their serum concentration. High levels of ALT is the most specific indicator of liver damage (25). It is proved that necrosis of hepatocytes is not necessary the main reason for the release of serum amino-transferases and that the correlation between the levels of liver aminotransferases and degree of hepatocyte damage is weak (26). While some studies detected normal histological findings in patients with normal ALT levels (27), other studies have shown that viral load in patients with normal ALT levels was associated with liver injury (28). On the other hand, increased serum transaminase activity is present especially in patients in which case infection was associated with other diseases. Generally, the AP is usually not changed in HCV infection, but there was a significant increase in γGT in liver cirrhosis, which was also detected in our study (29). Liver ultrasonography showed the prominent and coarser structural changes in HBV compared to HCV patients.

In all patients there were observed the cirrhotic changes in the liver, so the enzyme's activity is in correlation with the degree of the cell's integrity. Therefore, it is evident presence of correlation between the anatomic changes of hepatocytes and the enzyme's activity. Previous studies had shown (22) that bilirubin AST, ALT and ALP correlate with liver damage, while their values correlated with serum HCV RNA were present only in the case of severe stages of fibrosis, but not in

inflammatory processes. Elderly people who are positive for HBV have higher levels of serum bilirubin, transaminases, and a significantly higher prevalence of negativity in HBsAg and anti-HBs (23). Increased levels of bilirubin (particularly indirect) and transaminases (particularly γGT) may suggest to hepatitis, but its type can be identified only by using immunological markers.

The positive correlation between serum AFP and the other biochemical parameters refers to the role of AFP in the pathogenesis of chronic liver disease. Until today many markers were identified and used in the diagnosis and monitoring of disease progression, but serological test cannot clearly diagnose infection. For example, positive HBsAg is a sign of HBV infection but also negative HBsAg can not exclude HBV infection. So far, definitive diagnosis of HBV liver infection relies on a combination of serologic, biochemical and histologic tests. Invention of new hepatic markers will contribute to reduction the number of biopsies. During HBV infection, clearance of hepatitis B surface antigen (HBsAg) is a key event, which implies that the host is not immune tolerant for a long time and enters the low phase of replication (24). Therefore, most of HBV patients (89.28%) have present this antigen, and it could be considered as major marker in HBV positive patients. The presence of HBsAg is a sign of HBV infection and is considered as the first serological marker of acute hepatitis. Its serological presence of more than six months suggests the beginning of chronic HBV infection. The simultaneous presence of this antigen with other clinical and biochemical features usually indicates the occurrence of acute infection in patients with low endemic area, but not in patients with high or medium-endemic regions (20). Chronic HBV infection begins as an acute infection and is characterized by the presence of HBsAg and HBeAg in the serum. The presence of HBeAg indicates continuous viral replication and also indicates a high viral load and infectivity and can last several months or even years. People who are HBeAg positive better respond to antiviral drugs. Our results are comparable with previously presented studies whose results pointed to a large percentage of HBsAg and anti-HBe patients (15). All HBV patients had detected anti-HBc, whereas only 57% of HCV patients had a positive anti-HBc and HBsAg in 30% of patients. Therefore, diagnosis of HCV based on immunological markers is very difficult and unreliable.

Increased levels of serum bilirubin, AST, ALT, gGT can lead to the diagnosis of hepatitis. However, AFP together with γGT have greater diagnostic significance and they can be considered as the most important biochemical markers of hepatitis. For the diagnosis of HBV, anti-HBc and HbsAg are sufficient, while HCV diagnosis requires the combination of biochemical markers and immunological tests, especially anti-HBc.

Conflicts of interest: None declared.

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Alcohol use among adolescents and young adults in Albania

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Abstract

Aim: Alcohol use during adolescence and young adulthood remains a prominent worldwide public health problem. The aim of this study was to provide an overview of the frequency and volume of alcohol consumption, drinking habits and beverage preferences among adolescents and young adults in Albania.
Methods: During the period 2007-2008, 583 adolescents (31 boys and 252 girls) and 755 young adults (402 boys and 353 girls) from different regions of Albania completed a standardized self-administered questionnaire concerning the overall frequency of drinking on a weekly basis during the last 12 months, the number of drinks per drinking day, drinking habits and beverage preferences.
Results: The prevalence of alcohol consumption increased with age, from adolescents (63%) to young adults (77%). Daily drinking was also more common in young adults (11%) compared with adolescents (5%), $p<0.001$. Also, girls seem less likely than boys to drink frequently. The proportion of moderate drinkers, heavy drinkers and binge drinkers was higher in young adults than adolescents, respectively 28%, 9%, 34% and 20%, 4%, 28%. The most common type of alcohol beverage preferred by adolescents and young adults was beer, followed by spirits.
Conclusion: Alcohol drinking among adolescents and young adults in Albania is widespread and worrisome. These findings should raise the awareness of health professionals and policymakers in Albania and other transitional countries.

Keywords: adolescents, alcohol consumption, drinking patterns, heavy drinkers, young adults.

Introduction

Alcohol consumption is a worldwide problem and a major risk factor for alcohol-related diseases, with a high morbidity and mortality (1,2). Epidemiological findings on adolescents and young adults alcohol use indicate not only the high rates of alcohol consumption among those age groups, but also the fact that many adolescents and young adults engage in drinking practice (e.g. binge drinking, daily drinking) associated with a considerable number of fatalities on roads or suicide, a broad social impact, such as violence, hooliganism, crime, sexual assaults, with a range of other risky behaviours including tobacco or illicit drugs and with disruption in significant contents (e.g. school, family, work) (3). On the other hand, studies conducted in various countries have demonstrated that both frequency of drinking alcoholic beverages and the amount of alcohol consumed per person or per occasion vary greatly among different countries and cultures, among different population groups within given country, and for each population over time. These differences are found not only for adult drinkers but also for adolescents and young adults (4).

Alcohol has been consumed in Albania for centuries. Because of political and social restrictions after the Second World War, its use before 1990 has been limited for occasional and festive events. The number of regular and heavy drinkers was restricted almost entirely to the elderly and adults. Moreover, the use of alcohol by women has been nearly inexistent. Rapid political, economic, social and cultural transformation after 1990 has deeply changed habits of alcohol use and alcohol-related phenomena. This paper reports the results of an anonymous self-administered survey, undertaken among adolescents and young adults from different regions of Albania.

More specifically, the objective of this study is to provide factual information about of the overall frequency of alcohol consumption, volume of alcohol intake, drinking habits and beverages preferences among adolescents and young adults in Albania.

Methods

Study population

The study population comprised a random sample of 583 adolescents (331 boys and 252 girls) and 755 young adults (402 boys and 353 girls) from different districts of Albania. The study was conducted during the period 2007-2008.

Examination procedure

The participants filled in a standardized self-administered questionnaire concerning drinking habits. The survey questions addressed to the overall frequency of drinking on a weekly basis during the last 12 months, volume of pure alcohol consumption (i.e. the usual number of drinks per drinking day), on drinking habits and beverage preferences.

Alcohol intake

In order to calculate the amount of alcohol in grams for each "drink", participants were informed about the "drink size" and alcohol content of the drinks they had consumed. A standard "drink size" was defined as a volume of various alcohol beverages that contains approximately 12-13 gram of pure alcohol, i.e. 10 ounces of light beer (a bottle of beer = 340 ml), 4 ounces of regular wine (a glass of wine = 142 ml) and 1.5 ounces of strong drink (a shot of raki = 43 ml).

Individuals were classified according to the prevalence of drinking behaviour, such as rates of abstinence or current drinking (once a week, 3-4 times a week or every day) and according to the volume of alcohol consumption, i.e. the number of drinks consumed in a single day (grams/ethanol per day). As abstainers were defined people who had never consumed more than 12 alcoholic drinks in one year, light drinkers those who consumed 1-13 alcoholic drinks per month, moderate drinkers persons who consumed 4-14 alcoholic drinks per week and heavy or harmful drinkers respondents who consumed five or more alcoholic drinks per day for men and four or more alcoholic drinks per day

for women. Binge drinking was considered having five or more alcoholic drinks in one occasion during the past 30 days.

Finally, participants were asked to indicate the drinking habits and the number of servings of beer, wine, spirits or mixed drinks they consumed on a drinking day. Those who obtained less than 50% of their alcohol consumption from one type of beverage were classified as having no specific preferences.

Results

Table 1 shows the frequency of total alcohol consumption by two groups. The lowest proportion of abstainers was found in young adults (22.8% of responders). Nearly half of adolescents (47.9%) and more than half of young adults (51.0%) used to drink once a week. The proportion of responders who consumed alcoholic beverages 3-4 times a week was 10.8% among adolescents and 15.4% among the young adults. Daily drinking seemed also to be more common in young adults (10.8%) compared with adolescents (4.7%). The percentage of girls who consumed alcohol increased from adolescents (60.8%), to the young adults (73.6%). Girls were also less likely than boys to drink frequently. The majority of adolescents and young adults consuming alcohol 3-4 times a week were males ($p<0.001$). Likewise, adolescents who drank every day were almost exclusively of male gender.

Table 1. Classification of respondents according to the frequency (in percent) of drinking

Groups	Total	Abstainers		Once week		3-4 times a week		Every day	
		Total		Total		Total		Total	
		Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Adolescents	583	36.6		47.9		10.8		4.7	
		47.3	52.7	53.6	46.4	58.0	42.0	63.0	37.0
Young Adults	755	22.8		51.0		15.4		10.8	
		46.4	53.6	39.4	60.6	57.1	42.9	57.1	42.9

Table 2 shows a constant increase of moderate alcohol intake from adolescents (20.0%) to young adults (28.5%), $p<0.001$. Also, the percentage of heavy drinkers (more than 60g/day) was higher in young adults (9.3%). Even the proportion of heavy drinkers (more than 60g/day) was higher in young adults (9.3%) than adolescents (4.2%). Binge drinking was found among 28% of adolescents (70.8% boys and 29.2% girls) and in 34% of young adults (68.4% boys and 31.6% girls).

Table 2. Classification of respondents (in percent) according to the volume alcohol consumption

Groups	Total	Abstainers <12/year		Light Drinkers 1-13/month		Moderate Drinkers 4-14/week		Heavy Drinkers ≥5/day	
		Total		Total		Total		Total	
		Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Adolescents	583	36.6		39.2		20.0		4.2	
		47.3	52.7	34.5	65.5	64.3	35.7	66.8	33.2
Young Adults	755	22.8		39.4		28.5		9.3	
		46.5	53.5	26.6	73.4	69.8	30.2	57.2	42.8

Table 3 provides an overview of the overall frequency of habits and type of alcohol beverage preferences. The most common type of alcohol beverage preferred by adolescents and young adults was beer, followed by spirits and wine. There were no significant differences regarding the drink

preferences between boys and girls. Girls were proportionally less likely to prefer wine or distilled spirits and more likely to drink beer. About a third of respondents did not report any specific preferences.

Table 3. Overall frequencies (in percent) of drinking habits and beverage preferences.

Drinking habits Beverage preferences	Adolescents (370)	Young Adults (583)
Drinking place		
At home	18.1	14.0
Outside at public place	81.9	86.0
Companion		
Alone	7.0	13.0
With friends	93.0	87.0
Drinking time		
During the meal	6.2	18.0
During the dinner	27.0	17.0
No regular time	66.8	65.0
Beer	54.9	42.0
Wine	13.1	15.8
Spirits	16.9	18.2
Mixed beverage	15.1	24.0

Discussion

This survey focussed on the prevalence of current drinking, mean level of alcohol consumption, as well as drinking habits and types of alcohol beverages and is one of the first study on alcohol consumption among adolescents and young adults in Albania.

Trend of alcohol consumption by adolescents and rates of alcohol-related problems had remained stable or had risen until 2003, particularly in some Eastern European countries, followed by a decreasing trend observed in the last years (5). Our findings indicate that 63.4% of teenagers use alcoholic beverages regularly and the percentage of 10.8% of the alcohol use with frequency of 3-4 times a week, lists Albania between in the middle of various countries in Europe (5). On the other hand, 28% of adolescents (68% boys and 32% girls) were engaged in binge drinking. Besides the known factors, related to the physiological, personality and life-style change, such as the tendency to emphasize independence, changing beliefs, habits and performance, influence of peers, risk taking, rebelliousness on actions outside the social rules, the alcohol use by adolescents in Albania was strongly influenced by profound social and cultural changes during the last two decades (6,7).

The worrisome problem of alcohol abuse by adolescents turn them into regular consumers and progressive heavier drinkers, with alcohol problems in their future (8,9). Individuals who begin drinking at the age of 15 years are four times more likely to become alcohol-dependent than those who do not use alcohol before the age of 15 years (10).

In our study, teenage boys had a higher prevalence of alcohol use than girls, especially concerning the category of 3-4 times a week and daily drinking (Table 1). Such models of differences between boys and girls are encountered in the majority of European countries, except Sweden, Finland and Norway (5).

When adolescents start to drink, they generally tend to consume high quantities of alcohol, thus undermining the safety of themselves and others (11,12). Specifically, 20.0% of the teenagers

interviewed (64.3% boys and 35.7% girls) were included in the group of moderate drinkers and 4.2% of them as heavy drinkers.

The survey shows that drinking is common among young adults. Concretely, 51.0% of students interviewed used to drink alcohol once a week. A higher frequency of alcohol use, more than three times a week was found in 15.4% of students, less than those in developed countries of Europe, such as the Netherlands, Austria, Belgium or England (20%-25%), but higher than in Finland, Iceland, Norway or Sweden (13). The transitory period from adolescents to young adults is marked by frequent change and exploration and also of increased alcohol use and abuse (14). We consider that freedom from family ties and control, life in the dormitories, freedom from social control and the freedom to purchase alcohol, make students easier to embrace alcoholic beverages. The full-time college students are more likely to drink heavily and get drunk than those part-time or non-college peers (15). As in other post-communist countries, a major impact on Albanian students has the natural tendency of globalization, which leads to the trend of reducing differences between cultures through the use of alcohol. Comparative qualitative studies have shown some surprising similarities between youth drinking practices in Northern and Southern Europe (16). Binge drinking was found in 34% of the students (65% boys and 35% girls). Students are in fact able to consume much longer amounts of alcohol than adolescents before experiencing the negative consequences of drinking. This unusual tolerance may help to explain the high rate of binge drink among students.

Alcohol consumption in students is characterised by tendencies of the use of large quantities of it. Excessive youthful drinking, thus, may be seen as part of an overall tendency toward experimentations and risk-taking and not as an isolated and bizarre behaviour specific to a certain type of alcohol drink. The data of our study show that while 39.4% of students are included in the group of light drinkers, almost one third of them (28.5%) are considered moderate drinkers and 9.3% of them as heavy drinkers. Our data on the heavy drinkers are in fact lower than the 15.6% prevalence found in students of 35 different countries of Europe (15).

Preferences for specific types of alcohol drinks vary between different population groups. In our study, the most common type of alcohol beverage preferred by adolescents and young adults was beer, followed by spirits and wine (Table 3). In fact, distilled spirits, rather than wine, are the second most common beverage of choice (17). Finally, the study showed that adolescents and young adults consume alcohol in a variety of contexts, mostly outside at public places (bars, restaurants, parks, beaches), as well as homes of friends or acquaintances.

In conclusion, our survey indicates that alcohol drinking among adolescents and young adults in Albania is both widespread and harmful. In addition to the high rates of alcohol use among these age-groups, our data indicate the particularly worrisome aspect concerning the high prevalence of moderate or heavy drinking and heavy episodic drinking. The information provided in this study should support the efforts and social policies regarding health and well-being of Albanian youth.

Acknowledgements

We would like to thank for their assistance the doctors that helped in data gathering from all over Albania: Nevila Tabaku, Hilmi Duhanxhi, Nehat Dumani, Vanedik Çallo, Vasil Kareco, Sotiraq Vangjeli, Albana Duni, Vigjilenca Demiraj.

Conflicts of interest: None declared.

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Use of molecular markers immunoreactivity can help stratify glioma's patients into groups within the same grade and histological entity

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Abstract

Aim: Glial tumors are evaluated pathologically, based on the World Health Organization (WHO) or the St Anne - Mayo system. In recent years, some molecular markers are used to stratify patients into groups within the same grade and histological entity, in order to determine in the best possible way, the prognostic factors for each patient and to give them a targeted treatment. The most used markers are: protein 53 (p53), Isocitrate dehydrogenase-1 (IDH1), Internexin-Alpha (INA), Synaptophysin-syn, and Her-1 (EGFR).
Methods: We studied INA expression in 137 gliomas and correlated it with histology, IDH1, p53, EGFR and SYN expression by immunohystochemestry.
Results: INA was expressed by 71.1% of pure oligodentrogliomas versus 17.2% of non-pure oligodentrogliomas. In addition, INA was expressed by 45.1% of gliomas with IDH1 mutation versus 18.2% of gliomas without IDH1 mutation. Furthermore, INA was expressed by 27.4% of gliomas with P53 mutation versus 42.9% of gliomas without P53 mutation. Also, INA was expressed by 50.0% of gliomas with SYN expression versus 18.2% of gliomas without SYN expression. Finally, INA was expressed by 27.3% of gliomas with EGFR expression versus 34.0% of gliomas without EGFR expression. Our study has demonstrated that molecular markers used are very useful for determining the entity in glial tumor (P=0.0001) and even for determining the grade in these tumors (P=0.0001).
Conclusion: Use of molecular markers is a fast, cheap and reliable diagnostic and prognostic method, which helps identify patients of different prognostic groups in diffuse gliomas and should be used routinely in the pathologic diagnosis of glial tumors.

Keywords: alpha-internexin, EGFR protein, glial tumors, IDH1, P53, synaptophysin.

Introduction

Glial tumors, which include astrocytomas, oligodendrogliomas, mixed gliomas, and ependymomas, are the most common primary malignancy of the central nervous system (CNS) and account for 78% of cases globally in neurosurgery (1). From a therapeutic and especially prognostic point of view, the differential diagnosis among these entities and their histologic grades is of clinical importance to predict biological behavior and to determine the optimal treatment protocol. Distinguishing glial subtypes based only on morphologic criteria of the WHO system of 2007 as nuclear and cellular changes (2), is very subjective, with significant inter/intra observer variability, even among highly experienced neuropathologists (3,4). Furthermore, even in a same histological entity and tumor grade there are tumors harboring different molecular profiles (IDH1/2 mutations and 1p/19q codeletion) (5,6), which show different survival patterns. (7). It has been well demonstrated that 1p/19q codeletion, P53 and epidermal growth-factor-receptor (EGFR) gene amplification are mutually exclusive in gliomas (8-10). So are the IDH1 mutation and the EGFR amplification. Isocitrate dehidrogenase-1 mutation (IDH1) is founded in the major part of diffuse gliomas, but infantile gliomas and primary glioblastoma and is related with elevated overall survival and secondary glioblastoma (8,9). Internexin alpha (INA), a neuronal intermediate filament is found in most gliomas especially those with oligodendroglial features and 1p19q codeletion and seems to represent a valuable diagnostic, prognostic and predictive marker in clinical routine (12-13). EGFR is present in 30-40% of primary glioblastomas, is related with a worst prognosis and seems to predict e better reaction to avastine treatment (15). The aim of this study was to evaluate the possible relationship of INA with pure oligodendroglial phenotype, P53 expression, IDH1 mutation, SYN and EGFR immunoreactivity which can further help identifying and stratifying patients according to their clinical, pathological and survival characteristics. Another aim of this study is to show the value of these markers in optimizing the pathological diagnosis for glial tumors in daily routine.

Methods

Histological cases were selected from pathologically proven low grade and high grade gliomas operated at the University Hospital Center "Mother Teresa". From this database, only cases with thorough information on immunohistochemical expression of IDH1, P53, SYN, INA and EGFR were selected. A total of 137 patients who underwent a neurosurgical operation from 2010-2014, were included when complete clinical information and tissue paraffin blocks were available. Tumor histology was classified according to the 2007 WHO classification.

Each tumor tissue sample was fixed with formalin and embedded in paraffin. Representative paraffin blocks were selected and mounted on slides with hematoxylin and eosin (H&E) staining before they were prepared for the tissue microarray. Cores from representative areas of each tumor were marked on both an H&E stained tissue section and an original donor block. 4-mm diameter tissue cores were extracted from the marked area of each donor block and placed in tissue cores. Five 4-μm thick sections were cut from each array block.

Immunohistochemistry was performed on these sections. The immunolabelling technique was performed by a Bench Mark XT automated tissue staining system. The markers used, their clones, manufacturers and dilutions are shown in Table 1.

Table 1. The markers used in this study, their clones, manufacturers and dilutions

Antibody	Clone	Manufacturer	Dilution
P53	318-6-11	DAKO	1:50
IDH1	H09	DIANOVA	1:50
INA	ID2	ACRIS	1:1000
SYN	SP11	VENTANA	ready to use
EGFR	3C6	VENTANA	ready to use

Immunoreactivity of p53 was expressed in the percentage of immunostained nuclei. Immunoreactivity of INA, IDH1, SYN and EGFR was classified as positive if >10% cells were positive, and negative if <10% cells were positive. Immunoreactivity for INA was considered positive if intracytoplasmic crescents or paranuclear dots were present.

Presentation of data is done through tables and diagrams. Data processing was done with the statistical program SPSS 20. Statistical techniques selected were method of X2 (chi-square test), correlation methods by Pearson, Spearman and Cramer's.

Results

The histology, INA, IDH1, p53, SYN, EGFR expression of the 137 gliomas are reported in Table 2. INA was expressed in 72.2% of grade II oligodendrogliomas (n=22), 62.5% of grade III oligodendrogliomas (n=16), 57.2% of grade II oligoastrocytomas (n=7), 66.7% of grade III oligo-

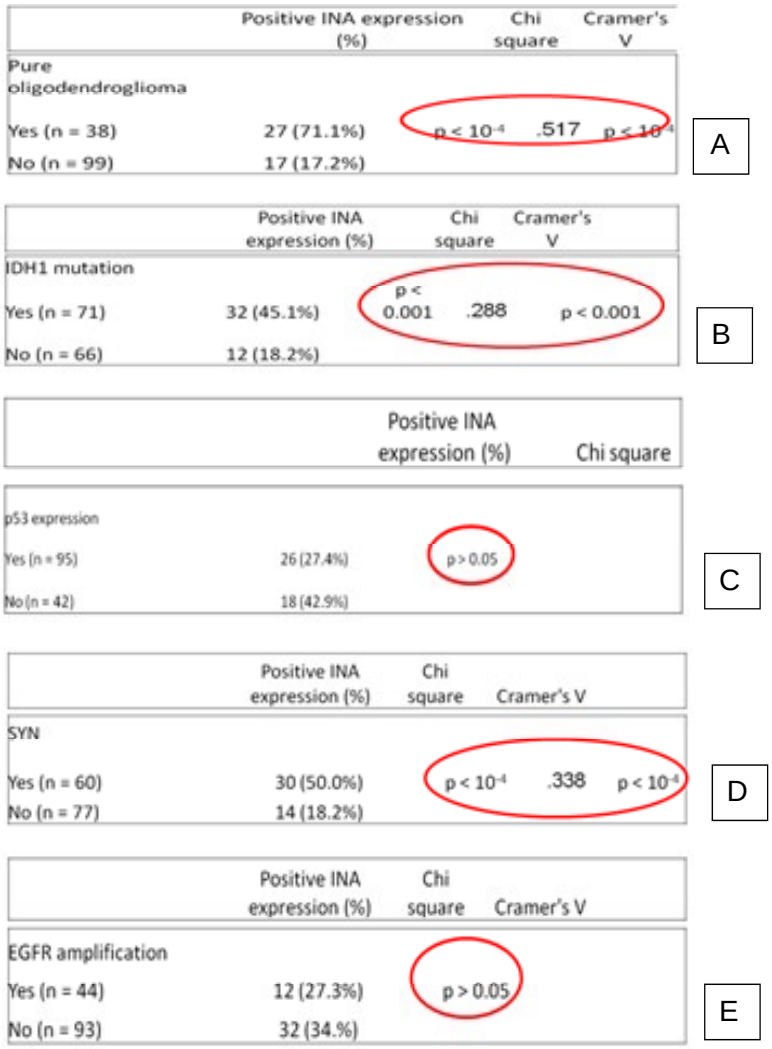
Table 2. The histology, INA, IDH1, p53, SYN, EGFR expression of the 137 gliomas

	INA	IDH1	P53	SYN	EGFR
Pilocytic astrocytoma (n=13)	0/13 (0%)	0/13 (0%)	2/13 (15.4%)	1/13 (7.7%)	0/13 (0%)
Grade2 astrocytoma (n=4)	0/4 (0%)	3/4 (75%)	3/4 (75%)	1/4 (25%)	1/4 (25%)
Grade 3 astrocytoma (n =12)	0/12 (0%)	10/12 (83.3%)	12/12 (100%)	2/12 (16.7%)	2/10 (16.6%)
Glioblastoma (n =40)	1/40 (2.5%)	14/40 (35%)	40/40 (100%)	11/40 (19%)	22/40 (55%)
Glioblastoma with oligo component (n =12)	8/12 (66.7%)	5/12 (41.7%)	12/12 (100%)	8/12 (66.7%)	6/12 (50%)
Grade2 oligodendroglioma (n = 22)	17/22 (77.2%)	16/22 (72.7%)	2/22 (18.2%)	16/22 (72.7%)	2/22 (9.1%)
Grade3 oligodendroglioma (n =16)	10/16 (62.5%)	10/16 (62.5%)	6/16 (37.5%)	11/16 (68.8%)	9/16 (56.3%)
Grade2 oligoastrocytoma (n =7)	4/7 (57.2%)	5/7 (71.4%)	7/7 (100%)	4/7 (57.2%)	0/7 (0%)
Grade3 oligoastrocytoma (n = 6)	4/6 (66.7%)	5/6 (83.3%)	6/6 (100%)	3/6 (50%)	1/6 (16.7%)

astrocytomas (n=6), 66.7 % of glioblastomas with oligodendroglial component (n=12), 0% of grade I astrocytomas (n=13), 0% of grade II astrocytomas (n=4), 0% of grade III astrocytomas (n=12) and 2.5% of glioblastomas and gliosarcomas (n=40).

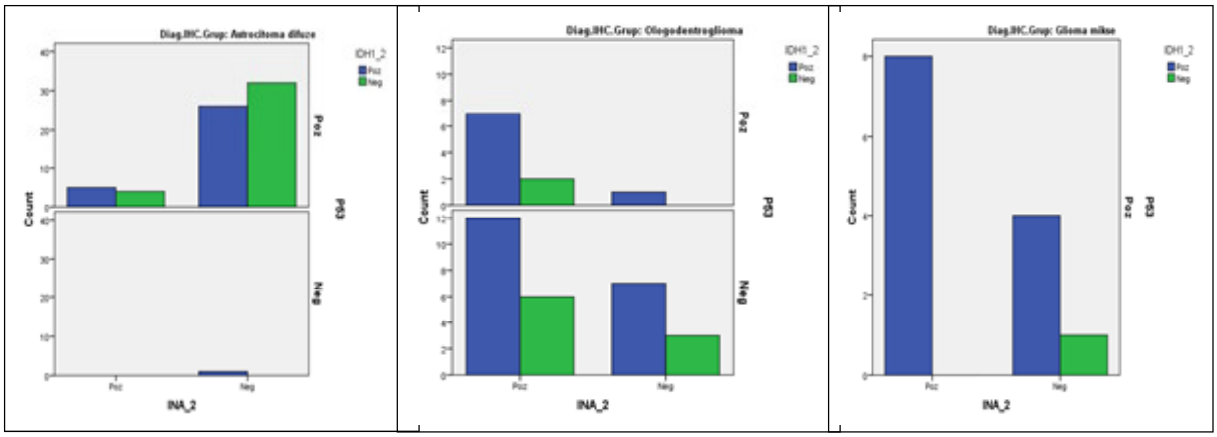
INA was expressed by 27 (71.1%) of pure oligodentrogliomas (n=38) versus 17 (17.2%) of non pure oligodentrogliomas (n=99). INA was expressed by 32 (45.1%) of gliomas with IDH1 mutation (n=71) versus 12 (18.2%) of gliomas without IDH1 mutation (n=66). INA was expressed by 26 (27.4%) of gliomas with P53 mutation (n=95) versus 18 (42.9%) of gliomas without P53 mutation (n=42). INA was expressed by 30 (50.0%) of gliomas with SYN expression (n=60) versus 14 (18.2%) of gliomas without SYN expression (n=77). INA was expressed by 12 (27.3%) of gliomas with EGFR expression (n=44) versus 32 (34%) of gliomas without EGFR expression (n=44).

Figure 1. Correlations of alpha-internexin (INA) with oligodendrogliomas (A), IDH1 mutation (B), P53 expression (C), SYN immunoreactivity (D) and EGFR immunoreactivity (E)



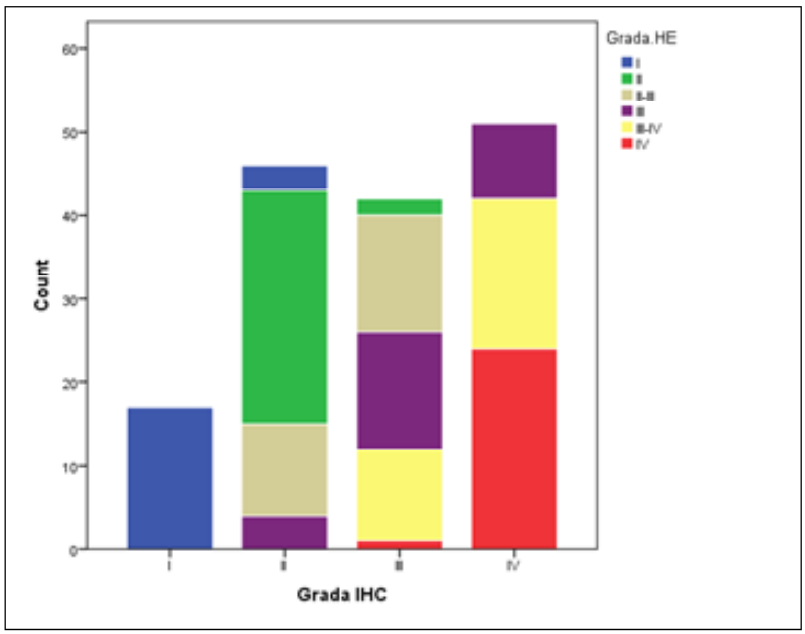
INA expression was tightly related to pure oligodendroglial phenotype (Chi square was $p < 10^{-4}$; Cramer's V was 0.517; $p < 10^{-4}$), to IDH1 mutation, (Chi square was $p < 0.001$; Cramer's V was 0.288; $p < 0.001$), whereas it was negatively correlated with p53 expression ($p = 0.05$). In the diagrams below are shown the immunoreactivity profiles of INA, IDH1 and P53 in oligodentrogliomas, astrocytomas and mixed gliomas in our study. Combining INA, DH1, P53 we identified the likelihood of pure oligodendrogliomas with the presence of 1p/19q co-deletion and showed which profile predominates in each entity.

Figure 2. Distribution of immunoprofiles IDH +/p53-/INA-; IDH +/p53 +/INA-; IDH +/p53-/INA+; and IDH -/p53-/INA- in diffuse gliomas



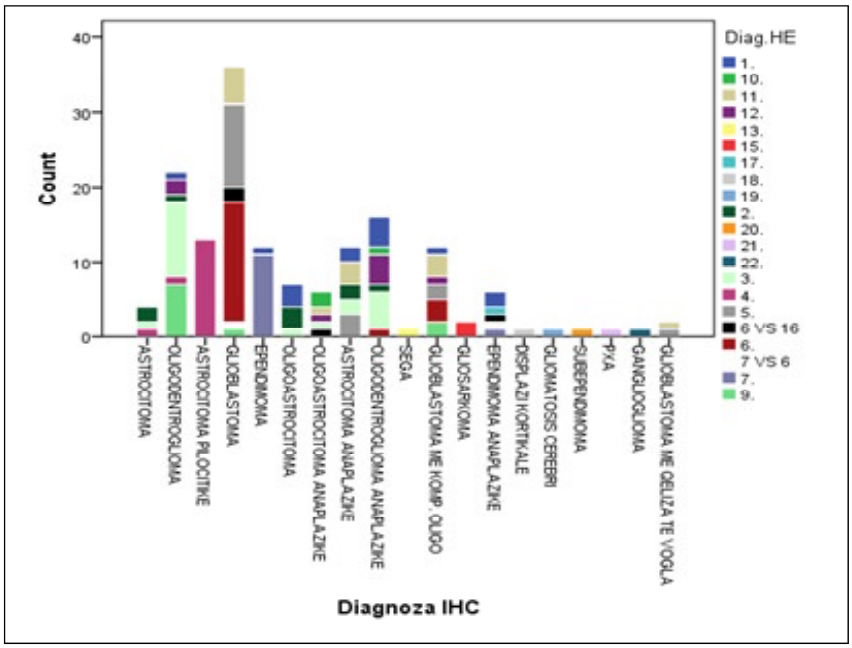
In conclusion, the following charts will show how the tumors entity is changed and the histological grade of tumors in the cases included in the study, after application of molecular markers, in comparison with the conventional method with H-E.

Figure 3. The distribution of tumors by grade in H-E compared with distribution of tumors by grade in IHC after the use of molecular markers



The graph shows that the tumor markers, used in the study through IHC method, are very useful for determining the tumor grade in glial tumors, with a Cramer's V = 0761 and P = 0.0001.

Figure 4. The distribution of tumors by entities in H-E, compared with the distribution by entities in IHC



The figure shows that tumor markers used in the study are very useful for determining the entity in glial tumors, with a Cramer's V = 0761 and P = 0.0001.

Discussion

For the prognostic and predictive values of the adult gliomas, oligodendroglial phenotype is sufficient to determine a treatment option (11). The oligodendroglial phenotype indicates a better prognosis and more chemosensitivity than astrocytic tumors, but the histological diagnosis is subjective and suffers from interobserver variability and discrepancies (12,13). In the other hand, the 1p19q codeletion, the MGMT promoter methylation and the IDH1 mutation are currently the most important prognostic biomarkers in adult gliomas. However, their assessment requires molecular biology techniques that in contrast to immunohistochemistry are not available worldwide and not always feasible. 1p/19q codeletion status, which is related to an unbalanced t(1;19) (q10;p10) translocation, is generally mutually exclusive with P53 mutation and EGFR gene amplification (14) and is a diagnostic, prognostic, and predictive marker for ODGs. Comparative genomic hybridization array analysis (15), loss of heterogeneity analysis, multiplex ligation-dependent probe amplification (16,17), and FISH are available to identify a 1p/19q deletion (13,18), but they have been rarely performed in clinical practice. Moreover, these are complex, sophisticated and expensive techniques, and all have limitations, such as contamination with normal cells or poor sensitivity and specificity (10,19). Therefore, diagnostic, prognostic, and predictive markers are needed that can replace 1p/19q deletion. INA, which is mainly studied in further studies leads to the accumulation of neurofilaments and is tightly related to oligodendroglial histology and to 1p19q codeletion. INA

expression can be assessed quickly from a simple biopsy, is reliable and inexpensive and does not need any special equipment (11). In our study we demonstrate that INA expression is overrepresented in tumors with IDH1 mutation (45.1%, $p< 0.001$) and underrepresented in tumors with p53 expression (27.4%, $p> 0.05$) and EGFR amplification (27.3%, $p> 0.05$). We also noted that INA expression was overrepresented in tumors with Syn expression (50.0%, $p < 10^{-4}$). The absence of INA expression in an oligodendroglial tumor makes the 1p19q codeletion very unlikely particularly if the tumor is p53 positive. In contrast, a tumor expressing INA has a 70% chance to be 1p19q codeleted and an 80% chance if p53 is negative (11). In our study 11 cases of ODG were negative for INA 29.9 % and 8 of 38 ODG were positive for P53 that is 21.05 % of them. In our study, INA overexpression cases were also present among the EGFR amplification cases. INA overexpression is common in ODGs (62.5-77.2%) but not in astrocytomas and GBMs, which have a lower frequency of INA overexpression (2.5%). INA was overexpressed in Glioblastomas with oligodendroglial component (66.7%). In contrast, EGFR overexpression is common in GBMs, which have a lower frequency of INA overexpression (27.3%), and is low in grade III ODGs (16.7%). Our study has demonstrated that molecular markers used are very useful for determining the entity in glial tumor with a Cramer's V = 0761 and P = 0.0001 and even for determining the tumor grade in these tumors, with a Cramer's V = 0761 and P = 0.0001.

Conflicts of interest: None declared

Conclusion

Our study has some limitations including the relatively small group, a high percentage of oligodendroglial vs. astrocytic tumors (bias selection or intra-observer bias), unavailable data about 1p/19q co-deletion and subsequent lack of evidence of this interesting correlation (INA and 1p/19q), but beside this we can confirm that INA expression is tightly related to oligodendroglial histology. We demonstrate that INA expression is overrepresented in tumors with IDH1 mutation and SYN expression (complementary information), INA expression is underrepresented in tumors with p53 expression and EGFR amplification. We also show that combining INA, IDH1, P53 may help identify the likelihood of oligodendrogliomas with 1p/19q co-deletion with a higher sensitivity and specificity. We demonstrated that in astrocytomas predominates the group with P53+/INA-, in oligodentrogliomas predominates the group P53+/INA- and in mixed gliomas predominates the group P53+/INA+. We also demonstrated that molecular markers are very useful in optimizing the diagnosis of glial tumors in daily routine, in terms of tumor entity and tumor grade, with a Cramer's V = 0761 and P = 0.0001.

Acknowledgements

This research would not have been possible without the support of many people. The authors wish to express their gratitude to Prof. Dr. Felice Giangaspero who was abundantly helpful and offered invaluable assistance, support and guidance.

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Improvement of biochemical parameters due to non-invasive ventilation in patients with chronic obstructive pulmonary disease and acute respiratory failure

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Abstract

Aim: Our objective was to compare selected biochemical parameters including the pH level and the bicarbonate (HCO_3) level in patients with chronic obstructive pulmonary disease (COPD) and acute respiratory failure with and without administration of non-invasive ventilation (NIV).
Methods: Our study included 250 patients diagnosed with COPD and acute respiratory failure who were hospitalized at the University Hospital of Lung Diseases "Shefqet Ndroqi" in Tirana during 2011-2014. Patients were divided into two groups: 125 patients were administered NIV, whereas 125 patients underwent the standard (conventional) treatment procedure. Mann-Whitney U-test was used to compare the mean values of pH and HCO_3 between the two groups of patients both upon hospital admission and hospital discharge.
Results: Upon hospital admission, there were no statistically significant differences in the biochemical parameters between the two groups of the patients. Upon hospital discharge, patients who were administered NIV had a pH less acid compared with their counterparts who underwent the standard/conventional therapy (mean pH level: 7.39±0.03 vs. 7.31±0.02, respectively; $P<0.001$). Similarly, the blood level of bicarbonates (HCO_3) – which has a crucial role in the physiological pH buffering system – was significantly lower in patients undergoing NIV (25.6±1.7 compared with 33.9±2.1 in patients with conventional therapy, $P<0.001$).
Conclusion: Our study indicates that NIV improves important biochemical parameters in patients with COPD and acute respiratory failure. Hence, it is important to identify in the clinical practice patients who need to undergo NIV in order to benefit as much as possible from this treatment strategy.

Keywords: bicarbonates (HCO_3), chronic obstructive pulmonary disease (COPD), lung diseases, non-invasive ventilation, pH.

Introduction

The benefit of non-invasive ventilation (NIV) for the treatment of acute respiratory failure in chronic obstructive pulmonary disease (COPD) patients has been well-documented in many studies including also meta-analyses (1,2). Hence, several randomized controlled trials have indicated that NIV improves arterial blood gas tensions and dyspnea and may prevent the need for intubation in patients admitted to hospital with an exacerbation of COPD associated with decompensated respiratory acidosis (3-7). In two large trials, there was also reported a significant reduction of in-hospital mortality (6,7). Therefore, it has been convincingly argued that it is important to identify in the clinical practice patients who need to undergo NIV in order to benefit as much as possible from this treatment strategy (3). Identification of suitable patients would enable an effective management in a higher dependency area or an intensive care unit with ready access to mechanical ventilation (3). Otherwise, the absence of mechanical ventilation may lead an increase in mortality, as already reported in several studies (3,8).

Albania emerged in 1991 from the most rigid communist regime which was characterized by a Semashko-type health system. In the past few decades, the transition towards a market-oriented system has been characterized by a significant shift in the disease burden from infectious diseases to non-communicable diseases, in particular cardiovascular diseases, cancer and COPD (9,10). However, the information about the prevalence of COPD and management of this condition in Albanian patients with the presence of acute respiratory failure is scant.

In this framework, our objective was to compare selected biochemical parameters including the pH level and the bicarbonate (HCO_3) level in patients with COPD and acute respiratory failure with and without administration of NIV.

Methods

A case-series study was conducted including 250 patients diagnosed with COPD and acute respiratory failure who were hospitalized at the University Hospital of Lung Diseases "Shefqet Ndroqi" in Tirana during the period 2011-2014.

Patients were divided into two groups: 125 patients were administered NIV, whereas 125 patients underwent the standard (conventional) treatment procedure.

Furthermore, information about demographic and socioeconomic characteristics [age, sex, place of residence (urban areas vs. rural areas), marital status (dichotomized in the analysis into: married vs. widowed/divorced/single), employment status (categorized into: employed, unemployed and retired), educational level, social status and income level (all trichotomized into: low, middle and high)] and lifestyle/behavioral factors [current smoking (no vs. yes), alcohol consumption (categorized into: never, occasionally and regularly) and physical activity (trichotomized into: low, moderate and high)] of study participants was collected through the medical charts and a structured interview.

The study was approved by the Faculty of Medicine in Tirana. All patients who agreed to participate in this study gave their informed consent.

Mann-Whitney U-test was used to compare the mean values of pH and HCO_3 between the two groups of patients (individuals with NIV vs. those without NIV) both upon hospital admission and upon hospital discharge. In all cases, a p-value of ≤ 0.05 was considered as statistically significant. Statistical Package for Social Sciences (SPSS, version 17.0) was used for the data analysis.

Results

Mean age of the patients with NIV was very similar to those without NIV (64.4±6.8 vs. 64.6±5.0, respectively). Likewise, the sex distribution was quite similar in both groups (34% women in patients with NIV compared to 33% in patients without NIV). Furthermore, there were no statistically significant differences regarding the other socio-demographic characteristics (place of residence, marital status, educational attainment, employment

status, income level and social status) between patients with NIV and their counterparts without NIV (data not shown in the tables).

The distribution of selected biochemical parameters in patients with NIV and those without NIV upon hospital admission is presented in Table 1. Mean value of pH was 7.27±0.04 in the NIV group compared with 7.29±0.03 in the group without NIV (P=0.11). On the other hand, the mean values of HCO_3 were 34.3±2.3 and 34.8±2.3, respectively (P=0.38). Therefore, given these findings, there were no statistically significant differences neither for the pH level nor for the HCO_3 level at the baseline of the study (i.e., before allocation of the patients into different treatment regimens).

Table 1. Selected biochemical parameters in patients with and without NIV upon hospital admission

PARAMETER	Without non-invasive ventilation (NIV) [N=125]		With non-invasive ventilation (NIV) [N=125]		P
	Mean±SD	95%CI of the mean	Mean±SD	95%CI of the mean	
pH	7.29±0.03	7.28-7.30	7.27±0.04	7.26-7.28	0.113
HCO_3	34.8±2.3	34.4-35.2	34.3±2.3	33.9-34.7	0.379

Upon hospital discharge (Table 2), patients who were administered NIV had a pH less acid compared with their counterparts who underwent the standard/conventional therapy (mean pH level: 7.39±0.03 vs. 7.31±0.02, respectively; P<0.001).

Table 2. Selected biochemical parameters in patients with and without NIV upon hospital discharge

PARAMETER	Without non-invasive ventilation (NIV) [N=125]		With non-invasive ventilation (NIV) [N=125]		P
	Mean±SD	95%CI of the mean	Mean±SD	95%CI of the mean	
pH	7.31±0.02	7.30-7.32	7.39±0.03	7.38-7.40	<0.001
HCO_3	33.9±2.1	33.5-34.3	25.6±1.7	25.3-25.9	<0.001

Similarly, the blood level of bicarbonates (HCO_3) – which has a crucial role in the physiological pH buffering system – was significantly lower in patients undergoing NIV (25.6±1.7 compared with 33.9±2.1 in patients with conventional therapy, P<0.001) (Table 2).

Discussion

Main findings of this study comprising a sample of Albanian patients with COPD and acute respiratory failure include a significant improvement of two important biochemical parameters such as the pH level and the HCO_3 due to administration of NIV. Hence, upon hospital discharge, compared with patients treated with conventional therapy, those who underwent NIV had a pH less acid and a significantly lower level of HCO_3 in the blood. These findings bear important clinical implications which should be taken into consideration in the routine medical practice.

Our findings are in line with many previous reports from the international literature (1-7). Hence, our finding regarding sustained clinical stability due to NIV is compatible with several previous studies where a reduction in the number of hospital admissions after three months of therapy was observed (2,11). Furthermore, it has been shown that domiciliary administration of NIV in COPD patients

with recurrent admissions for acute-on-chronic respiratory failure requiring NIV reduces hospital admissions (12). Nevertheless, the particular strength of our estimates may be partly explained by our selection of patients with a high probability of recurrent respiratory failure. Future studies should be conducted in Albania to confirm and expand our findings.

This study may have some limitations including the sample representativeness and the validity of the information obtained. The sample included in this study included only patients hospitalized at the University Hospital of Lung Diseases "Shefqet Ndroqi" in Tirana. As such, this study sample may not represent the overall patients with COPD and acute respiratory failure in Albania. Therefore, findings from this study should be generalized only to patients who are admitted at the University Hospital in Tirana. The instruments used for data collection consisted of standardized and valid tools which tend to argue in favor of absence of information bias. However, there might have been some sorts of differential reporting between patients pertinent to different demographic and socio-economic backgrounds (based on age, sex, educational attainment, employment status, economic level and social status). As such, findings of this study should be interpreted with caution.

In conclusion, regardless of its potential limitations, this study indicates that NIV improves important biochemical parameters in patients with COPD and acute respiratory failure. Thus, it is important to identify in the clinical practice patients who need to undergo NIV in order to benefit as much as possible from this treatment strategy. Health professionals and policymakers in Albania should be aware of the clinical importance and benefits of NIV in patients with COPD and acute respiratory failure.

Conflicts of interest: None declared.

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A clinical study of Kaposi Sarcoma among HIV/AIDS patients in Albania

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Abstract

Aim: This descriptive study aims to characterize the epidemiological and clinical features of Kaposi Sarcoma (KS) among HIV/AIDS patients in Albania.
Methods: This is an observational retrospective study of 32 adult patients diagnosed with AIDS-associated Kaposi's sarcoma between January 2007 to December 2015 at the Ambulatory HIV/AIDS Clinic, Infectious Disease Service, University Hospital Centre of Tirana.
Results: The median age at diagnosis was 43 years (range: 26-79 years), with HIV-KS cases more common among patients of age 41-50 years (32%). Men comprised 91% of the study population, while men who had sex with men comprised 12% of the cases. The most common lesions of the KS in AIDS patients were the nodule (46%), patch (31%) and plaque (15%). The most common tumour locations were the trunk (47%), lower extremity (36%), oral (34%) and face (28%). The most common oral lesions were nodule (54%) and exophytic (36%). 24 patients (75%) had T0 stage of the disease, while 8 cases (25%) had T1 stage of the disease. The median CD4 level at baseline was 142 cell/mm^3. All the cases were treated with combined antiretroviral therapy (ART). The most used ART regimens were zidovudine + lamivudine + efavirenz. Seven patients (22%) received chemotherapy. Mortality rate during the study period was 22%.
Conclusion: In more than two thirds of the cases (69%), the diagnosis of KS was the HIV/AIDS presenting or defining illness/diagnosis, which indicates a very late HIV diagnosis in Albania.

Keywords: ART, HIV/AIDS infection, Kaposi Sarcoma.

Introduction

Kaposi's sarcoma (KS) is the most common malignancy seen in the setting of HIV infection. In the current era of treatment, KS is generally identified as a late manifestation of HIV infection, occurring when immunosuppression is severe (1). Treatment with effective antiretroviral therapy (ART) has led to a dramatic decline in KS incidence. A prescription for ART may reduce the likelihood of KS by 50% (2). In a multivariate analysis, the Multicentre AIDS Cohort Study demonstrated an 81% reduced risk of death for KS patients treated with combination ART (3). Although Albania is considered a low prevalence country for HIV infection, reported cases are increasing every year. Until December 2015 there are 870 HIV cases reported in Albania, with 87 cases reported during 2015, where more than half of them were at AIDS stage (4). The ART was introduced in 2004 in Albania, with currently more than 450 cases being under treatment at Infectious Disease (ID) Service and ID Paediatric Service at University Hospital Centre of Tirana (UHCT).

This is the first study carried out in Albania which aims to characterize the epidemiological and clinical features of KS and HIV/AIDS patients followed at Ambulatory HIV Clinic at Infectious Disease (ID) Service, University Hospital Centre of Tirana (UHCT) "Mother Teresa".

Methods

This is an observational retrospective study conducted at the Ambulatory HIV/AIDS Clinic, Infectious Disease Service, University Hospital Centre of Tirana. This is the only centre in Albania which provides care and treatment for all HIV infected adult patients since 2007. In order to select the patients included in the study, we reviewed the database of the HIV cohort adult patients who are receiving care and are followed at the Ambulatory HIV clinic from January 2007 to December 2015. There were 429 adult patients with HIV diagnosis whose data were available. The study population consisted of all HIV adult patients diagnosed with Kaposi Sarcoma, either by clinical or histological diagnosis. In the database, there were 32 cases with KS diagnosed clinically or histologically which were included in our study. For the purpose of this analysis, a KS diagnosis was defined by the presence of a clinical-only diagnosis of KS, or a clinical KS diagnosis accompanied by definitive or indeterminate pathologic confirmation. Patients with a clinical diagnosis and a negative biopsy were not considered to have KS.

Information obtained included patient's age, gender, risk of HIV transmission, time of KS diagnosis related to HIV diagnosis, CD4 cell count at time of HIV diagnosis and when HIV-KS was diagnosed, other HIV associated opportunistic diseases, general area of KS lesion, tumour localization, type of lesion, oral involvement, antiretroviral treatment regimens, and systemic chemotherapy. KS stage was defined per AIDS clinical trials group (ACTG) tumour staging classification as T0 if disease was confined to the skin and/or lymph nodes or oral involvement was confined to the hard palate, or T1 if there was pulmonary or gastrointestinal involvement, tumour associated oedema or ulceration, or extensive oral involvement.

Results

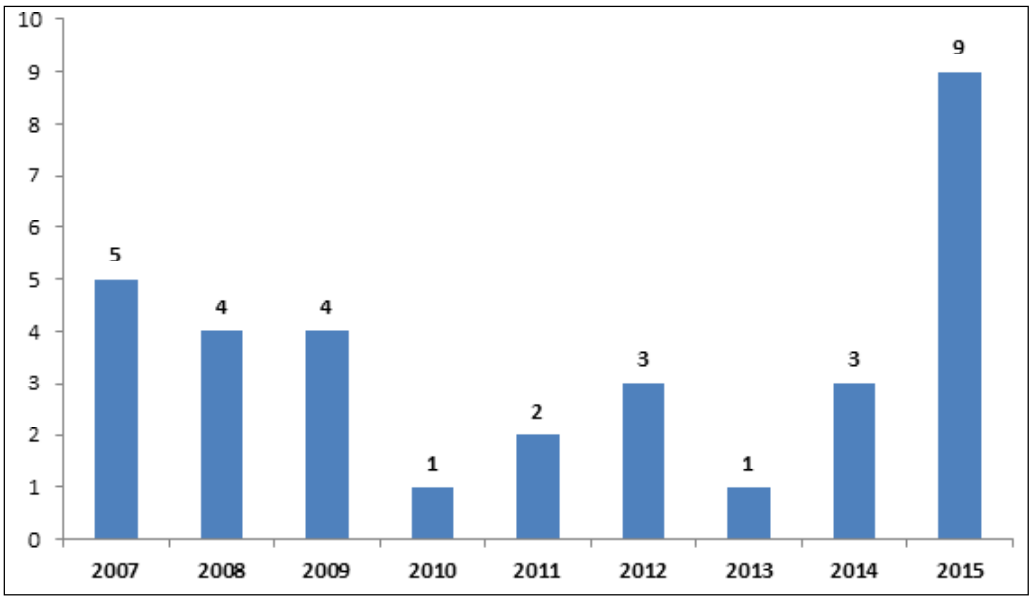
This study comprised 32 patients diagnosed with HIV related KS (Table 1). In majority of cases (22 cases, 69%), diagnosis of KS was the first HIV/AIDS presenting or defining illness/diagnosis. For those KS cases diagnosed after HIV diagnosis being established, the median time of diagnosis of KS was 4.3 years (range 0.8-15 years). Diagnosis of KS was clinically based in 24 cases (75%) and histopathologically based in 8 cases (25%). Number of KS cases diagnosed among HIV patients at our Service each year is shown in Figure 1, with the highest number diagnosed during 2015. The median age at the time of KS diagnosis was 43 years (range = 26-79), with HIV-KS cases more common among patients of age 41-50 years (32%). Men compri-

sed 91% of the study population with male to female ratio was 9:1, while those who declared as men who have sex with men comprised 12% of cases.

Table 1. Epidemiological characteristics of the patients

Characteristics	Numbers (Percentages)
Number of patients	32
Male	29 (91%)
Female	3 (9%)
Age (mean years)	43
<30 years	8 (25%)
31-40 years	61 (8%)
41-50 years	10 (32%)
>51 years	8 (25%)
Civil and risk status (n=27)	
Married	16 (48%)
Single	7 (21%)
Divorced	2 (6%)
MSM	4 (12%)
Number of patients with concomitant HIV-KS diagnosis	22 (69%)
Number of patients with KS diagnosed after HIV diagnosis	10 (31%)
Time from HIV to KS diagnosis, mean (range)	4.3 years (1-15 years)

Figure 1. Number of new AIDS-KS cases by year



Clinical characteristics of the patients are summarized in Table 2. The most common lesions of the KS in AIDS patients are the nodule (46%), patch (31%) and plaque (15%). The most common tumour locations were the trunk (47%), lower extremity (36%), oral (34%) and face (28%). The most common oral lesions were nodule (54%) and exophytic (36%) with palate and tongue as the

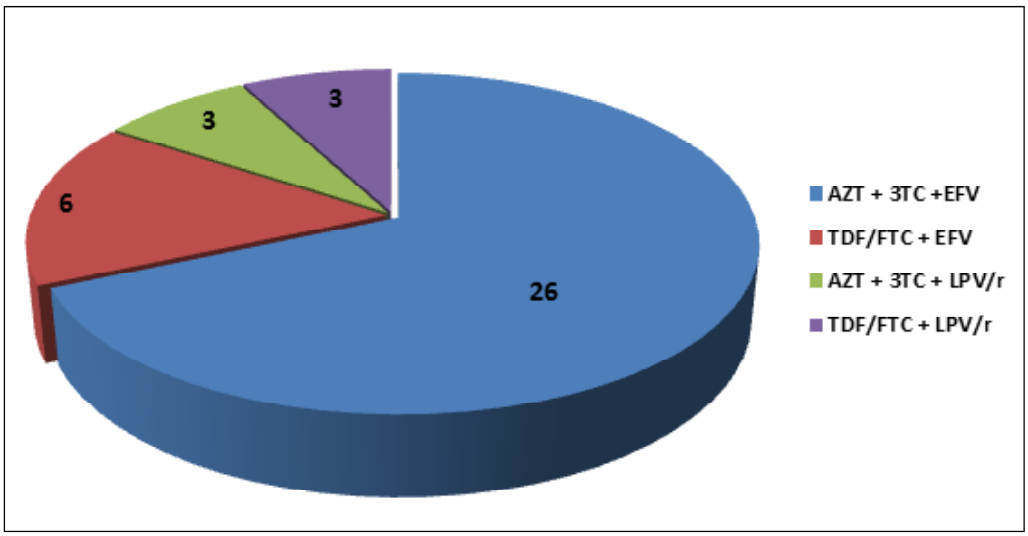
most common site location. 24 patients (75%) had T0 stage of the disease, while 8 cases (25%) had T1 stage of the disease (7 cases with extensive oral involvement and 1 case with visceral and oral involvement). About one third of cases presented with anaemia, leukopenia and thrombocytopenia. The median CD4 level at baseline was 142 cell/mm^3 (range 7- 530 cells). The most common concomitant opportunistic infections were wasting syndrome (59%), oral-pharyngeal candidiasis (50%), pneumonia (34%), syphilis (17%), and dermatitis (13%).

Table 2. The clinical characteristics of AIDS-KS cases

Characteristics	Numbers (Percentages)
Average CD4+ T-cell count at KS diagnosis (cell/mm^3) (range)	142 cell/mm^3, (7-530)
Type of lesions	
Patch	8 (31%)
Plaque	4 (15%)
Nodule	12 (46%)
Edema	1 (4%)
Erosion	1 (4%)
Tumor localization	
Upper extremity	7 (22%)
Lower extremity	12 (36%)
Upper and lower extremity	8 (24%)
Trunk	15 (47%)
Face	9 (28%)
Oral (+ skin)	11 (34%)
Oral type: Nodule	6 (55%)
Exophytic	4 (36%)
Patch	3 (27%)
Oral site: Gingival	3 (27%)
Tongue	4 (36%)
Palatum	6 (55%)
Visceral	1 (3%)
Prognosis (mortality)	5 (16%)
Anemia	9 (28%)
Leukopenia	10 (31%)
Thrombocytopenia	9 (28%)
Other concomitant OI	
Oro-pharyngeal candidiasis	16 (50%)
Pneumonia	11 (34%)
Wasting syndrome	19 (59%)
Syphilis	5 (17%)
Genital warts	5 (17%)
Dermatitis atopic	4 (13%)

All cases were treated with combined antiretroviral therapy (ART) (Figure 2). 25 patients (78%) with KS were under first line ART therapy while less than a quarter of cases (7 cases, 22%) have been prescribed second line regimens. The most used ART regimens were zidovudine + lamivudine + efavirenz (81%) and then TFD/FTC/EFV (18%). Seven patients (22%) received chemotherapy, which was based on doxorubicin regimen in the majority of time. The median time of follow up was 3.97 years (range 0.3 to 11 years). Mortality rate during the study period was 22% (7 patients died).

Figure 2. ART regimens



Discussion

The estimated prevalence of HIV-associated KS at our cohort of HIV patients was about 7.5%. At the beginning of the HIV epidemic, Kaposi sarcoma was one of the most common manifestations of AIDS (5), present during the mid-1980s in 25% of individuals at the time of AIDS diagnosis in the United States, but decreased steadily through the late 1980s and mid-1990s, down to 2% after the advent and widespread use of highly active antiretroviral therapy (HAART) in 1996 (6). Most of HIV-KS cases at our Service are diagnosed clinically, due to limited diagnostic pathology availability especially for this type of patients at our facility. Skin punch biopsy and histological examination was performed in 8 cases (25%). All the eight cases with confirmed histological diagnoses were diagnosed very recently during year 2015. There is discrepancy in the data regarding high proportion of male gender (91%) and low proportion of MSM risk group (12%) which underscores the low level of sexual orientation disclosure among HIV male patients and under-reporting phenomenon of this risk behaviour in Albania. According to the Institute of Public Health in Tirana, MSM route of transmission is reported to be around 11% for 2015, while other neighbouring countries report rates of 40 to 60% (7). Different cohorts of HIV patients in North America and Europe have revealed an elevated prevalence of HIV associated KS in men who have sex with men, which goes till 90% of cases (8,9). In more than two third of the cases (69%) the diagnosis of KS was the HIV/AIDS presenting or defining illness/diagnosis which indicates a very late HIV diagnosis in Albania. This is also confirmed by the fact of very low average CD4 level at HIV-KS diagnosis time. Late HIV diagnosis is common

in Albania, with 75% of patients being diagnosed with a CD4 count <350 cells/mm^3 and 52% with a CD4 counts <200 cells/mm^3, representing severe immunosuppression (7). The data from our study, together with late HIV diagnoses trends in Albania, indicates that a large proportion of Albania's HIV population are not being tested and diagnosed promptly and suggests a relatively large undiagnosed population within the country.

The clinical characteristics of KS lesions of our cases were an early patch and late stage nodule. The most common locations of the lesions were the trunk and the lower extremities. In more than two third of cases there was an oral cavity location of the lesions, with hard palate, gingiva and dorsum of the tongue as the most commonly affected sites. It has been reported that in 22% of HIV-seropositive subjects with KS, the initial presentation of HIV-KS is in the mouth, and that in up to 70% of subjects with HIV-KS, the mouth will sooner or later be affected (10,11). All patients in our study received ART while only one fifth received chemotherapy. While HAART is recommended for virtually all patients with a KS diagnosis, individuals with advanced and/or symptomatic KS should receive some form of local or systemic therapy specific for KS (12). Any therapeutic choice for treatment of patients with AIDS and KS in the HAART era should take into consideration several parameters, such as the extension of mucous-cutaneous KS lesions, the presence of visceral involvement that may be life-threatening (e.g., symptomatic lung KS), KS involvement at a location that could compromise a specific organ function, and the intensity of immunosuppression (13).

Furthermore, regression with cART alone has been well-documented, and a pooled analysis of patients with early disease (T0 = KS with low tumour volume, no associated ulceration or oedema, and no visceral disease) who had not received cART previously suggest that approximately 80% will have disease regression with cART alone, with median time to response ranging from 3-9 months (14). Several modalities, including radiotherapy, topical therapy, cryotherapy, or intralesional injection historically have been employed for control of localized KS, but their use has been largely replaced by cART. However, systemic therapy remains indicated for the bulky, rapidly progressing, symptomatic, or life-threatening disease (14). The most common chemotherapy used among our cases was doxorubicin. Also the literature data shows that liposomal doxorubicin is currently the chemotherapeutic agent of choice for advanced KS (15). In a study of moderate to advanced KS, those receiving liposomal doxorubicin in addition to ART had a markedly better response rate at 48 months than did those on ART alone (76 versus 20%) (16). Majority of our patients (81%) were under non-nucleoside based ART regimens, which is according to the National Guidelines for ART (Albanian Guideline 2006). Effective combination of antiretroviral therapy consists of a combination of either a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) with two nucleoside reverse transcriptase inhibitors (NRTI). In the Chelsea and Westminster Cohort of 8640 patients with HIV, 1240 patients with KS were identified, and NNRTI and PI based regimens had the same protective effect against the development of KS (17).

In summary, we found that the number of cases of HIV-associated KS being diagnosed yearly in Albania is increasing, especially among middle-aged male patients. KS remains an important clinical problem in terms of early diagnosis and management in Albania.

Conflicts of interest: None declared.

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Traumas on dentition during childhood and their consequences

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Abstract

Aim: The aim of this study was to estimate the prevalence of dental traumas and their consequences among the patients at a dental clinic in Albania, and assess some of the consequences that traumas can cause on the primary dentition, also determining the overjet higher than 3 mm as a risk factor for dental traumas.
Methods: The material for this study was collected from 307 subjects aged 2-15 years who presented at the dental clinic "Matis" in Tirana during 2013-1015. Out of these 307 subjects, 71 individuals with complete records had experienced trauma to the dental system and were considered to represent the "trauma group". The data of "trauma group" were compared to the data of the "no-trauma group" which consists of the remaining 236 subjects. The variables were the age at the moment of examination, gender and dental protrusion (overjet higher than 3 mm).
Results: The prevalence of dental traumas was 23%. The ratio male/female resulted 1.2:1. The age in which dental traumas were most frequent was 3.5-6 years old. It was found a statistically significant difference between the two groups for the variable of overjet higher than 3 mm ($p<0.01$).
Conclusion: Dental traumas are a common problem among the pediatric patients. Dental injuries to primary dentition occur during early childhood, in the age of 3-5 years old, but can also affect the further development of dentition causing different consequences on permanent dental system.

Keywords: childhood, dental trauma, overjet.

Introduction

Dental trauma are an important issue and of great interest in terms of oral pathologies. Based on statistical data of different authors, it can be said that dental trauma are an important cause of tooth loss in modern times. Anterior teeth dental trauma in preschool children is a very unfortunate situation, but often ignored (1).

Children with dental injuries and their parents represent a challenge to the dentist. Loss or damage of to the teeth, especially the anteriors, in children represents not just a dental problem, but they have a huge psychological impact on the child and his parents, especially if the injury causes significant loss of tooth structure (2).

Dental traumas can happen at different ages, during different periods of the development of the dentition, and in different regions of the mouth, giving the respective consequences.

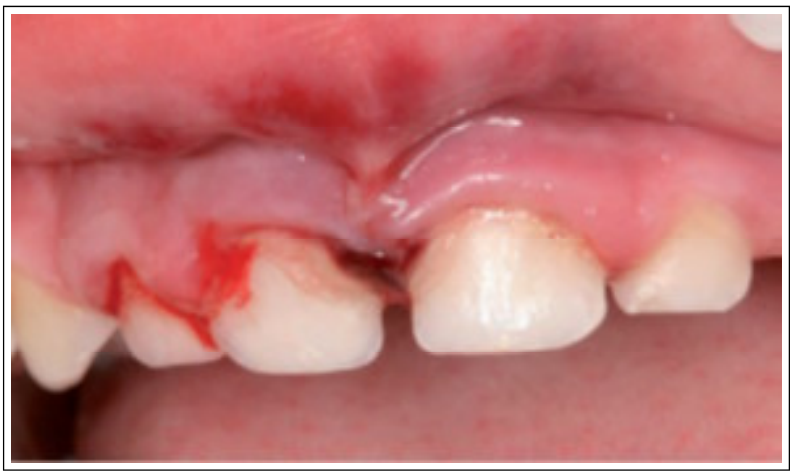
Although it may be difficult to predict an exact prognosis for permanent teeth, the doctor and parents should be prepared for the consequences. One of the major problems associated with dental trauma, is the fact that many times the consequences are not immediate and the real will show years later. Different studies show that in up to 25% of cases children have different problems in the development of permanent teeth (3).

Dental trauma can lead to a malocclusion through three ways: i) Indirectly damaging the permanent germ through the trauma of the primary tooth; ii) Causing the displacement of the permanent germ through the early loss of primary tooth, and; Direct damage to the permanent tooth (4).

The consequences on the dentition depend on: i) The direction and displacement of the apex of the primary tooth; ii) The direction and intensity of the traumatic factor, the type of injury suffered and the presence of alveolar bone fracture, and; iii) The age of the child at the time of traumatic injury (follicles are particularly sensitive in the early steps of their development, which occurs in the age between 4 months and 4 years old), the stage of formation and development of the permanent tooth germ (2,5-7).

Some of the consequences of the trauma on the primary dentition are: i) Pulp necrosis of the primary tooth: this can happen in cases of tooth intrusion, lateral luxation, tooth fractures involving the pulp and alveolar bone fractures. Clinically the tooth presents a grey, red or blue discoloring. The pulp necrosis if not treated, leads to periapical inflammation with loss of periapical bone; ii) Loss of the primary tooth without damaging the successive germ: there are two possible ways a traumatic avulsion of the primary incisive can affect the permanent dentition: delayed eruption, or early eruption of the permanent tooth.

Figure 1. Traumatized primary central and lateral incisive in a 4-year old child



The consequences that dental traumas on primary teeth cause on permanent dentition

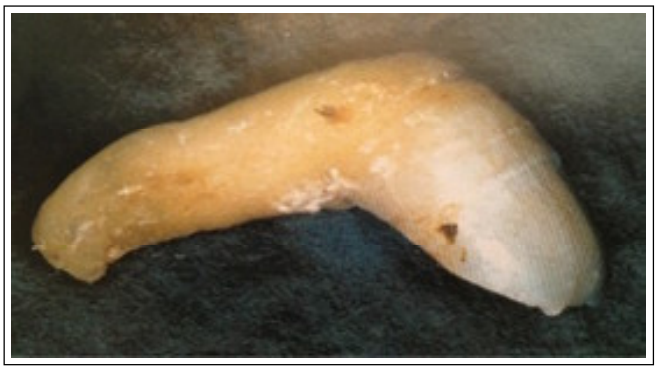
The consequences of dental trauma depend on their severity, the age of the child and the stage of the formation and development of permanent germs. They can affect the process of hystogenesis, morphogenesis or can cause the displacement of the germ. They can affect the coronary region, the radicular region or the whole permanent germ (8).

- Some of these consequences can be found in the coronar region:
- White or yellow-brown discoloration of enamel;
- Yellow-brown discoloration of enamel, and circular hypoplasia, which represent the border between the hard tissue formed before and after the injury (9);
- Crown dilaceration, which is described as an acute deviation of the long axe of the crown, that has its origin due to an acute non-axial displacement of the formed hard tissue against the not calcified tissue (crown dilacerations can be as a result of the intrusion of the primary tooth at the age of 2 years, when almost half of the crown of the permanent tooth is formed) (5,6).

Consequences in the radicular region:

- *Root dilaceration,* these lesions present a curvature in radicular region and can be caused by the intrusion of the primary incisive after the complete formation of the crown of the permanent tooth, between the age of 2 and 5 years;

Figure 2. Radicular dilaceration



- Partial or total interruption of root formation, which is a rare complication of traumas on primary incisives between the ages of 4 and 7 years (5,6);
- Root duplication.

In cases when the whole germ is involved, there can be alterations in the erupting of permanent tooth:

- Delayed eruption may occur, as a result of early loss of the primary tooth and the formation of a thick fibrous gingival tissue;
- Early eruption, when the loss of the primary incisive happens after the age of 5, especially if it is accompanied with bone resorption due to the inflammatory reaction to the dental trauma; (8)
- Ectopic eruption, in cases when there has been an inflammatory process with fistula forming, the permanent tooth tends to erupt in a position higher than normal (10);
- Malformation of the permanent germ;
- Impaction of the permanent tooth;
- Odontoma-like malformations;
- A rare consequence may be the sequester of the permanent germ.

The aim of this study was estimate the prevalence of dental traumas and their consequences among the patients at a dental clinic in Albania, and assess

some of the consequences that traumas can cause on the primary dentition, also determining the overjet higher than 3 mm as a risk factor for dental traumas.

Methods

The material for this study was collected from 307 subjects of age 2-15 years who presented at the dental clinic "Matis" in Tirana during 2013-1015. Out of these 307 subjects, 71 individuals with complete records had experienced trauma in the dental system and were considered to represent the "trauma group". Their dental records included complete anamnesis, objective examination and other needed records, radiographs, cephalometric analysis. The diagnosis of previous dental trauma was determined from dental records and from the detailed anamnesis.

The data of "trauma group" (n=71) were compared to the data of the "no-trauma group" which consisted of the remaining 236 subjects (n=236). The variables were the age at the moment of examination, sex and dental protrusion (overjet higher than 3 mm).

Results

Table 1 presents selected characteristics of the patients by study group.

Table 1. Characteristics of the patients by study group

Variables	The trauma group n=71 (%)	No-trauma group n=236 (%)	P-value
Males	39 (54.9)	126 (53.4)	0.236*
Age at the time of examination (years)	9.9±3.47	7.8±4.20	0.107†
Age at the time of trauma	4.2±1.63	-	-
Overjet>3 mm	28 (39.4)	44 (18.6)	<0.01*

* Hi – square test.

† Student's test for two independent samples.

Using the chi-square test, it was found a statistically significant difference ($p<0.01$) concerning the presence of an overjet higher than 3 mm between the two groups (39.4% of the subjects in the "trauma group" vs. 18.6% of the subjects in the "no trauma group").

Conversely, no statistically significant difference was found concerning gender ($p=0.236$).

As shown in Table 2, most of dental traumas had occurred in the age group 3-6 years.

Table 2. Age at the moment of dental trauma

Age group	No. of subjects	Percentage
0-3 years	7	9.6
3-6 years	39	54.8
>6 years	25	35.6

There was a statistically significant correlation between the overjet > 3 mm and the presence of a dental trauma (Kendall's rank correlation coefficient tau =0.41, $p=0.013$)

The dental problems verified from the examination, which resulted from previous trauma on the primary dentition are listed in Table 3.

Table 3. Dental problems verified during the examination procedure

Dental problems	No. of subjects
Delayed eruption	8
Anomalies of tooth shape	6
Dilaceration	4
Ectopic eruption	8
Hypoplasia of enamel	7
Impaction	2
Necrosis	5
Transposition	1
Total	*41*

The remaining 30 subjects presented traumas on permanent dentition, different types of fractures of permanent teeth.

Discussion

Large discrepancies exist in reports on the prevalence of dental trauma. Reported prevalence ranges from less than 6% to nearly 40%.

In our study, there were examined 307 patients of age 2-15 years and 71 of them resulted to have dental trauma experience. This group consists of 23% of the total number of the examined patients.

The variation in the given prevalence may be as a result of factors such as: the classification of dental traumas, the dentition studied, geographical and behavioral differences between study locations and countries.

In our study, the patients with dental trauma records were subjects who had traumatized teeth at the time of examination, and those subjects who presented in the permanent dentition consequences of previous dental traumas of the primary dentition.

According to different studies, the differences between males and females with dental trauma experience are not significant in the age 2-5 years, both girls and boys are equally exposed to dental traumas at this age.

Males experience more dental traumas then females with the eruption of permanent teeth in most of international studies. The male/ female ratio varies from 1.3-2.3 : 1.4 (11-15).

In our study the male/female ratio in the "trauma group" resulted 1.2:1 (39 males and 32 females), while in the "no trauma group" this ratio was 1.1:1 (126 males and 110 females. No significant difference between males and females was found. Other authors too have found no significant differences between both genders. Bijella et al. observed an insignificant difference between males and females 1.3:1 (16), whereas Onetto et al. reported that the male/female ratio was 0.9:1.0 in children younger than seven years (17).

It has been shown that if a child or a teenager experiences a dental trauma, it is more important the type of trauma and its treatment then the gender as a risk factor.

In our study, the age when the trauma experience was most frequent resulted 3-6 years. This result is similar to results of other studies of different authors. The reason is the specific characteristics of this age concerning the motoric and psychological development. During this age, the conscience and muscular coordination are not yet fully developed. The children of this age have a lower psychomotor development and motoric capabilities, and this can lead to injuries from accidents. Andreasen reports another peak at the age of four years, age in which the physical activity of the child increases (18,19).

Many authors have reported the presence of an overjet higher than 3 mm to be a risk factor for dental trauma (20,21).

In our study, we found a statistically significant

correlation between the overjet >3 mm and the presence of a dental trauma (Kendall's rank correlation coefficient tau =0.41, p=0.013).

Others have reported a strong correlation of a higher overjet to dental trauma. The risk increases when the labial incompetence is present too. Furthermore, children with increased overjet seem more predisposed to experience crown fracture. There are other studies which have not found this correlation. These different results may occur due to differences of the population and the year in which the research is conducted.

Conclusion

The percentage of the subjects who had experienced a dental trauma in our study was 23%. Traumas on primary dentition not only are accompanied with pain, aesthetic and functional issues, but also can lead to anomalies in the permanent dentition. In our study, 41 subjects showed dental anomalies of the permanent dentition as a result of traumas on primary dentition experienced at the age of 3.5-6 years. This age resulted the age in which the traumas were more frequent.

It is important for the dentists to treat the traumatic injuries in the proper mode, and it is also important to inform the patients and the parents for eventual consequences that they can lead to. Having an increased overjet can increase the probability of experiencing dental trauma. The only benefit for providing early orthodontic treatment for patients with class II malocclusions was a reduction in the incidence of incisal trauma. It is important to perform an early orthodontic treatment of the class II malocclusion in order to reduce the risk of these individuals for experiencing a dental trauma.

Conflicts of interest: None declared.

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Is the left lateral sphincterotomy a necessity during the Milligan-Morgan hemorrhoidectomy in patients with hemorrhoids prolapse?

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Abstract

Aim: The alteration of the Milligan-Morgan (MM) technique in order to reduce the number of postoperative complications and enhance rehabilitation of patients continues to attract attention. We aimed to compare the classic and altered MM techniques in a group of patients in Albania.

Methods: This cross-sectional study included 152 patients suffering from anal fissure requiring surgical treatment in Tirana during September 2009-October 2013. Patient suffering from any pathology of anal canal, colon and other disease affecting the intervention outcome were excluded. 92 patients (group A) were intervened with the classic MM technique and 60 patients (group B) were intervened with MM accompanied by left lateral sphincterotomy. Chi-square test and student's t-test were used to compare study groups regarding dependent variables.

Results: Average post-operative and first defecation pain level were significantly higher in group A patients. A significantly higher proportion of group A patients required three morphine vials. Beyond ten days after intervention a significantly higher proportion of group A patients suffered from rectorrhagia. Also, the wound lasted for a month or less in 85% of group B patients compared to 53.2% in group A patients ($P<0.001$). Prevalence of urinary retention and any complication up to six months after intervention was significantly higher in group A patients.

Conclusion: Lateral sphincterotomy accompanying open hemorrhoidectomy in patients with prolapsed hemorrhoid, reduces the level of postoperative pain, helps in faster healing of wounds and rehabilitation of the patient, without damaging the continence and reduces the frequency of postoperative complications, such as anal stricture.

Key words: Albania, anal fissure, hemorrhoids, Milligan-Morgan, sphincterotomy.

Introduction

Hemorrhoid prolapse was described by Hippocrates in 460 BC (1). According to statistics it is a pathology that affects 10 million Americans annually (2). It occurs mostly in women aged 40-65. It is common in pregnant women (3). Hemorrhoids are highly vascular submucosal cushions that generally lie along the anal canal in three typical columns – the left lateral, the right lateral and anterior (2). According to Thomson, the hemorrhoid disease is a pathophysiological and anatomic damage characterized by the decline of elasticity and increase in the volume of hemorrhoid structures (4). There are two main types of hemorrhoids: internal and external (5), depending on the localization, and they are classified into four grades by their degree of prolapse (6). Hemorrhoid prolapse is characterized by bright red blood defecation, hemorrhoid nods prolapse, pain during prolapse, anal pain and straining during defecation. Surgical treatment techniques are classified into two groups: open hemorrhoidectomy and closed hemorrhoidectomy (7) always referring to the presence or not of postoperative wounds. The "Milligan-Morgan" technique is known as the open hemorrhoidectomy and it is considered as the golden standard for the surgical treatment of hemorrhoid disease (8). This technique has been applied widely in our clinic since 1979, but it was firstly implemented in 1937 (8).

The left lateral sphincterotomy is the most common technique for the surgical treatment of benign anal fissures. It consists on cutting the internal anal muscle up to the cryptal level. Its purpose is the internal anal muscle relaxation resulting in better blood supply in the anal canal, reduction of pain, and fissure recovery (9,10). It is performed in two ways: open and closed (9,10). We have mainly used the open technique.

There is limited information about the results of surgical treatment with and without sphincterotomy of benign anal fissures in Albania. Therefore, the purpose of this study was to compare the results of the surgical treatment for this pathology by comparing the two above mentioned techniques.

Methods

This is a cross-sectional study conducted during September 2009 - October 2013.

Study population

All patients presenting at the 3rd Surgical Clinic, Hospital number 2 in the premises of University Hospital Center "Mother Teresa" in Tirana during this time period with signs and symptoms of benign anal fissure requiring surgical treatment were included in the study. The exclusion criteria comprised any accompanying pathologies of the anal canal, colon and any other systemic disease that could affect the result of surgical treatment. In total 152 patients meeting the inclusion and exclusion criteria participated in the survey. Of these, 92 patients were treated through the open surgical technique Milligan-Morgan without sphincterotomy (hereafter referred to as group A) whereas the remaining 60 patients were treated through the open procedure but without sphincterotomy (hereafter referred to as group B).

Data collection and procedures

Diagnosis was based on the use of anoscopy and the clinical signs and symptoms of the disease. None of the patients presented anemia resulting from rectorrhagia. Before surgery all patients had undergone colonoscopy, abdominal ultrasound, routine examinations and biochemical laboratory. Anamnesis of the patients resulted as follows: right herniotomy (6 patients), hypertension stabilized on medication (5 patients), rhinoplasty (1 patient), removal of the cyst of the thyroid gland (1 patient), right hydrocele drainage (1 patient), excision of the mammary gland fibroadenoma (1 patient) and varicectomy (2 patients).

All interventions were performed with spinal anesthesia with 2% of lidocaine solution. The preoperative preparation consisted in light hydric

diet, ciprinol (2x500 mg) flagyl (2x250mg) and omeprazole (2x20mg) 24 hours before the intervention and two rectal enemas two hours before the intervention. Interventions were performed by the same surgeon.

Technically, we attempted to minimize the use of electro lancet, which was at level 40 in all cases. Parks Retractor was used by exerting minimal dilating effect in all cases. Left lateral sphincterotomy was realized by excising the internal anal muscle with scissors. Hemorrhoid peduncles were saturated by using vicryl 2-0. At the end of the intervention we did not use intra-anal but perianal swab. The pain level was evaluated using the Visual Analog Scale (VAS) for pain system (11). The VAS scale ranges from zero (no pain) to 10 (worst imaginable pain) (11). The subject is asked to indicate his/hers pain level according to his/her perception.

At the same time, we registered the number of analgesic vials applied to each patient. At the end of the intervention, all patients were injected an s/c morphine vial. Later, morphine was applied only on patient's request, also referring to the pain level evaluation. When the effect of anesthesia wore off we started a fiber diet and two hours later we used 5 ml Dulcolax Pico Liquid per os every six hours until the first defecation.

The patients were discharged from the hospital after first defecation, when they had no complications and the level of pain was tolerable. The postoperative follow up lasted six months after the surgery and it consisted in notifying all patients for a visit every 10 days in the first month and every month for the next three months. The last visit was made six months after the surgery. Incontinence was assessed six months after the surgery. The type and nature of incontinence is measured after the "Pescatori score" scale (12). This method divides the patients into three groups: gas incontinent patients, liquid or solid defecation patients and continent patients. At the same time, it determines the type of incontinence: permanent or intermittent (12). We have not evaluated the type of incontinence.

We assessed the following indicators: the postoperative pain level, pain level during the first defecation, quantity and duration of postoperative rectorrhagia, duration of the surgery wound closure, urinary retention, anal sepsis. The incontinence grade was evaluated six months after surgery.

Statistical analysis

For categorical values we calculated the respective absolute number and frequency (in percentage) was reported. The chi-square test was used to compare categorical variables. The Student's t-test was used for both techniques for the comparing mean values of continuous variables (postoperative pain and pain during first defecation) by type of technique used (open vs. closed). A p-value ≤ 0.05 was considered as the acceptable level of statistical significance.

Results

This study included 152 patients: 101 males (66.4%) and 51 females (33.6%). The male:female ratio was 58 men and 34 women for group A, 43 men and 17 women for group B. The average age of patients was 46.8 years for group A and 52.5 years for group B.

Table 1 presents the average pain level, measured by VAS scale by study group. The average pain level for group A patients was significantly higher compared to group B patients.

Table 1. Average pain level by type of surgery

Variable	Average pain level according to VAS scale	T-test	P-value
Study group			
Group A (n=92)	8.18	4.96	<0.001
Group B (n=60)	6.21		

Table 2 presents information about the average pain level during first defecation when the patients are still hospitalized, by study group. The average pain level during first defecation was significantly higher for group A patients.

Table 2. Average pain level during first defecation

Variable	Average pain level according to VAS scale	T-test	P-value*
Study group			
Group A (n=92)	9.32	5.26	<0.001
Group B (n=60)	7.23		

* P-value according to student's t-test.

Table 3 displays the information about the number of morphine vials applied during hospitalization, by study group. The proportion of group A patients using three morphine vials is significantly higher compared to respective proportion in group B patients (82.6% vs. 16.7%, respectively). On the other hand, 60% of group B patients did not require the application of any morphine vial compared to none in group A patient not needing morphine. These differences are significant (Table 3).

Table 3. Number of morphine vials during hospitalization

Variable	Study group		P-value †
	Group A (n=92)	Group B (n=60)	
Number of morphine vials			
Three vials	76 (82.6) *	16 (16.7)	
Two vials	16 (17.4)	14 (23.3)	<0.001
One vial	0 (0.0)	36 (60.0)	

* Absolute number and column percentage (in parenthesis).
† P-value according to chi-square test.

Table 4 displays the duration of rectorrhagia and operative wound closure by study group. All patients experienced rectorrhagia for at least ten days, after this many days the proportion of patients suffering from it is significantly lower in group B patients than in group A patients (except for duration of rectorrhagia over 30 days, where a borderline significance was achieved) (Table 4). Regarding wound closure, a significantly higher proportion of group B patient experienced wound closure within 30 days of intervention compared to group A patients (85.0% vs. 53.2%, respectively) (Table 4).

Table 4. Duration (in days) of rectorrhagia and operative wound closure by study group

Variable	Study group		P-value†
	Group A (n=92)	Group B (n=60)	
Duration of rectorrhagia 1-10 days			
Yes	92 (100.0)*	60 (100.0)	-
No	0 (0.0)	0 (0.0)	
Duration of rectorrhagia 11-20 days			
Yes	76 (82.3)	35 (58.3)	<0.001
No	16 (17.7)	25 (41.7)	
Duration of rectorrhagia 21-30 days			
Yes	31 (33.7)	2 (3.3)	<0.001
No	61 (66.3)	58 (96.7)	
Duration of rectorrhagia >30 days			
Yes	5 (5.4)	0 (0.0)	0.066
No	87 (94.6)	60 (100.0)	
Duration of wound closure			
≤30 days	49 (53.2)	51 (85.0)	<0.001
>30 days	43 (46.7)	9 (15.0)	

* Absolute number and column percentage (in parenthesis).
† P-value according to chi-square test.

Table 5 displays the distribution of urinary retention and wound sepsis evaluated one month after intervention by study group. Significantly lower proportion of group B patients experienced urinary retention and wound sepsis compared to group A patients (Table 5).

Table 5. Distribution of urinary retention and wound sepsis by study group

Variable	Study group		P-value†
	Group A (n=92)	Group B (n=60)	
Urinary retention (catheterization)			
Yes	33 (35.9)*	4 (6.7)	<0.001
No	59 (64.1)	56 (93.3)	
Wound sepsis			
Yes	7 (7.6)	0 (0.0)	0.029
No	85 (92.3)	60 (100.0)	

* Absolute number and column percentage (in parenthesis).
† P-value according to chi-square test.

Table 6 displays the distribution of incontinence grade (according to Pescatori score) 6 months after intervention. Differences are not significant.

Table 6. Incontinence grade by study group

Variable	Study group		P-value†
	Group A (n=92)	Group B (n=60)	
Incontinence grade			
Continent	91 (98.9) *	59 (98.3)	
Gas incontinence	1 (1.1)	1 (1.7)	
Liquid defecation incontinence	0 (0.0)	0 (0.0)	0.759
Normal defecation incontinence	0 (0.0)	0 (0.0)	

* Absolute number and column percentage (in parenthesis).
† P-value according to chi square test. The chi square test was performed taking into consideration only the first two categories of incontinence grade, since the two last categories are zero.

Table 7 presents the information about complications identified up to six months after intervention, by study group. In general there are no significant differences regarding the proportion of subjects with various complication in both groups, except for anal stricture which was significantly higher in group A patients (Table 7).

Table 7. Distribution of complications up to six months after intervention by study group

Type of complication	Study group		P-value†
	Group A (n=92)	Group B (n=60)	
Serious rectorrhagia	3 (3.3)	1 (1.7)	0.548
Anorectal fecaloma	2 (2.2)	0 (0.0)	0.250
Hemorrhoidal thrombosis	4 (4.4)	0 (0.0)	0.102
Anal canal secretions	5 (5.4)	0 (0.0)	0.066
Perianal sentinel	2 (2.2)	1 (1.7)	0.826
Unclosed wounds	5 (5.4)	0 (0.0)	0.066
Anal fissure	5 (5.4)	0 (0.0)	0.066
Anal stricture	8 (8.7)	0 (0.0)	0.019
No complications	58 (63.0)	58 (96.6)	<0.001

* Absolute number and respective percentage within the total of the group (in parenthesis).
† P-value according to chi-square test.

Discussion

Findings of this study suggested that the average level of postoperative pain, as measured by VAS scale, in group A was significantly higher than in group B patients. Likewise, the average pain level during the first defecation was higher for group A compared to group B patients. Post-hemorrhoidectomy pain is a known complication, which is due to the wounds created, the use of electro coagulation, suture placement and high sensitivity of the anal canal (13-15). Post-hemorrhoidectomy pain is a complex phenomenon. Damaged tissues release mediators such as TNF-α, interleukin, cyclooxygenase, histamine and chemokine (13-15). All these cause a cascade of predominantly pro-inflammatory effects. These conditions cause the disruption of electrolytic cell balance resulting in its depolarization. These mediators, not only amplify

and distribute the pain, but also turn the painless incentives into painful ones. Based on these data, we understand that by relaxing the anal intern muscle, the sphincterotomy creates opportunities for better supply of blood and rejects postoperative edema and spasm, resulting in reduction of pain (16-18).

The present study also found that patients who were applied sphincterotomy had their first defecation faster than the patients in who sphincterotomy was not applied, a finding reported in international literature as well (19-21).

Difference in the pain level is also evident by the number of morphine vials applied. Around 83% of group A patients were applied three ampoules of morphine versus 16.7% of group B patients. Postoperative wounds epithelized faster in patients of group B. Also, 30 days after intervention, 85% of group B patients had epithelized wounds, versus only 53% of group A patients. Urinary retention was identified in 35.6% of patients of group A versus 6.7% in group B. In all cases urinary dischargers were used, instead of the catheter. The catheter was removed immediately after bladder was discharged. No patient left the hospital with urinary catheter on. Local sepsis was observed only in 7 patients of group A. All patients were treated with local treatment in hospital. Urinary retention is referred to as a complication, which occurs in 15% of patients after the benign anorectal surgery. Urethral reflex, benign prostate hypertrophy, excessive amounts of postoperative liquid, use of morphine analgesics, and pain, are the main causes of urinary retention (8,21,22). Incontinence evaluated on both groups was not significant in our research.

Literature has documented cases where sphincterotomy during open hemorrhoidectomy is accused as causing incontinence (9,23,24). The incontinence grade ranges from 1.3% to 2.9%. In our study total postoperative complications within 6 months resulted higher in group A, however in general the differences were not significant. The four rectorrhagia cases in both groups were resolved with saturation in hospital, with local anesthesia. In both cases of fecaloma, removal was conducted manually. Hemorrhoid thrombosis was treated with thrombus removal with local anesthesia. Unhealed wounds lasted until the 45th day. To speed up the closure of wounds they were provoked several times. 5 cases with anal fissures were treated with Antrolini, a cream with nifedipine and lidocaine content. Anal stenosis was identified in 8 cases, only in group A. It is referred that this complication occurs in up to 6% of patients treated with the "Milligan-Morgan" technique (group A patients in our study). In our research the difference had statistical significance.

Conclusion

The left lateral sphincterotomy accompanying the open hemorrhoidectomy in patients with hemorrhoid prolapse influences on: reducing the postoperative pain, faster epithelization of postoperative wounds, reducing the number of catheterized patients, reducing the number of patients with wound surgery sepsis, reducing various types of complications outlined. In particular, it helps avoiding complications like the anal stricture, a pathology seriously damaging the patient's life quality and that in most cases requires surgery.

Conflicts of interest: None declared.

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The adjuvant treatment of breast cancer human epidermal growth factor receptor (HER2) positive in Albania

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Abstract

Aim: Our aim was to identify and compare the use of Trastuzumab in adjuvant therapy in breast cancer patients who are human epidermal growth factor receptor (HER2) positive in various combinations of chemotherapy regimens.
Methods: The data were retrieved from the medical records of the Chemotherapy Unit at "Mother Teresa" University Hospital Center in Tirana for two consecutive years 2009-2010 including 90 women diagnosed with breast cancer HER2-positive and treated with adjuvant chemotherapy (Trastuzumab). Demographic data, tumor characteristics and disease outcomes for all patients included in this study were also collected.
Results: Chemotherapy was completed in 98% of the patients, while trastuzumab was used in 28 patients, or 31% of them. After 52 months of median follow-up, there were five disease events in the Trastuzumab group (17.8%) and 40 events in the non-Trastuzumab group (64.5%). The hazard ratio (HR) was 0.22 (95%CI: 0.05-0.86; P=0.01).
Conclusion: Our study provides useful evidence on clinical benefits related to the combination of chemotherapy regimens with Taxane and Trastuzumab which indicated an improvement of the disease recurrence compared to regimens without Trastuzumab in this sample of cancer patients in Albania.

Keywords: adjuvant chemotherapy, breast cancer, combination chemotherapy regimens.

Introduction

Breast cancer is a molecularly diverse disease with several defined molecular subgroups. Clinically, however, three therapeutic groups are used: those classified as hormone receptor positive (estrogen receptor and progesterone receptor with normal HER2 expression), those classified as HER2-positive as defined by HER2 amplification, with variable expression of hormonal receptors, or those classified as triple negative by low or absent hormonal receptor and HER2 (1).

More than 1.5 million breast cancer cases are reported each year worldwide and more than 50% are hormone receptor positive, 20%-25% of the cases are HER2-positive and 15% are triple negative (1).

New classes of molecularly targeted therapy can affect the natural history of some groups of breast cancer such as HER2-positive disease both in adjuvant and metastatic disease. Approximately 15%-20% of invasive breast carcinomas have amplification of the human epidermal growth factor receptor 2 gene (HER2-neu) and over-express the HER2 protein (1,2). Before the development of the anti-HER2-targeted therapies, women with early HER2-positive breast cancer faced a worse prognosis in terms of both disease-free (DFS) and overall survival (OS) (1). Three large randomized trials produced convincing evidence that the anti-HER2 monoclonal antibody trastuzumab, administered with adjuvant chemotherapy for 12 months, increases DFS and OS of women with HER2-positive operable breast cancer (2-5). In two of the studies, the NCCTG N9831 and the NSABP B-31, a remarkable increase in 10-year OS from 75% to 84% was recently reported (6). Similarly, significant clinical benefit with adjuvant trastuzumab after chemotherapy was confirmed in a previous report of the HERA trial (2).

The aim of this study was to identify and compare the use of Trastuzumab in adjuvant therapy in breast cancer patients in Albania who are human epidermal growth factor receptor (HER2) positive in various combinations of chemotherapy regimens.

Methods

The data were collected from the cancer registry at the Chemotherapy Unit of the University Hospital Center "Mother Teresa" in Tirana for two consecutive years 2009-2010 including 90 women diagnosed with HER2-positive breast cancer and treated with adjuvant chemotherapy Trastuzumab. Notably, 99% of the patients received adjuvant treatment with chemotherapy and some but not all with Trastuzumab concurrent or sequential with chemotherapy. Women aged 18-75 years old with histologically confirmed invasive early breast cancer with HER2 over-expression were eligible. The HER2-positive status was determined by local pathology laboratories using immunohistochemistry testing. Patients were required to have undergone either lumpectomy or modified radical mastectomy with tumor-free surgical margins plus axillary node dissection, and the tumor had to be invasive carcinoma. Adequate hematologic, hepatic, renal parameters and cardiac function were mandatory. Cardiac function was assessed by an echo-cardiogram procedure, or the left-ventricular ejection fraction (LVEF). All women received epirubicin (75 mg/m^2 by i.v. infusion over 5-15 min), cyclophosphamide (700 mg/m^2 by i.v. infusion over 30-60 min), and 5-fluorouracil (700 mg/m^2 by i.v. infusion over 5-15 min) every two weeks for four cycles followed by docetaxel 75 mg/m^2 by i.v. infusion over 1 h every two weeks for four cycles. Trastuzumab was administered intravenously over 30-90 min every three weeks (loading dose 6 mg/kg; 4 mg/kg thereafter), starting concurrently with docetaxel. Thereafter, trastuzumab 6 mg/kg was administered every three weeks until the completion of 12 months therapy. Radiation therapy was decided by the treating physician and followed the current standards of the "Mother Teresa" University Hospital Center in Tirana.

Patients' follow-up consisted of medical history and physical examination, with laboratory and imaging

studies as indicated, every three months for the first two years, every six months for the next three years and yearly thereafter. Cardiac monitoring was carried out by history, clinical examination, and LVEF assessments every three months and, at the end of treatment, with trastuzumab. Thereafter, cardiac function was assessed only if clinically indicated (e.g. dyspnea, peripheral edema, chest pain, palpitations and the like). Cardiac toxicities were assessed with several indicators including symptoms, a decrease of LVEF under 50% (independent from the baseline value) or an absolute drop of LVEF of more than 15% from baseline.

The primary end point of the study was to compare the 4-year DFS rates between the treatment groups. The DFS was defined as the time from randomization to the date of breast cancer recurrence (either locoregional or distant), contralateral breast cancer diagnosis, non-breast second primary cancer, or death from any cause, whichever occurred first. Patients alive without any predefined event were censored at the time of the last assessment. Secondary end points were to compare the OS (defined as the time from the date of randomization to death from any cause) and toxicity.

Stratification parameters for the random assignment were the number of infiltrated axillary lymph nodes (0 versus 1-3 versus 4-10 versus >10) and hormone receptor status [estrogen (ER) and/or progesterone receptor (PR) positive versus both negative]. All patients who received at least one cycle of treatment were included in the analysis. The DFS and OS rates were calculated using the Kaplan-Meier method. The comparison of treatment arms was assessed using the log-rank test. The independent effect of treatment and other prognostic factors on DFS and OS was analyzed by Cox's proportional hazards model. Quantitative factors were compared by Pearson's $\chi 2$ contingency table analysis or Fisher's test whenever appropriate. P-values ≤ 0.05 were considered statistically significant for all comparisons. The statistical analysis was done in SPSS, version 17.0.

Results

About 32% of the patients received Trastuzumab in addition to chemotherapy in a total of 97 patients (100%) patients. After a median follow-up of 48 months, 5 (17.1%) patients had disease recurrence in the Trastuzumab group and 40 patients (64.5%) in the non-Trastuzumab group (P=0.0001). During this period of time, Trastuzumab was not available for all patients hospitalized at the "Mother Teresa" Hospital in Tirana. The patients treated with Trastuzumab had few events 5 (17.1 %) compared with treatment regimens without Trastuzumab where the number of events was 40 (or, 64.4%). The group treated with CAF consisted of 36 patients and 27 of them had disease recurrence (75.0%). We studied the localization of relapse in this group. The rate of visceral relapse was higher in the HER2-positive group with 32 (64.2%) patients, compared with other localizations of relapse Osseo and loco-regional.

Table 1. Characteristics of HER2-positive patients according to the number of lymph nodes, size of tumor, grade and local treatment (N= 90)

Mastectomy	85 (94.4)
Breast conserving	5 (5.5)
Radiotherapy	23 (25.5)
N0	27 (30.0)
N1-3	18 (20.0)
N 3-10	23 (25.5)
N >10	22 (24.4)
G I	0 (0.0)
G II	42 (46.6)
G III	48 (53.3)
T 1 < 2cm	10 (11.1)
T 2 2-5 cm	71 (78.8)
T3 > 5 cm	9 (10.0)

Table 2. Treatment regimen used in the group of patients HER+/HR

Regimen used	Recurrence	Chi-square	Hazard ratio	95%CI	P-value
AC/T H (4+4) +H N=28	5 (17.8.)*		1		
AC/T (4+4) N=26	13 (50.0)	6.152	0.22	0.05 to 0.86	0.01
CAF (6) N=36	27 (75.0)	22.4	0.07	0.02 to 0.28	0.0001
Total: 90 cases (100%)	45 (50%)				

*Reference group is the regimen AC/T H

Most of the patients undergoing surgical treatment had mastectomy which, under these circumstances, is a commonplace procedure at the University Hospital "Mother Teresa" in Tirana. These cases involved high grade and node-positive tumors in an advanced stage. There were more disease events in the non-Trastuzumab group and they mostly occurred during the first and second year after the diagnosis.

Table 3. The localization of relapse according to regimen used

Recurrence	Loco regional N (%)	Visceral N (%)	Osseo N (%)	Brain N (%)	Total N (%)
AC/T H (4+4) +H N=28	3 (50.0)	3 (9.3)	0 (0.0)	1 (33.3)	7 (100.0)
AC/T (4+4) N=26	2 (30.3)	12 (37.5)	1 (25.0.)	2 (66.7)	17 (100.0)
CAF (6) N=36	1 (16.6)	17 (53.1)	3 (75.0)	0 (0.0)	21 (100.0)
Total	6 (13.3)	32 (71.1)	4 (8.8)	3 (6.6)	45 (100.0)

There was evidence of a high rate of visceral metastases in this group (around 71%) and most of them occurred in the non-Trastuzumab Group. There were some recurrent cases (6.6%) involving the brain.

Discussion

Our findings do not point to an optimal duration of treatment with adjuvant trastuzumab. Hence, this issue remains unclear for our clinical practice. Some observations support trastuzumab administration for a long period. Conversely, in early breast cancer, two small studies have suggested that when trastuzumab is administered concomitantly with chemotherapy for nine weeks to six months, the reduction in the risk of relapse is similar to a longer treatment (7,8). The HERA trial compared 1-year versus 2-year trastuzumab added sequentially to adjuvant chemotherapy and found no additional benefit for the 2-year regimen (4). After a median follow-up of 52 months, DFS events occurred in five patients in the Trastuzumab group (17.4%) and 40 (64.5%) patients in the non-Trastuzumab group. Two-year DFS was 93.8% in the Trastuzumab group and 70% in the non-Trastuzumab group. Fewer patients in the Trastuzumab group had distant recurrences (6.4% versus 8.3%, HR=1.33, 95%CI: 1.04-1.71). Among patients with ER-negative tumors who received only chemotherapy and DFS was significantly shorter in patients of the CAF group compared with those of the Taxane group (HR=1.57, 95%CI: 1.08-2.28). Therefore, in this group of 'pure' HER2-positive tumors, most of the adjuvant effects might occur early during therapy, possibly during the concomitant administration of chemotherapy and trastuzumab (9,10). On the contrary, in the PHARE trial, patients with ER-negative tumors who received trastuzumab sequential to chemotherapy experienced more benefits from the prolonged trastuzumab administration. In our study, the benefit of longer trastuzumab administration almost reached statistical significance (HR=2.20, 95%CI: 0.91-5.31) for patients with ER-negative tumors.

The number of patients enrolled in our study was relatively small and the non-inferiority margin set by the statistical hypothesis was relatively large. Moreover, the study was hampered by a slow accrual and eventually took four years to be completed instead of the planned three years and would have been benefited by an independent review committee which unfortunately was not involved. The two study arms were well-balanced for all the stratification parameters; however, although age was not a stratifying factor, older patients were actually randomized.

Nonetheless, our results indicate that women with small, node-negative tumors have a favorable long-term outcome and if adjuvant therapy is prescribed, then six months of trastuzumab might be a reasonable option. Moreover, in a PHARE trial's date the treatment effect was assessed according to tumors' characteristics and four prognostic groups were defined (very-low, low, intermediate and high) (2).

In conclusion, our results support the current standard of care of 12 months adjuvant trastuzumab for women with early HER2-positive breast cancer. Whether a shorter course of trastuzumab is enough for specific subgroups (e.g. ER-negative tumors, small tumors) needs to be addressed in future clinical studies.

Conflicts of interest: None declared.

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Effectiveness of combined Measles, Mumps and Rubella vaccine in Albania: An analysis based on health impact surveillance

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Abstract

Aim: In this paper we aim to provide an overview about the efficiency of combined measles-rubella (MR) and MMR (measles-mumps-rubella) vaccines in use in Albania during the last 15 years.
Methods: We used the Albanian epidemiological surveillance system on infectious diseases to estimate the health impact of the vaccination strategies in Albania, which has been historically using single and multi-antigen vaccines.
Results: Despite the limitations of our methodology that does not permit a firm conclusion about the effectiveness of the single antigen measles vaccine used in Albania before the year 2000, there seems to be a clear impact of the combined antigen vaccines MMR applied in the country since 2000. There is a clear time relation between the introduction of MR vaccine in 2001 and the virtual stop of circulation of measles in Albania. Few sporadic cases encountered in 2006-2007 remained isolated and no more cases were reported later. Similarly, parotitis cases, which have been reported by thousands per year in the 2000-2005 period, dropped immediately after 2006 and especially after 2007, where only 20-50 cases per year were reported in all Albanian districts.
Conclusion: It seems that, beyond any reasonable doubt, the dramatic decrease of the disease in the population was due to the block of virus circulation by effectively applying induced immunity among vaccinated children. This is equally true for measles and parotitis.

Keywords: effectiveness, immunization, impact, surveillance.

Introduction

Most of infectious diseases preventable-with-vaccination are transmitted through air, specifically known as measles, rubella, diphtheria, pertussis, epidemic parotitis (1).

Measles is a highly communicable disease caused by a morbillivirus and it may seriously threaten the health of the child because of complications such as pneumonia, croup, encephalitis and death. Prior to widespread immunization it was a common infection in childhood, so that more than 90% of the people had been infected by the age of 20 years.

Global measles deaths have decreased by 79% from an estimated 546,800 in 2000 to 114,900 in 2014 (2).

The measles vaccine has been in use for 50 years. WHO recommends immunization for all susceptible children and adults for whom measles vaccination is not contraindicated. Covering all children with two doses of measles vaccine, either alone, or in a measles-rubella (MR) or measles-mumps-rubella (MMR) combination, is considered as a standard for all national immunization programs (3).

In the pre-vaccination era, mumps constituted the main cause of viral encephalitis in many countries. By 2002, mumps vaccine was included in the routine immunization schedule of 121 countries. In countries where vaccination was not introduced, the incidence of mumps remains high, mostly affecting children aged 5-9 years (4).

Rubella, a mild febrile viral disease caused by *Rubella Virus*, is important for public health because of its ability to produce anomalies in the developing fetus. Congenital rubella syndrome (CRS) occurs in up to 90% of infants born to women who are infected with rubella during the first trimester of pregnancy. Vaccine is recommended for all susceptible non pregnant females without contraindications (4).

In Albania, measles vaccine has been in systematic use since 1971 as a single antigen (5). Because of the measles epidemic of 1970-71, an urgent mass vaccination of all birth cohorts 1956-1970 was carried out for the first time in Albania within three weeks throughout the country with the imported B55 attenuated live measles vaccine. In 1971, the routine mandatory vaccination against measles was introduced in the national immunization calendar for all new birth cohorts. Measles routine vaccination was based on the B55 attenuated live vaccine, that was first imported and then in 1977 started to be produced locally (5).

In 1992, the local production of B55 vaccine was interrupted, being substituted by the imported Schwarz measles vaccine in the routine mandatory vaccination. Since January 2001 Albania has introduced measles and rubella bi-vaccine in the calendar of mandatory immunization, giving a basal dose at 9th month of age and the booster dose at the age of 5 years. The mumps antigen was added to the National Immunization Program since the 1st of January 2005 introducing the combined tri-vaccine MMR, which is still in use today in Albania (5).

This vaccination strategy was implemented in the framework of the National Strategy for the elimination of measles and congenital rubella syndrome (5).

There are still some debates about the effectiveness of combined vaccine as compared to use of single vaccine (6-8).

In this paper we aim to provide facts about the effectiveness of combined MR and MMR vaccines in use in Albania during the last 15 years, based on data provided from the surveillance of vaccine controlled diseases in Albania.

Methods

We have used the Albanian epidemiological surveillance system on infectious diseases to estimate the health impact of the vaccination strategies in Albania, which as mentioned above, has been historically using single and multi-antigen vaccines.

The Albanian epidemiological surveillance system on infectious diseases has been and it continues to be a statutory one, the infectious diseases included in that system must be reported by law.

In 1997, the Department of Epidemiology (DE) of the Institute of Public Health in Albania initiated and carried out conspicuous quantitative and qualitative modifications of the statutory notification system thus compiling the new Major Disease-Based Epidemiological Surveillance System (MDBSS). The new highly improved reporting system was officially approved by the Albanian Ministry of Health and put into practice starting from January 1, 1998. In the compilation of the new statutory reporting system all of the attributes of an epidemiological surveillance system were taken into account such as flexibility, sensitivity and acceptability. The actual notification system contains 73 nosological entities of infection diseases presented in a standard official Form (named 14/Sh). The infection diseases are divided into three groups (namely A, B, C) in that Form according to the degree of their public health importance, based on the respective measuring parameters such as the magnitude of the problem, indices of disease severity, socio-economic impact and preventability. The aggregated data in the monthly 14/Sh Form are presented for each infectious diseases according to place (urban and rural), specific case definition (suspect and confirmed case), and age groups. The 14/Sh monthly Form of the actual reporting system is obligatorily by law to be accompanied by the Individual Forms for each Group, which contain detailed epidemiological information about the case-patient thus increasing, first of all, the specificity of the surveillance system and quantitatively and qualitatively enriching the system's epidemiological evidence (5).

Mandatory reporting system on Measles/Rubella Case-Based Surveillance represent in itself an addendum of the statutory reporting system of infectious diseases. These diseases are enlisted in the Group B of the 14/Sh Form and are of a rapid notification (within 1-3 days) from data sources to the local level and of a monthly notification from local level to the national one if their occurrence is represented as sporadic cases.

Data flow structure of the Alert System implies the weekly mandatory notification from the basic level (data sources) to the national one (DE of IPH) of the surveillance system through the local level (district epidemiological service).

Data handling includes data collection, check, aggregation and analysis in order to produce the weekly Alert Form, the monthly 14/Sh Form and yearly epidemiological report, which should be sent to the IPH.

Actually, all data sources are public. Anyhow, the private health services are by law under the mandatory notification, concerning the epidemiological surveillance of infectious diseases. We have used the surveillance data since year 2000 till the latest available year (2013, 2014) for all 36 districts of Albania (5).

The data are presented in a way that unfolds the impact on health differences before and after the introduction of the combined vaccine. Regarding parotitis, the available data belong to the years 2001 to 2013, helping us realize that the number of cases in the districts of Albania have decreased after the application of the vaccine (5).

The surveillance on birth defects in Albania since 2007 does not have the necessary specificity to identify cases with Congenital Rubella Syndrome (CRS) and we have limited our health impact analysis based only on measles and parotitis based surveillance. Due to the insufficiency of data, the study of rubella is not included.

Data were analyzed in Stata and Excel software.

Results

In Albania, Measles, from 1945 till 1955 had its usual endemic circulation among country population, with two epidemic peaks: epidemic of 1947-48 with 40,106 cases, and epidemic of 1954-55, spread all over the country, with 190,020 cases. In

1956, immediately after epidemic of 1954-55, a strategy of a total quarantine toward each eventual imported measles case was established throughout Albania. As a result of such strategy a total elimination of indigenous measles over a 15 year period (1956-1970) was achieved.

Measles epidemic of 1970-1971 started in November 1970 when the period of quarantine was accidentally interrupted. In 1971, the routine mandatory vaccination against measles was introduced in the national immunization calendar for all new birth cohorts. Measles routine vaccination was based on the B55 attenuated live vaccine that was first imported and then in 1977 started to be produced locally.

July 1971 marked the beginning of an 18 year period (1971-1989) of indigenous measles elimination in Albania, due to the primary prevention through the specific vaccine prophylaxis. Episodes of imported cases (1981-1984) were not able to support the survival of measles circulation among the population because of such levels of the total herd immunity. Following the 18 year period of measles elimination, a nation-wide measles epidemic took place (April 1989-March 1990), being distributed in all country districts, with an attack rate of 5,374 cases per 100,000 population, but with a low case fatality. In 1992, the local production of B55 vaccine was interrupted, being substituted by the imported Schwarz measles vaccine in the routine mandatory vaccination (5). The period 1992-2000 was characterized by measles circulation at sporadic levels with small and limited outbreaks, with an annual average of 700-800 reported cases and zeros deaths. The pediatric age groups (1-14 years old) showed the highest incidence rates, more than 80-85% of the total annual reported cases.

Table 1. Measles cases and incidences over the years in Albania

Measles	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Cases (n)	662	18	0	0	0	0	68	22	0	0	0	0	0	0
Incidence	21.1	0.6	0	0	0	0	2.2	0.7	0	0	0	0	0	0

Since 2003, Albania had been classified as a measles free country until two epidemic outbreaks emerged in 2006 and 2007 mainly among Roma population in the districts of Elbasan, Saranda (2006) and Shkodra (2007). They were monitored and efficiently stopped due to the interference of immediate mass vaccination in these populations. Based on the studies conducted by genotyping in the laboratory of virology, it was proved that the epidemic emerged in Elbasan and Saranda had a single source, originally coming from Greece, while the one emerged in Shkodra district had its source in Italy. An immediate reduction of measles cases was noticed and for nearly 4 years Albania was considered "free" from measels (11). Through ALERT system, maculopapular rash cases are reported in epidemiology sector, but so far none has been confirmed to be measles.

Rubella

The average annual number of reported rubella cases over the period 1964-2001 is 9.6 cases, but there are even years with zero cases such as 1980 and 1981. The epidemic peaks were recorded over the period: 1969 with 3,676 reported cases, epidemic of 1985, the largest one, with 78,594 reported cases, and epidemic of 1994 with 3,432 reported cases. Age groups of 5-14 years old represent 60-70% of rubella cases, both in each epidemic peak. Meanwhile, older age groups (25-44 years old) are not exempted from rubella virus infection: they represent 1-2% of the total annual rubella reported cases over the period 1964-2001. There are not statistically significant differences of rubella incidence among country districts and incidence between urban or rural areas. The real weight of rubella infection in Albania is given by

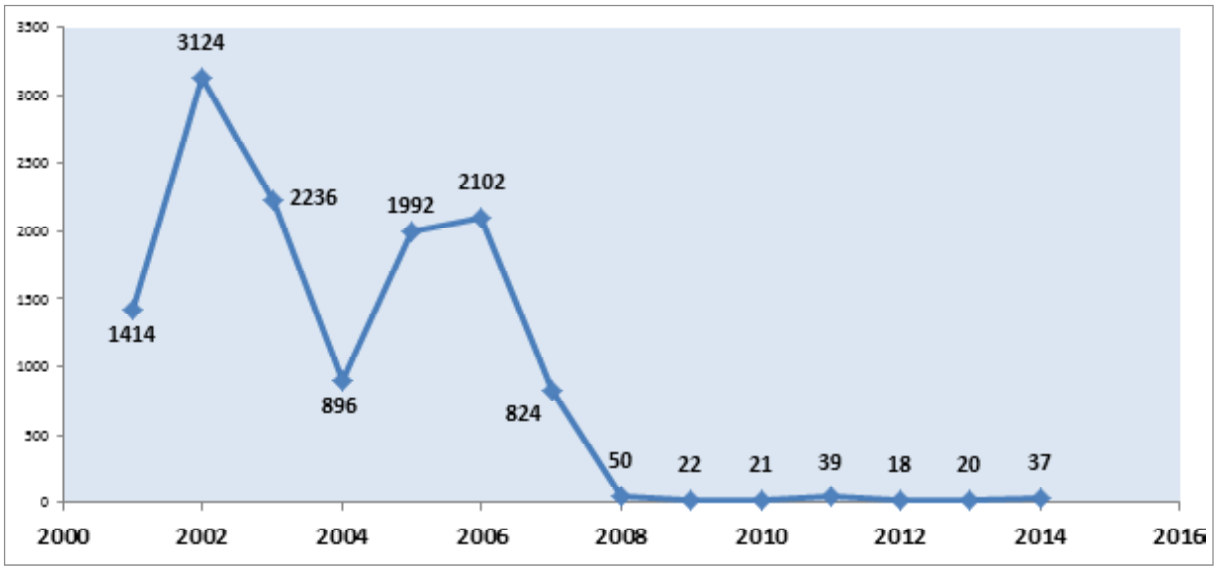
cross-sectional sero-epidemiological surveys, conducted in 1981, 1983, 1989 and 1995-1996 by the Institute of Public Health. The total rubella seroprevalence rate varies from 48% to 58% in inter-epidemic intervals, being increased up to 86-87% immediately after rubella epidemic circulation. Meanwhile, there are significant differences in age specific immune profiles, resulting to be: generally low from 10-30% at pre-puberty age groups, at puberty age increased to 30-70% and over 90% at post-puberty age groups. Such a strong relationship of rubella seropositivity levels with age essentially represents the specific feature of rubella epidemiology in Albania, quite contrary to other European countries during the pre-vaccine era.

Though there is not any evidence on Congenital Rubella Syndrome (CRS) occurrence in Albania yet, it might be evaluated as an annual expected number of around 70 cases. Furthermore, there is a significant pool of susceptible cases in puberty and post-puberty age groups just because of the above age specificity of rubella incidence, which overemphasizes the high risk of CRS in Albania. A mass vaccination (with measles-rubella bi-vaccine) of pediatric age was carried out in Albania in November 2000. On 1 January 2001 it was introduced in the calendar of mandatory immunization in Albania.

Results for the period after the introduction of MMR vaccine

A considerable (high) number of cases were noticed in the year 2002. More than 700 cases were reported to be encountered in our capital city in 2002. In 2003 the city of Shkodra counted about 400 cases, followed by a decrease in the number of cases in 2005 in Korca, Gjirokastra, Vlora and Shkodra. Referring to Figure 1, we can say that the peak with 3,124 cases was reached in 2002 followed by 2,236 cases in 2003 and 2,102 cases in the year 2006. It is obviously noticed that the situation remained constant starting from 2008 onwards.

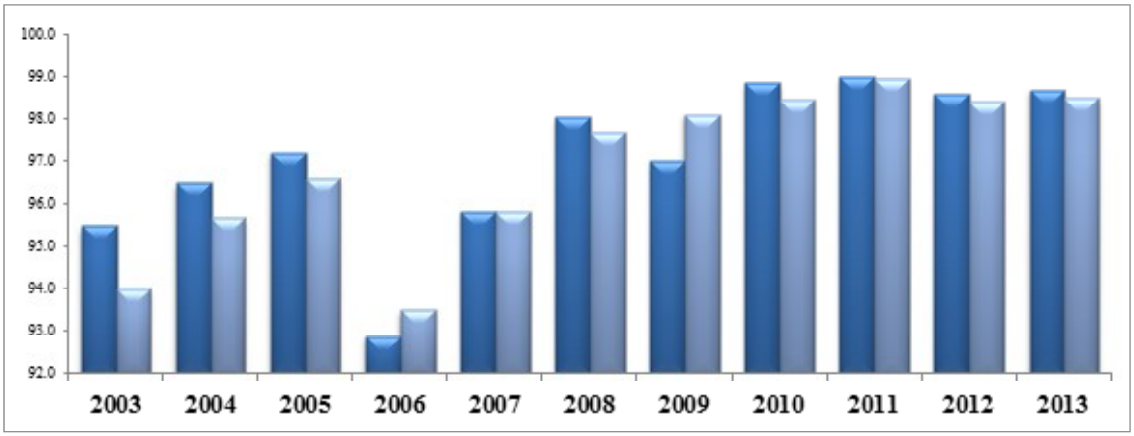
Figure 1. Distribution of parotitis cases over years



Considering these data, we noticed that from 2,000 verified cases encountered in 2006, the number of cases during the recent years varies from 20 to 30. The disease incidence has gradually decreased throughout Albania from the year 2006 onwards. Figure 2 presents cases that have been identified over the years in the largest districts of the country (9).

The situation after 2006 appears to have taken another trend and we notice a steady decline in the reported cases and, therefore, we conider that the combined MMR vaccine is effective in Albania (10).

Figure 2. Immunization coverage for the two doses of vaccine containing measles component (MR/MMR)



For the first dose of vaccine, the average coverage value is estimated to be around 98% which shows that the vaccination coverage is also high at the district level. Examination of Figure 2 and Figure 1 (which presents the distribution of the cases for parotid component over the years, from 3,124 cases in 2002 to 20-30 cases in 2014), indicates a steady decline in the reported cases and, therefore (9), we consider that the combined MMR vaccine is effective in Albania, despite some small geographic differences.

Discussion

The history of vaccination for measles, parotitis and rubella has been going through phases where different approaches have been applied. The period of full isolation was followed by massive vaccination of various single antigen vaccine against measles. Obvious health improvement has been noticed but nonetheless, the virus has been circulating in the limited groups of the population and has even caused (somehow mild) large-scale epidemics.

The situation may become problematic in cases when parents refuse to immunize their children, and if the number of refusals increases, our country may face measles epidemic as it has happened in the United States, England and France in the last years. Vaccination in Albania is still optional, i.e., parents themselves decide if they want to vaccinate their child. Their skepticism would endanger other children's life. So, the need to increase parents' awareness on vaccination becomes a necessity; they must never neglect the vaccination of their children as the current need in Albania is to maintain the highest vaccine coverage possible.

When measles is endemic, routine monthly reporting of aggregated data on clinical measles cases is recommended by district, age group and immunization status. In situations of low-incidence should be conducted a Case-based surveillance and every case should be reported and investigated immediately. Suspected measles outbreaks should be confirmed by conducting serology on the first 5-10 cases only (12).

In case of endemic mumps, it is recommended routine monthly reporting of aggregated data of clinical mumps cases by district. Only outbreaks (not each case) should be investigated. Suspected mumps outbreaks should be confirmed by con-

ducting laboratory investigation on 5-10 cases only. In specific situations, viral isolation can be attempted to differentiate meningitis cases that could be related to the wild virus, the vaccine strain or other factors (13).

Despite the limitations of our methodology that does not allow us to firmly conclude about the effectiveness of the single antigen measles vaccine used in Albania before the year 2000, there seem to be a clear impact of the combined antigen vaccines MMR applied in the country since that year. There is a clear time relation between the introduction of MR vaccine in 2001 and the virtual stop of circulation of measles in Albania. Few sporadic cases encountered in 2006-2007 remained isolated and no more cases are reported in the later years.

Similarly, parotitis cases which have been reported by thousands per year in the 2000-2005 period, dropped immediately after 2006 and especially after 2007, where only 20-50 cases per year were reported in all Albanian districts.

It seems that beyond any reasonable doubt, the reason for that dramatic decrease of the disease in population is the block of virus circulation by effectively applying induced immunity among vaccinated children. This is equally true for measles and parotitis as well.

For rubella the limits in methodology hindered us to prove the same fact.

Our analysis focused mostly on the effectiveness of vaccine among those undergoing routine appropriate immunization. Nevertheless, it is equally important to verify other components of a vaccination programme, such as vaccination coverage, clustering of problems in some large areas, cold chain quality, and the like. In our study, we were able to include some of them but not all. For the data related to vaccine coverage, some time correlation can be seen among the increase of the vaccination coverage during the two periods; 2003-2007 and afterwards. The only reported cases of measles in Albania belonged to that period (exactly the end of it) and practically the natural circulation of parotitis, despite its smaller scale, seemed to continue for two years after the introduction of the parotitis vaccine, and there were still some hundred cases in 2007.

In addition, we verified and proved that there might be some conditions that potentially affect the circulation of the virus of parotitis in Albania. To achieve the maximum effectiveness, the vaccination program should ensure the equality of the coverage among all populations. It seems that in Albania living in a region geographically positioned in a more western district or lower sea level altitude could increase the chances to be more exposed to parotitis. One of the reasons for that could be the higher density rate of the communities living in those areas and the increased possibility for contacts between infected sources and susceptible individuals. It should be noted that an effective vaccination programme must be accompanied by an effective public health surveillance of diseases to be controlled.

It is assumed that, while the ongoing vaccination programme based on MMR is achieving the objectives, it can be easily jeopardised by a lowering of vaccination coverage, especially in certain geographical areas.

Conflicts of interest: None declared.

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Effects of blood transfusion in patients with upper gastrointestinal bleeding

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Abstract

Aim: Acute upper gastrointestinal bleeding (AUGB) could pose numerous threats to patient's health and life. Blood transfusion strategy is still controversial whereas its effectiveness draws considerable scientific interest. Our aim was to evaluate the effectiveness of blood transfusion in AUGB patients in Albania.
Methods: This cross-sectional study was carried out in Tirana during January 2014-December 2014 among 92 patients experiencing AUGB (66% males, average age 51 years old). Laboratory (number of erythrocytes, haemoglobin (Hb) and haematocrit (Hct) level) and clinical parameters (cardiac frequency, respiratory frequency and arterial pressure) were measured before and 12 hours after blood transfusion. Patients were divided into three groups based on number of blood units being transfused.
Results: After the transfusion, mean values of clinical parameters across study groups were significantly different. Mean number of erythrocytes and mean values of Hb and Hct increased significantly with the number of blood units being transfused. Transfusion of one blood unit was almost as effective as the supply of two blood units in terms of clinical parameters. After transfusion, the laboratory parameters significantly improved in all study groups but differences across groups remained not significant.
Conclusion: Transfusion of one unit of blood in patients experiencing acute upper gastrointestinal bleeding might significantly improve their clinical and laboratory parameters. The transfusion of more than one unit of blood could result in further improvements of these parameters, but in this case the financial costs should be considered as well.

Keywords: Acute upper gastrointestinal bleeding, Albania, blood transfusion, haematocrit, haemoglobin.

Introduction

Acute upper gastrointestinal bleeding represents a serious emergency condition that requires prompt intervention to avoid potential complications and save patient's life (1,2). The clinical manifestations of patients with acute gastrointestinal bleeding might include hematemesis, melena, and hematochezia (3-5). Because of the potential for life threatening blood loss, severe acute gastrointestinal bleeding might require the transfusion of red blood cells (RBC) (6). There is a debate regarding the optimal threshold of haemoglobin (Hb) for starting the transfusion of red blood cells in patients with acute GI bleeding (6). The two prevailing modalities are the restrictive and liberal transfusion strategies. In restrictive transfusion strategy the Hb threshold for initiation of red blood transfusion is $\leq$7 g/dL whereas in liberal transfusion strategy the Hb threshold is $\leq$9 g/dL (6,7), even though the thresholds of Hb, haematocrit (hCT) or other parameters used to decide upon red blood transfusion vary quite a lot (8-10). The decision whether restrictive or liberal strategy is best depends on the patient's conditions (7). However, restrictive transfusion strategy seems to be safe in most clinical settings (11) and, in patients with acute gastrointestinal bleeding it significantly improves outcomes such as reducing the risk of further bleeding and length of hospital stay, reducing other serious adverse events and improving the probability of survival 6 weeks after the transfusion (6,12). Regardless of patients' conditions, in general the number of red blood units transfused is lower with restrictive transfusion strategy (11). The thresholds of Hb for indicating blood transfusions in patients without active bleeding are published by the American Association of Blood Banks (AABB) (13).

A recent cluster randomised feasibility trial comparing restrictive versus liberal blood transfusion for acute upper gastrointestinal bleeding suggested that mean levels of haemoglobin concentration after the transfusion did not differ significantly between the study groups (116 g/L for the restrictive strategy group and 118 g/L for the liberal strategy group) and also no significant differences in red blood cell units transfused (14). However, the haemoglobin threshold for the initiation of blood transfusion in the restrictive group was <80 g/L whereas for the liberal group was <100 g/L (14). In this perspective, the increase of Hb level after the transfusion was higher (in absolute number) in the restrictive than in liberal group (a mean increase of 36 units vs. 18 units, respectively) (14). Usually, transfusion of red blood cells aims to reach a Hb level of at least 100 g/L or a haematocrit level of at least 30% (15).

The improvement of Hb and Hct levels is expected to occur after the administration of RBC units, as this is the main objective of such a procedure. For example, a study reported that 24 hours after the administration of two units of RBCs there was an increase in Hb level of 22.4 g/L whereas the level of Hct increased by 6.4% percentage points (16).

In Albania the change in Hb and Hct levels after the administration of RBCs units in patients suffering from upper gastrointestinal bleeding is not documented. In this context, the aim of this study was to evaluate the effectiveness of blood transfusion in patient with upper gastrointestinal bleeding based on the selected clinical and laboratory parameters.

Methods

This was a cross-sectional study carried during January 2014 - December 2014 at the University Hospital Center "Mother Theresa" in Tirana, Albania.

Study population

The study involved 92 patients: 61 males (66%) and 31 females (34%) with acute anaemia due to acute upper gastrointestinal bleeding. The age of the patients varied from 28 years to 62 years old, with a mean age of 51 years old. All patients were diagnosed with upper gastro-intestinal bleeding. No patient complained of pre-existing cardiovascular or pulmonary diseases.

Data collection and procedures

All included patients suffering from upper gastro-

intestinal bleeding were subjected to sclerotherapy. The procedure resulted successful in all of them. Every patient was catheterized by a central venous catheter in order to give the proper amount of crystalloid and colloid solutions and on the other hand to measure the central venous pressure (CVP). Every patient had oxygen supply given by a nasal catheter (4-5l/min).

The following laboratory parameters were evaluated: number of erythrocytes (Er), haemoglobin (Hb), haematocrit (HCt). The following clinical parameters were evaluated: cardiac frequency (fC) and respiratory frequency (fR), mean arterial pressure (MAP).

All clinical and laboratory parameters were measured in the moment of admission and again 12 hours after blood transfusion.

After sclerotherapy the patients did not have active haemorrhage. Patients were treated with crystalloid, colloid solutions and blood transfusion. After the treatment patients were normovolemic.

Based on the number of blood units being received, patients were divided into three groups:

- Group I = 66 patients: 44 males (66%) and 22 females (34%) = one unit transfused;
- Group II = 18 patients: 12 males (65%) and 6 females (35%) = two units transfused;
- Group III = 8 patients: 5 males (62%) and 3 (38%) female = three units transfused.

Statistical analysis

For describing the continuous variables included in the study the measures of central tendency (mean values) and measures of dispersion (respective standard deviations) were calculated and reported.

For comparing continuous variables across patients' groups the ANOVA procedure was used.

A p-value of ≤ 0.05 was considered as statistically significant. All analysis was carried out through the Statistical Package for Social Sciences (SPSS), version 20.

Results

Table 1 presents the mean values and standard deviations of laboratory parameters under study according to study group, before and after the transfusion of RBCs units.

Mean values of number of erythrocytes, percentage of Hct and level of Hgb before transfusion were not statistically different across the three study groups but after the transfusion these differences were significant (Table 1). In general, the higher the number of RBCs units transfused the higher the mean value of erythrocytes, Hct and Hgb after transfusion.

Table 1. Changes in the laboratory parameters before and after transfusion

Time of measurement	Laboratory parameter	Study group			P-value
		Group 1	Group 2	Group 3	
Before transfusion	Erythrocytes (in millions)	2.47 ± 0.48 *	2.16 ± 0.42	2.30 ± 0.20	>0.05 †
	Haematocrit (in %)	23.0 ± 4.3	20.6 ± 3.9	22.0 ± 2.2	>0.05
	Haemoglobin (in g/dL)	6.44 ± 0.67	6.34 ± 0.33	6.45 ± 0.45	>0.05
After transfusion	Erythrocytes (in millions)	2.80 ± 0.45	2.84 ± 0.38	3.32 ± 0.35	<0.05
	Haematocrit (in %)	27.0 ± 4.3	27.9 ± 2.8	34.0 ± 2.1	<0.05
	Haemoglobin (in g/dL)	7.40 ± 0.07	7.76 ± 0.40	9.20 ± 1.1	<0.05

* Mean value and standard deviation (in parenthesis).

† P-value according to the ANOVA procedure.

Table 2 presents the mean values and standard deviations of clinical parameters under study according to study group, before and after the transfusion of RBCs units.

It can be noted that the mean values of cardiac frequency, respiratory frequency and mean arterial pressure were not significantly different across study groups both before and after the transfusion of RBC units (Table 2).

Table 2. Changes in the clinical parameters before and after transfusion

Time of measurement	Clinical parameter	Study group			P-value
		Group 1	Group 2	Group 3	
Before transfusion	Cardiac Frequency	120 ±4.6 *	122.5 ± 7.5	124.7 ± 4.4	>0.05 †
	Respiratory frequency	29.9 ± 2.5	31.4 ± 1.7	31.4 ± 1.8	>0.05
	Mean Arterial Pressure	82.4 ± 6.3	83.2 ± 8.2	82.1 ± 5.6	>0.05
After transfusion	Cardiac Frequency	98.7 ± 4.2	96.8 ± 8.1	94.0 ± 4.3	>0.05
	Respiratory frequency	17.5 ± 1.5	18.6 ± 1.1	19.3 ± 0.5	>0.05
	Mean Arterial Pressure	83.1 ± 6.4	85.0 ± 5.7	85.0 ± 7.8	>0.05

* Mean value and standard deviation (in parenthesis).
† P-value according to the ANOVA procedure

Mortality in the three groups of patients included in this study was zero.

Discussion

The current study is an effort to contribute to the definition of transfusion effects based on the certain clinical and laboratory indicators. The clinical and laboratory evaluation was carried out 12 hours after the transfusion in order to acquire the most real values for the effectiveness of the treatment. Our study showed that the mean increase of the number of red cells after this interval in the patients belonging to the first group was about 430.000, the increase of haematocrit level was about 4 percentage points and the increase of haemoglobin level in this group was approximately 0.9 g/dl (Table 1).

Failure to achieve the expected laboratory parameters after transfusion of one unit of RBCs should orient us toward the possibility of an unidentified haemorrhage. These situations demand a change in the treatment strategy for these patients, and the identification and domination of the haemorrhage source becomes a priority, since it can be a threat to the life of the patient and make the transfusion therapy ineffective.

After the transfusion the laboratory parameters of the second and third group of patients showed statistically significant increases in comparison with the patients of the first group. This might be an indication that the transfusion of two or more units of blood could be more effective and helpful to the patients. Meanwhile, clinical parameters improve in all the three groups of patients after the transfusion of RBC units compared to before-transfusion values (Table 2) but no significant differences were observed between groups before and after transfusion. As a result, the clinical effects are similar, independently of the number of the blood units transfused.

Patients suffering from acute anaemia face a high life-risk. Therefore the substitute treatment with

blood and liquids should be very accurate and decided upon in a very short time. The consensus conferences on the blood transfusion problem have set the laboratory parameters which serve as limits for starting transfusion. The current guideline for red blood cell transfusion derives from recommendations of the WHO published in 2003 (17) and AABB published in 2012 (13). ABB guidelines strongly recommend adhering to a restrictive transfusion strategy when the Hb level is 7-8 g/dl in hospitalized, stable patients (13). In patients with pre-existing cardiovascular disease, the transfusion should be considered for patients with symptoms and Hb level $\leq$8 g/dl (13). The AABB does not make recommendations for or against liberal or restrictive transfusion threshold for hospitalized, hemodynamically stable patients with an acute coronary syndrome, but it suggests that transfusion decisions be influenced by symptoms as well as Hb concentration (13).

There is still a controversy regarding the safest and most cost-efficient strategy for treating patients suffering from acute anaemia (6). In this perspective two issues might be of interest: the optimal number of RBCs units to be transfused and the cost of blood transfusion compared to alternative therapy.

Is one unit of blood always sufficient?

Results of the current study showed that transfusion of one unit of blood was sufficient to improve both the laboratory parameters and the clinical ones, and 92.5% of them didn't need a second transfusion. For the five patients (7.5%) whose clinical improvement was not satisfactory, the transfusion of one unit of blood provided time to evaluate the need for a second transfusion. Therefore, the transfusion of one unit of blood does not always optimize the clinical situation of the patients, but in most of the cases it is sufficient.

This might come as a result of the high tolerance that the human organism has towards low levels of haemoglobin (18). Blood transfusions aim to improve oxygen supply to tissues (19). All the patients included in our study experienced profound anaemia or circulatory shock, implying a critical impairment of tissue oxygenation. If the patient is normovolemic a normal Hb concentration is not critical for adequate tissue oxygenation (19). In our study all patients were firstly treated with crystalloid and colloid solutions and blood transfusion until CVP was in normal ranges. This engaged compensatory mechanisms such as the increase of cardiac input and oxygen extraction, decrease of haematocrit and reduction of blood viscosity (19). As a consequence, venous return to the heart and left ventricular preload increase, while systemic vascular resistance and thus left ventricular afterload decrease, resulting again in increase of left ventricular performance and cardiac output (20). The decrease of haematocrit will ultimately lead to a decrease of oxygen delivery to tissues but body's oxygen demand can still be met (19) since oxygen transporting capacity is several times greater than the demand in calm conditions (17). This can explain the similar clinical effects achieved after blood transfusion in the three groups of patients. Also this could be due to the restoration of the plasma volume through liquids, which not only improves the cardiac debit and increases the amount of oxygen dissolved in plasma, but at the same time lowers the blood viscosity, resulting in considerable improvement of perfusion and tissue oxygenation (20).

Mortality in three groups of the patients included in this study was 0. It may be result of the small number and the young age of patients included in the study. A literature review suggested that transfusion strategy does not affect mortality measures (21).

The cost of blood transfusion

According to the data given by the National Centre of Blood Transfusion in Tirana, one unit of blood costs $75, a figure that is much higher if compared to the therapy with solutions. Obviously the

increase of the number of RBC units transfused is accompanied by a considerable increase of costs and therefore the most cost-effective strategy to treat acute anaemia patients is by transfusing one unit of blood.

The decision for transfusion is taken after a precise evaluation of both laboratory and especially the clinical parameters (17). After the transfusion of the first unit of blood, the parameters are evaluated again. Only then the decision for a second possible transfusion is taken. However this procedure has not been always followed in the patients included of our study. Often the decision for blood transfusion, or about the number of RBCs to be transfused, is taken without evaluating the clinical parameters or without re-evaluating these parameters after the transfusion of the first unit of blood.

Conflicts of interest: None declared.

Conclusions

Based on the findings of this study, the transfusion of one unit of blood in the patients suffering from acute anaemia results in improvement of clinical parameters (mean number of RBCs, mean level of Hb and Hct), as well as decrease of cardiac and respiratory frequency. On the other hand, the transfusion of two or three units of blood in these patients is accompanied by considerable further improvement of laboratory parameters (as well as financial costs) compared to the transfusion of one unit of blood. Finally, no significant differences were observed between study groups before and after transfusion regarding clinical parameters. However, the transfusion of two or more blood units implies considerable economic costs and should be carefully assessed against potential additional benefits.

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Treating keratoconus disease with the cross-linking method

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Abstract

Aim: Keratoconus is a degenerative disease, starting generally at the age of 14-25 years and causing progressive thinning of the cornea. Nowadays, cross-linking is the only procedure used to stop the natural progression of keratoconus. Studied and applied for the first time at Dresden University, a great number of clinical studies have supported its efficacy in halting the progression of keratoconus. The aim of this study was to evaluate the treatment of keratoconus disease with the cross-linking method in Albanian patients.
Methods: This study was conducted at the American Hospital in Tirana and included 81 eyes (75 patients) with progressive keratoconus. Mean age was 23.5 years (range: 15-38 years). A rotating Scheimpflug camera (Pentacam HR, Oculus) was used to diagnose and follow-up the keratoconus before and post cross-linking treatment. The parameters measured included corneal elevation, pachymetry and keratometry.
Results: Central and thinnest values of pachymetry decreased. Central pachymetry values continued to decrease until three years after cross-linking. Thinnest pachymetry values followed the same trend. Flattest, steepest and maximal keratometry significantly reduced six months after cross-linking and continued to reduce even after three years. There was a tendency of stabilizing UCVA after cross-linking. Also, there was a tendency of continuous increasing of BCVA, especially six months after the procedure.
Conclusion: Cross-linking procedure seems effective in reducing corneal radius (flattest, steepest and maximal). Having a flatter cornea in a progressive keratoconus means that the progress of keratoconus is stopped and there is also a remodeling of its surface. Remodeling the cornea also stabilizes visual acuity and even improves best spectacles visual acuity.

Keywords: cornea, cross-linking, keratoconus, keratometry, pachymetry.

Introduction

The cornea is a transparent interface covering the front of the eye. It has the function of protecting the eyeball and also is a powerful refracting surface, providing 2/3 of the eye's focusing power. The adult cornea has a thickness of 500 μm and is comprised of 5 layers: epithelium, Bowman's membrane, stroma, Descemet's membrane and the endothelium. The stroma is the thickest layer composed of collagen fibrils oriented parallel to each-other. It has also transversal fibrils which bond the parallel ones to each-other, giving to the cornea its natural strength. This phenomenon is known as natural cross-linking and it is responsible for the cornea's resistance against deformation. Keratoconus is a bilateral non-inflammatory disease which causes progressive corneal thinning, leading to protrusion, distortion, and scarring of the cornea (1). It is a naturally occurring ocular condition which leads to steepening of the central cornea, increasing myopia, irregular astigmatism, and loss of best spectacle-corrected visual acuity. Corneal thinning normally occurs in the infero-temporal or in the central cornea (2). Exceptional case of superior localizations have also been described (3,4). Keratoconus becomes evident normally during puberty, although the disease has also been found to develop earlier (5) and latter in life (6). It potentially progresses until the fourth decade of life, when it usually stabilizes (6). A study has determined that 50% of non-affected eyes of subjects with unilateral keratoconus will develop the disease in 16 years (7). If left untreated, keratoconus frequently progresses to formation of Descemet's tears (known as Vogt's striae) and corneal perforation, seriously threatening the vision. At this point, corneal transplantation is required to restore useful vision and saving the eye. Corneal Collagen Cross-linking is a treatment for keratoconus and other corneal ectasia which was developed first at the University of Dresden in 1998 (8). In this procedure ultraviolet (UV) light and riboflavin (vitamin B2) drops are used to strengthen the cornea's structure, to slow or halt the progression of keratoconus, preventing deterioration of vision and the need for corneal transplantation. Firstly experimented in porcine and rabbits corneas, the results showed that riboflavin soaked and UVA irradiated corneas were stiffer and more resistant to enzymatic digestion. Investigations also proved that the treated corneas contained high molecular weight polymers of collagen due to fibril cross-linking. Others, *in vitro* investigations, on human and porcine corneas examined the best treatment parameters for standard cross-liking, such as riboflavin concentration, intensity, wavelength of UVA light, and duration of treatment (9). Also, it has been proved that UVA irradiation is not harmful for the endothelium, if the corneal thickness is above 400 μm (10). After the laboratory tests, clinical results were also encouraging. The pilot study included 16 patients with progressive keratoconus that were treated with cross-linking. All of them stopped the progression after treatment. About 70% had flattening of the steepest keratometry, decrease in average and maximum keratometric values and 65% had visual acuity improvement. No complications were reported (8).

After that, cross-linking became a worldwide used technique. Generally is applied by using Dresden protocol (8) requiring the removal of central 9 nm of corneal epithelium layer, followed by 30 minutes of riboflavin administration, subsequently, UVA light is applied for 30 minutes. The corneal epithelial layer is generally removed to increase penetration of the riboflavin into the stroma (11). During the UV light illumination, riboflavin acts further as a shield during irradiation to the cornea, protecting deeper ocular structures such as the endothelium, lens, and retina from UVA irradiances that are too high (12). Another important role of riboflavin is to prevent corneal dehydration during exposure (13). The combination of riboflavin and UVA light creates 80-95% absorption into the cornea during

cross-linking depending on the concentration and the corneal thickness (12). Given the simplicity and minimal costs of the treatment, cross-linking treatment is also well-suited for developing countries (8). As the Siena Eye Study (14), later studies (15,16), investigated long-term effects of standard cross-linking.

The aim of this study was to evaluate the treatment of keratoconus disease with the cross-linking method in Albanian patients.

Methods

In our institution, namely the American Hospital in Tirana, cross-linking technique is applied from 2009. The patients presented with complains such as: progressive changes in refraction, changing frequently the glasses and not feeling comfortable with them, high astigmatism and myopia, are suspected for keratoconus. These patients were advised to undergo topographic examination with Pentacam instrument which is based on the Scheimpflug working principle, taking 12-50 images of the cornea at different angles using a rotating camera. Anterior and posterior corneal elevations are then measured using topographic analysis, providing useful information in keratoconus diagnostic and grading the severity of keratoconus (15). IV-th grade of keratoconus with pachymetry lower than 360μm, Vogt's striae or corneal hydrops are immediately advised to undergo corneal transplant procedure. The patients, diagnosed in stage 1-3 of keratoconus, with no corneal changes are followed for six months to check the evidence of keratoconus progression and in this case, are advised to undergo cross-linking procedure. Others, already presenting clear evidence of progression in comparison of earlier topographic examination are immediately advised to the cross-linking procedure.

Overall, there were 81 eyes (75 patients) with progressive keratoconus that were included in this study. Average age was 23.5 years (the youngest patient 15 years old and the oldest 38 years old).

A rotating Scheimpflug camera (Pentacam HR, Oculus) was used to diagnose and follow-up the keratoconus before and post cross-linking treatment. Corneal elevation, pachymetry and keratometry were the parameters measured.

The inclusion criteria were as follows: progression of keratoconus resented as an increasing in maximum keratometry (steepest keratometry) at least 0,5 D in six months, preoperative corneal thickness above 400 μm, no corneal scar, no previous corneal surgeries.

Patients underwent cross-linking procedure according to Dresden protocol. After the cross-linking procedure, the patients were followed with three dimensional corneal topography (Pentacam HR Oculus). The parameters followed included: keratometry steepest, flattest, average, corneal pachimetry average and thinnest, uncorrected and best-corrected visual acuity. The follow-up time was 12 months.

Statistical Analysis of the data was performed using SPSS (Statistical Package for Social Sciences, version 20.0). For all numerical variables, measures of central tendency and dispersion were calculated. For variables following the normal distribution, arithmetic means and the respective standard deviations were calculated. Differences between groups were calculated with student's t-test. Correlation between variables was analyzed through coefficients of Kendal's tau. P-values ≤ 0.05 were considered as statistically significant.

Results

The parameters before the cross-linking treatment are presented in Table 1.

Table 1. Parameters before the cross-linking treatment

Variables	Average±SD	Minimum	Maximum
Pak_central_preop	467.09±33.70	341	554
Pak_thinnest_preop	444.83±33.52	313	526
Kerat_flatest_preop	46.68±4.41	39.2	62.4
Kerat_steepest_preop	50.58±4.93	42.3	67.9
Kmax_preop	56.46±6.30	45.4	78.2
UCVA_preop	0.20±0.18	0.01	1
BCVA_preop	0.41±0.21	0.01	1

The comparison of medium values of PAK after cross-linking is exhibited in Table 2.

Table 2. Comparison of medium values of PAK (central corneal thickness) after cross-linking

Comparison groups		Average±SD	P-value*
Comparison couple I	Pak_central_preop	466.99±34.14	<0.001
	Pak_central_1 week	457.54±32.26	
Comparison couple II	Pak_central_1 week	457.54±32.26	0.004
	Pak_central_1month	452.47±34.00	
Comparison couple III	Pak_central_1month	452.26±33.93	0.169
	Pak_central_3month	450.39±35.50	
Comparison couple IV	Pak_central_3month	450.48±35.27	0.970
	Pak_central_6month	450.53±34.12	
Comparison couple V	Pak_central_6month	450.53±34.12	0.541
	Pak_central_12month	451.25±34.19	
Comparison couple VI	Pak_central_12month	451.25±34.19	<0.001
	Pak_central_24month	445.05±36.57	
Comparison couple VII	Pak_central_24month	445.05±36.57	<0.001
	Pak_central_36month	440.61±35.65	

*Student's t-test for two couples

Analyzing the values through student's t-test for two couples, there is a statistically important difference between average values of PAK central before treatment and after the first week ($p<0.001$); the first week and the first month after treatment ($p=0.004$); the 12^{th} month compared to the 24^{th} month ($p<0.001$) and the 24^{th} month compared to the 36^{th} month after cross-linking ($p<0.001$), when a significant reduction in average values of central pachymetry is evident (PAK central) (Table 2).

Based on student's t-test for two couples, there is a statistically important difference between the average values of PAK thinnest before cross-linking and one week after cross-linking ($p<0.001$), first week and first month after procedure ($p=0.015$), first month and third month ($p=0.002$), 12^{th} and 24^{th} month ($p=0.030$), 24^{th} and 36^{th} month after cross-linking ($p=0.012$), resulting in a significant reduction of medium values of PAK thinnest (Table 3).

Table 3. Comparison of medium values of thinnest pachymetry (PAK thinnest) after cross-linking

Comparison parameters		Average±SD	P-value
Comparison couple I	Pak_thinnest_preop	444.99±34.00	<0.001
	Pak_thinnest_1week	436.06±34.86	
Comparison couple II	Pak_thinnest_1week	436.06±34.86	0.015
	Pak_thinnest_1month	431.52±33.74	
Comparison couple III	Pak_thinnest_1month	431.11±33.70	0.002
	Pak_thinnest_3month	427.39±34.75	
Comparison couple IV	Pak_thinnest_3month	427.63±34.58	0.449
	Pak_thinnest_6month	428.72±35.51	
Comparison couple V	Pak_thinnest_6month	428.72±35.51	0.432
	Pak_thinnest_12month	430.25±35.28	
Comparison couple VI	Pak_thinnest_12month	430.25±35.28	0.030
	Pak_thinnest_24month	426.43±37.73	
Comparison couple VII	Pak_thinnest_24month	426.43±37.73	0.012
	Pak_thinnest_36month	422.40±35.82	

Based on the student's t-test for two couples, there is a statistically important difference between the medium values of steepest keratometry before cross-linking and one week after cross-linking (p=0.005); first month and 3rd month after procedure (p<0.001); 3rd month and 6th month (p<0.001); 6th month and 12th month (p<0.001); 12th month and 24th (p<0.001); and 24th and 36th month (p<0.001), where a statistically important reduction is seen in medium values of steepest keratometry. There is no evidence of a statistically important reduction between first week and first month after treatment (p=0.596) (Table 4).

Table 4. Comparison of Steepest Keratometry after cross-linking

Comparison of keratometry		Average±SD	P-value
Comparison couple I	kerat_steepest_preop	50.70±5.04	0.005
	kerat_steepest_1week	51.19±5.12	
Comparison couple II	kerat_steepest_1week	51.19±5.12	0.596
	kerat_steepest_1month	51.07±5.15	
Comparison couple III	kerat_steepest_1month	51.03±5.05	<0.001
	kerat_steepest_3month	50.22±4.80	
Comparison couple IV	kerat_steepest_3month	50.18±4.78	<0.001
	kerat_steepest_6month	49.57±4.93	
Comparison couple V	kerat_steepest_6month	49.57±4.93	<0.001
	kerat_steepest_12month	48.71±4.51	
Comparison couple VI	kerat_steepest_12month	48.71±4.51	<0.001
	kerat_steepest_24month	47.75±4.43	
Comparison couple VII	kerat_steepest_24month	47.75±4.43	<0.001
	kerat_steepest_36month	46.34±4.39	

Based on the student's t-test for two couples, there is a statistically important difference between the average values of maximal keratometry (Kerat_kmax) before cross-linking and one week after cross-linking (p=0.008); first and third month (p<0.001); third month and 6th month (p<0.001); 6th month and 12th

month (p<0.001); 12th month and 24th month (p=0.002); and 24th and 36th month (p<0.001), where a statistically important reduction is seen in average values of maximal keratometry (Kerat_Kmax). There is no evidence of a statistically important reduction between first week and first month after treatment (p=0.917) (Table 5).

Table 5. Comparison of maximal keratometry after cross-linking

Parameters		Average±SD	P-value
Couple I	Kmax_preop	56.57±6.42	000.1
	Kerat_Kmax_1 week	57.09±6.23	
Couple II	Kerat_Kmax_1 week	57.09±6.23	0.917
	Kerat_Kmax_1 month	57.11±6.19	
Couple III	Kerat_Kmax_1 month	57.07±6.07	<0.001
	Kerat_Kmax_3 month	56.00±5.90	
Couple IV	Kerat_Kmax_3 month	55.94±5.89	<0.001
	Kerat_Kmax_6 month	55.08±5.90	
Couple V	Kerat_Kmax_6 month	55.08±5.90	<0.001
	Kerat_Kmax_12 month	53.14±5.15	
Couple VI	Kerat_Kmax_12 month	53.14±5.15	0.002
	Kerat_Kmax_24 month	52.24±5.00	
Couple VII	Kerat_Kmax_24 month	52.24±5.00	<0.001
	Kerat_Kmax_36 month	50.53±4.80	

Based on the student's t-test for two couples, there is a statistically important difference between the average values of UCVA (uncorrected visual acuity) in first month and third month (p<0.001) and first month (p=0.052); 3rd month and sixth month (p=0.214); sixth month and 12th month (p=0.135); and 12th month and 24th month (p=0.157) (Table 6).

and 24th month and 36th month (p=0.002), where a statistically important increasing is seen in average values of UCVA. There is no evidence of statistically important changes between first week

Table 6. Comparison of values UCVA (uncorrected visual acuity)

Comparing parameters		Average±SD	P-value
Couple I	UCVA_preop	0.19±0.18	0.754
	UCVA_1week	0.22±0.60	
Couple II	UCVA_1week	0.22±0.60	0.052
	UCVA_1month	0.23±0.59	
CoupleIII	UCVA_1month	0.17±0.14	0.001
	UCVA_3month	0.21±0.17	
Couple IV	UCVA_3month	0.22±0.17	0.214
	UCVA_6month	0.23±0.18	
Couple V	UCVA_6month	0.23±0.18	0.135
	UCVA_12month	0.25±0.18	
Couple VI	UCVA_12month	0.25±0.18	0.157
	UCVA_24month	0.26±0.19	
Couple VII	UCVA_24month	0.26±0.19	0.002
	UCVA_36month	0.29±0.18	

Table 7. Comparison of BCVA (best corrected visual acuity) after cross-linking

Comparing parameters		Average±SD	P-value
Couple I	BCVA_preop	0.41±0.20	<0.001
	BCVA_1week	0.27±0.19	
Couple II	BCVA_1week	0.27±0.20	<0.001
	BCVA_1month	0.33±0.20	
Couple III	BCVA_1month	0.33±0.20	<0.001
	BCVA_3month	0.43±0.20	
Couple IV	BCVA_3month	0.43±0.20	<0.001
	BCVA_6month	0.51±0.19	
Couple V	BCVA_6month	0.51±0.19	<0.001
	BCVA_12month	0.57±0.18	
Couple VI	BCVA_12month	0.57±0.17	<0.001
	BCVA_24month	0.60±0.17	
Couple VII	BCVA_24month	0.60±0.17	<0.001
	BCVA_36month	0.67± 0.15	

Based on the student's t-test for two couples, there is a statistically important difference between the average values of BCVA after cross-linking for all the comparison period (Table 7).

There is a tendency of continuous increasing of BCVA especially starting six months after procedure and continuing even after three years with 2/10 (Snellen chart).

Discussion

The main parameters which define the topographic corneal shape are the radius of corneal curvature. Generally, two of them, perpendicular to each-other, are used to topographically characterize a certain cornea (the flattest and the steepest keratometry). Another keratometry value, corresponding to the apex of the cone or the point of maximal corneal elevation is recorded in Pentacam examination referring as maximal keratometry (Kmax).

As the Siena Eye Study (14), later studies (15,16), investigated long-term effects of standard cross-linking. Three hundred and sixty-three eyes were treated and monitored over four years, producing reliable long-term results proving the efficacy of the procedure in terms of long-term stability of the cornea by halting the progression of keratoconus, and proving the safety of the procedure (14,17-19). The use of cross-linking was also employed in other forms of corneal ectasia, even in iatrogenic after refractive surgery (20).

In our study, the flattest, steepest and maximal radius of the cornea was taken from the anterior curvature sagittal map of the cornea. The corneal thickness values, central and thinnest, were taken also from this map. With the advancement of keratoconus: steepest, flattest and Kmax increase. Central and thinnest values of pachymetry decrease. Central pachymetry values continue to decrease until three years after cross-linking. This phenomenon is known as "Corneal shrinking". Cornea stiffens and becomes stronger, opposing to the deforming tendency of the keratoconus. Thinnest pachymetry values follow the same tendency as central pachymetry. They continue to decrease until three years after cross-linking. Cornea stiffens and becoms stronger, opposing to the deforming tendency of the keratoconus. Flattest keratometry significantly reduces six months after cross-linking and continues to reduce even after three years (flattening 3.8 D). Steepest keratometry significantly reduces six months after cross-linking and continues to reduce even after three years (flattening 3.36 D). Maximal

keratometry significantly reduces six months after cross-linking and continues to reduce even after 3 years (flattening 6 D). There is a tendency of stabilizing UCVA after cross-linking and even an increasing 1/10, three years after the procedure. Also, there is a tendency of continuous increasing of BCVA especially starting six months after the procedure and continuing even after three years with 2/10.

Conflicts of interest: None declared.

Conclusion

Cross-linking procedure seems effective in reducing corneal radius (flattest, steepest, maximal). Having a flatter cornea in a progressive keratoconus means that the progress of keratoconus is stopped and there is also a remodeling of its surface.
Remodeling the cornea also stabilizes visual acuity and even improves best spectacles visual acuity.

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Harmonised health indicators in the European Union: A brief introduction

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Abstract

To improve the public's health, policymakers have to know what the problems are and where changes need to happen. Here, health indicators can help by monitoring and describing health statuses and determinants. Therefore, health indicators are an important tool for effective policy making and health actions. Countries had established their own indicators, which introduces difficulties in comparing the data within the European Union (EU).
A narrative literature review was conducted to gain an overview of the development of harmonised health indicators in the EU countries over the past decades. The development of harmonised health indicators in the EU started over two decades ago. Since then, different health programmes and projects regarding European Community Health Indicators (ECHI) have been developed, introducing 88 core indicators, but not all indicators have been established yet. Effort is needed to implement and improve ECHI further. The main work is done by projects instead of long-term approaches. The implementation of harmonised indicators took a long road and did not achieve its goal yet. Formulating the right indicators for an overview of the health status and health determinants is a dynamic process and thus, effort is needed, to keep ECHI updated.

Keywords: ECHI, European Union, harmonisation, health indicators.

Introduction

Health is of great importance in the daily life. Whether people can enjoy their life depends on the health status, fulfil their duties and manage their work-life (1). Thus, the objective of public health policies is not only to maintain, but to improve the health of citizens (2). To develop effective health policies and other measures for health, and to assess their impact, reliable information about the public's health status are needed, which can be drawn from indicators (2,3). A health indicator is a measurement of a certain health aspect in a group or country, ranging from measures of life-expectancy, mortality and rates of certain diseases to determinants of health, such as smoking (4). Verschuuren et al. (2) state that information for health measures can only be reliably drawn, if "health indicators [are] based on representative population-based health data and [are] comparable between points in time, countries and areas". Such comparable data across the European regions exist for non-communicable diseases since the late 1970s, when the World Health Organisation (WHO) started the *Health for All Programme* and collected data in the HFA database (5). Additionally, health data of European countries was also collected by the Organisation for Economic Co-operation and Development (OECD), as well as from Eurostat, which is the most decisive health statistics collection of EU Member States (MS). However, these organisations implemented different methods of collecting and calculating data and thus, those are not generally comparable (6). Furthermore, harmonised and comparable health data, which aimed not only to be descriptive, but to make improvements of public health measures possible, were seldom (5).

Therefore, the establishment of harmonised health indicators in the Member States is creating a fundamental basis for health monitoring and reporting within the European Union (EU) and EU-wide public health policies (2,5). Because relevant health indicators, which are comparable between the EU Member States, play a huge role in identifying and overcoming health challenges (7) and resulting improving the overall health of the EU citizen, this paper aims to describe how the EU established comparable health indicators, why they should be comparable, and where there is still a need for improvement.

Methods

A narrative literature review was conducted to gain an overview over the development of harmonised health indicators in the EU over the past decades. The aim was to follow the actions taken of the EU over the past and thus, to understand where the impact, but also possible shortcomings lie and to investigate what has been achieved and where still some needs exist.

Databases such as PubMed, Science Direct, but also Google Scholar were searched for different keywords, using the Boolean operators «AND» and «OR». Key terms that were searched for included "health indicators", "health statistics", "ECHI (M)","health data", "health measures", "data needs", "health status", "European regions" and "evidenced-based actions/policies". Furthermore, the suggested MeSH terms were revised to include also controlled key terms.

Additionally, suggestions of the journals and databases were considered, as well as reference lists were scanned to complement the research. Journal articles, book chapters, reports, published papers of the European Commission, as well as the websites of the WHO and the EC were included as reference material. To acquire insight into the history of harmonised indicators in the European Union, articles from 1990 until 2015 were included. Reference material published in languages other than English has been excluded. After the titles and abstracts were scanned to investigate whether the identified articles matched with the research questions, the full articles were reviewed and a final selection was made.

Results

The foundation for harmonised health indicators and EU wide health monitoring was established in the 1990s (2). The European Parliament asked for steps towards this goal and the European Commission initiated the first Health Monitoring Programme in 1993 (2). Included in this Programme were also projects for developing joint health indicators. Nevertheless, the more extensive work on harmonised health indicators started with the basis of the Amsterdam Treaty (8) and thus, a commission working group was created which led to the presentation of a report on health monitoring and indicators in 1998 (5). Because the need of comparable health indicators received increased awareness, the European Community Health Indicators have been introduced to provide a frame for uniform data collection and to fill information gaps (9). The work on ECHI proceeded with different Health Programmes' of the European Commission (9), of which currently the third health programme - running from 2014 until 2020 - is in place (10).

The first version of the ECHI shortlist and long-list with core indicators was introduced in 2005 and two updated versions of the shortlist followed in 2008 and 2012 (11). Even though other data collections exist, ECHI is established especially as tool for policy support (2). ECHIM followed the first two versions of the ECHI projects and further tried to identified which health indicators should be included at EU level, what kind of data is needed to establish these health indicators and how these can be implemented by different actions (6). Moreover, ECHIM tries to build a bridge between the developed core indicators (ECHI) and a way of implementation in the Member States (6). The current shortlist of 2012 consists of 88 indicators (11), which aim to describe the overall health in its different facets and are clustered in five main areas, including demographic and socioeconomic factors, the health status, the determinants of health, and health interventions which are clustered in health services and health promotion (5,12). In 2013, ECHI was renamed the European Core Health Indicator (12).

For implementing ECHI in the Member States, two main activities are of great importance. On the one hand, the MS have to map and improve the data availability for their countries, which are provided for the ECHI indicators. On the other hand, these ECHI indicators should also be used in the nations themselves for monitoring and reporting the national status (6). The Joint Action for ECHIM has a big share in the implementation and in the improvement of comparability of the health indicators within the EU. Data that are comparable between Member States and are collected according to the ECHI shortlist are provided at the HEIDI Data Tool, which is to a great extent based on collected data of Eurostat and other databases. Moreover, there is an increased cooperation between the WHO, OECD and the EC with regard to health data and statistics (2). All the effort taken for comparable health data between the MS aims at evidenced-based policy making, as comparable data draws a comprehensive picture about current situations and allows identifying best practices (6). Nonetheless, unified data collection and comparability also adds value within a national context between regions and helps to improve the national health policies and not only the EU-wide policies (8).

Even though the history of unified health indicators in the EU reaches back for over two decades, the work on harmonised health indicators keeps going. While, for instance, data on mortality is routinely collected and mostly available in all countries, data for ECHI indicators on health care quality were only available in half of the Member States (13). In 2012, no country had reached to implement all ECHI indicators and Denmark and Finland performed best with 84% of indicators implemented. The average data availability in the EU-27 was 76% with every MS scoring more than half of all ECHI indicators. However, a gap between the availability in Eastern and Southern Europe compared with the availability in Northern and Central Europe exists. In general there is a significant difference between availability

of data in Member States, as well as a crucial difference between availability of the different types of indicators (6). However, Aromaa (5) suggested that the complete indicator system should have been adapted in most countries by 2014.

Discussion

In the following part two different sides of this paper are addressed. On the one hand, it analyses ECHI and the effort it will take further. On the other hand, it examines the reference material used for this overview.

The ongoing effort of ECHI

In the past two decades much has happened regarding harmonised health indicators and unified health monitoring in the EU. It became clear that comparable health indicators add value in different ways to the development of evidenced-based policy and thus, the improvement of the overall health. Many different stakeholders identify the importance of comparable indicators, which resulted in increased cooperation between different stakeholders, including the European Commission, the WHO, OECD, as well as the Member States. This increased cooperation leads to better and more comprehensive outcomes (2). The European Core Health Indicators have been developed over the past years and different funded projects and working groups tried to support the implementation and constantly improved them. However, even though it has been worked on ECHI for years, efforts with regard to maintaining a sustainable base with high quality data for policy makers are constantly needed (2). This concerns the European Commission and the EU level as well as the national level. Moreover, it can be criticised that the main effort for harmonised health indicators is taken by projects, which only last for a couple of years and no sustainable ad horizontal approach regarding health indicators has been taken yet. Thus, the constant improvement and development of ECHI depends on the established projects for that period of time (2).

A different aspect about the impact of comparable data, which was not addressed in the literature, is the chance of achieving improvements only due to 'peer pressure' instead of regulations and policies. When countries are compared in different health areas, to see how they are doing, it is more satisfying to be one of the top countries, than a subordinated country. This can lead to increased action on a field by the certain country, to improve the performance and to become as good as other countries. Thus, comparable indicators and health statistics have the ability to point on weaknesses and to make countries improving them without regulating it by hard law.

Furthermore, in 2012, there was still a substantial lack of the national implementation of ECHI. Tuomi-Nikula et al. (6) reason the slowed-down progress on the one hand with problems on the part of the EU with a lack of leadership. On the other hand, also a lack of commitment and funding personal at the national level hindered a quick implementation process (6). In addition, more work needs to be done, as by far not all core health indicators have been implemented yet.

Flaws of reference material

While studying the literature, it became clear that most documents have been published between 2008 and 2013 with regard to the implementation of ECHI, so before the third health programme came into place, which is why it is difficult to make a statement on how far ECHI is implemented in the MS in 2015. Therefore, the estimation of Aromaa (5) that most indicator systems are implemented by 2014 cannot be checked. More comprehensive reports on the overall implementation would be useful. However, some country focused articles exist, such as about the implementation status of the Netherlands, which indicates that in general, there is sufficient data availability (14).

A different point of interest would have been to investigate what was expected 20 years ago from the introduction of health indicators and what has been realised by now of these expectations.

Nonetheless, there was no general article found regarding this issue, despite the different objectives that have been named in different papers and developed over the past years. Therefore, future research should investigate what harmonised health indicators have been expected to change and in how far this change occurred 20 years later and what would still be missing.

Moreover, it stood out that, with regard to ECHI and the topic of health indicators, especially in the European context, it is mainly a concern of the same author groups. However, this is mainly because those authors belong to the joint action for ECHIM professionals.

Conclusion

This paper aimed at giving a brief overview over the ECHI development, what has been done and what is still needed. The implementation of harmonised indicators has come a long way and did not achieve the goal yet. However, it became clear that the health indicators should be improved constantly, because formulating the right indicators for an overview of the health status and health determinants is a dynamic process and thus effort is needed, to keep ECHI updated. Furthermore, the whole process of ECHI is a much more complex approach, with more different measurement tools and working groups than described in this paper, as it was the aim to simplify the understanding of the core indicators' objective.

Moreover, health indicators are an important tool for evidenced-based health policy, as well as for achieving improvement in the public's health. Even though there are implementation gaps and no country has established the full ECHI indicator set yet, all Member States achieved to implement more than half of the indicators. Hence, it can be stated that the EU is on its way to harmonised health indicators. Additionally, the importance of cooperation between the different stakeholders in this topic was shown.

Conflicts of interest: None declared.

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Prescription patterns of drugs used for peptic ulcer disease in primary health care in Albania during 2004-2014: International comparisons

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Abstract

Aim: To evaluate the out-of-hospital anti-ulcer drugs use in Albania (national level) during 2004-2014 along with international comparisons on drugs use.
Methods: All data were collected from the Albanian Health Insurance Institute (HII) and analyzed reflecting the ambulatory and outpatient use for the period 2004-2014. The data about the consumption of drugs were expressed as a number of Defined Daily Dose (DDDs) /1000 inhabitants/day. For all the period under study 2004-2014, data of import and domestic production of drugs were collected and analyzed, which represent the real consumption of drugs in Albania. These data were subsequently included in a comparative analysis with the utilization data according to the HII.
Results: The most prescribed drug was ranitidine: 0.97 – 0.25 DDD/1000 inhabitants/day, while the consumption of omeprazole is 0.18 -0.19 DDD/1000 inhabitants/day respectively 2004-2014. The reimbursement scheme provides a quite poor coverage of necessary alternatives of the proton pump inhibitors that are used for the treatment of the ulcerous disease. The reimbursement scheme offers only omeprazole. However, the consumption of omeprazole under the scheme is in much lower levels compared to the real data of omeprazole consumption coming from import figures. On the other hand, a consistent part of the sales of omeprazole is out-of-pocket expenditure.
Conclusion: There exists a significant decrease in anti-ulcer drugs use from HII covering, while there is a significant increase in their use from out-of-pocket expenditure during 2004-2014. The total consumption of these drugs is very low in comparison with the developed countries in Europe.

Keywords: anti ulcer drugs, DDD/1000 inhabitants/day, drug utilization.

Introduction

An "ulcer" is an open sore. The word "peptic" means that the cause of the problem is due to acid. Peptic ulceration commonly involves the stomach, duodenum, and lower oesophagus. The most important symptoms that ulcers cause are related to bleeding. The two most important causes of ulcers are infection with *Helicobacter pylori* and a group of medications known as NSAIDs.

Healing can be promoted by general measures, stopping smoking and taking antacids and by antisecretory drug treatment, but relapse is common when treatment ceases. Nearly all peptic ulcers will be treated with a proton pump inhibitor (PPI). PPIs are powerful acid blocking drugs that can be taken as a pill or given in an IV. Sometimes duodenal ulcers (not gastric ulcers) will be treated with H2 blockers. H2 blockers are another type of acid reducing medication.

Our study tries to evaluate the level of access to and benefit of the ulcerous patients from the drug reimbursement scheme. It does also evaluate the level of coverage by the scheme of the necessary alternatives for the treatment of peptic ulcer disease during 2004-2014 in Albania.

Methods

The data were obtained from the Health Insurance Institute (HII) (1). All data were collected and analyzed reflecting the ambulatory and outpatient use for the period 2004-2014. The analysis included the total number of prescriptions, and quantities of drugs. The data about the population were obtained from the Institute of Statistics (INSTAT) (2). The data about the consumption of drugs were expressed as a number of Defined Daily Dose (DDDs)/1000 inhabitants/day. All drugs were classified by groups of Anatomic Therapeutic Chemical Classification (ATC).

Data on the levels of morbidity

From the database of HII there were extracted the general number of patients reported for each diagnose, for each year. Following, there were calculated the respective levels of annual morbidity (based on the respective code-diagnoses) for 1000 inhabitants.

Data on real consumption (import and domestic production)

For all the period under study 2004-2014 there were collected and analyzed data from the import and domestic production of the drugs (3), which represent the real consumption of drugs in the country. It was noted that the increase in consumption from one year to another were small, e.g. the consumption from 2011 to 2014 (i.e. 4 years) was increased by only 2.57%. Consequently, in order to obtain an updated study, there were chosen the data of import and domestic consumption only for the last three years, 2012, 2013, 2014, and those were involved in a comparative analysis with the equivalent consumption data according to HII. In order to minimize the effect of variations consumption-inventory balances from one year to another, it was calculated and put to analysis the annual average value of the three chosen years (on one hand that of the import and domestic consumption, and on the other hand that of HII).

Presentation of the results and statistical elaboration

The database of HII was modified in Microsoft Office Excel 2007, whereas the statistical elaboration of the obtained results was conducted with the statistical package StatsDirect (version 2.7.2.). A descriptive statistics was used to report all data on drugs consumption and the results obtained were displayed in tabular form as well as through the histogram method.

Average annual values of consumption in the country level and for each district were used as a basis to generate the overviews and the graphics that illustrate the trends of consumption for each class of drugs during the 10-years period 2004-2014. The linear regression model was used to evaluate the trends of consumption of drugs relative to the time. A value

of $p \leq 0.05$ was considered as significant.
In order to asses if there exists a correlation statistically significant between the level of consumption of drugs and the level of morbidity, it was applied the Spearman correlation (with a significance level of ≤ 0.05).

Results

The anti-ulcer agents included in the reimbursement list during these years were cimetidine, ranitidine and omeprazole. The most prescribed drug was ranitidine: 0.97-0.25 DDD/1000 inhabitants/day. At last comes cimetidine: 0.07-0.00 DDD/1000 inhabitants/day, while the consumption of omeprazole is 0.18-0.19 DDD/1000 inhabitants/day, respectively for 2004-2014.
The data of ulcerous morbidity show that there exists a statistically significant correlation between this morbidity and the trend in consumption of anti-ulcer drugs (p=0.0048) (Figure 1).

Figure 1. Consumption of anti-ulcer drugs in the national level (DDD/1000/inhabitants/day, 2004-2014) versus ulcerous morbidity (number of cases/1000/inhabitants)

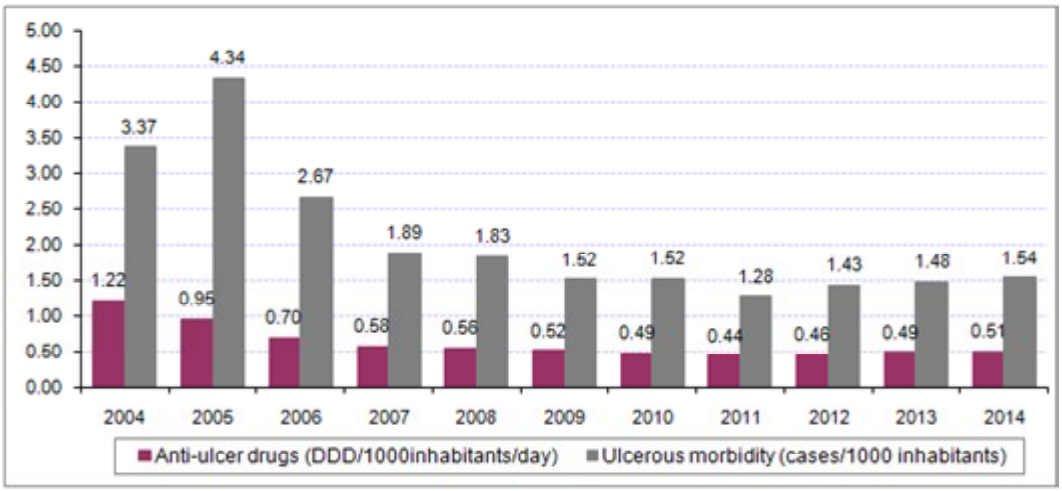
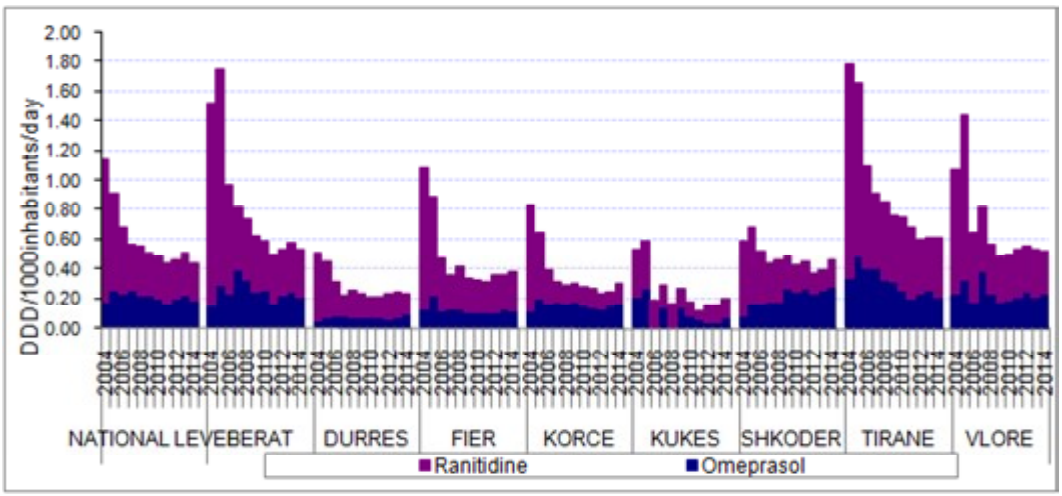


Figure 2. Comparative consumption of anti-ulcer drugs at the national level and in several country regions (DDD/1000 inhabitants/day)



The annual average value of anti-ulcer drugs consumption (PPI) and the annual average value of consumption of each PPI alternative, as a consumption from import (real actual consumption)

versus the consumption reported by HII (DDD/1000 inhabitants/day), are presented in Figures 3 and 4 respectively.

Figure 3. Annual average value of anti-ulcer drugs consumption (PPI): Consumption from import (real consumption) versus consumption from HII

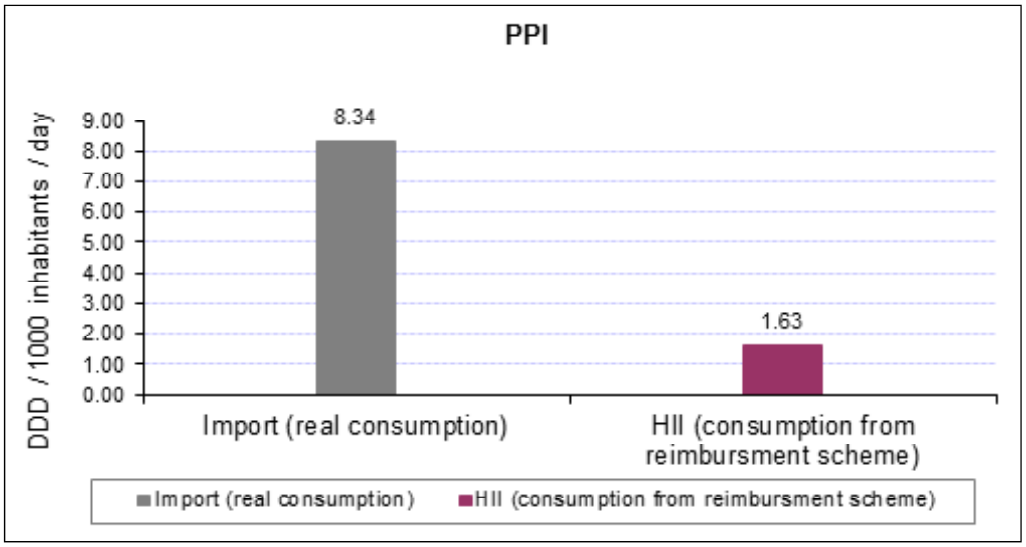
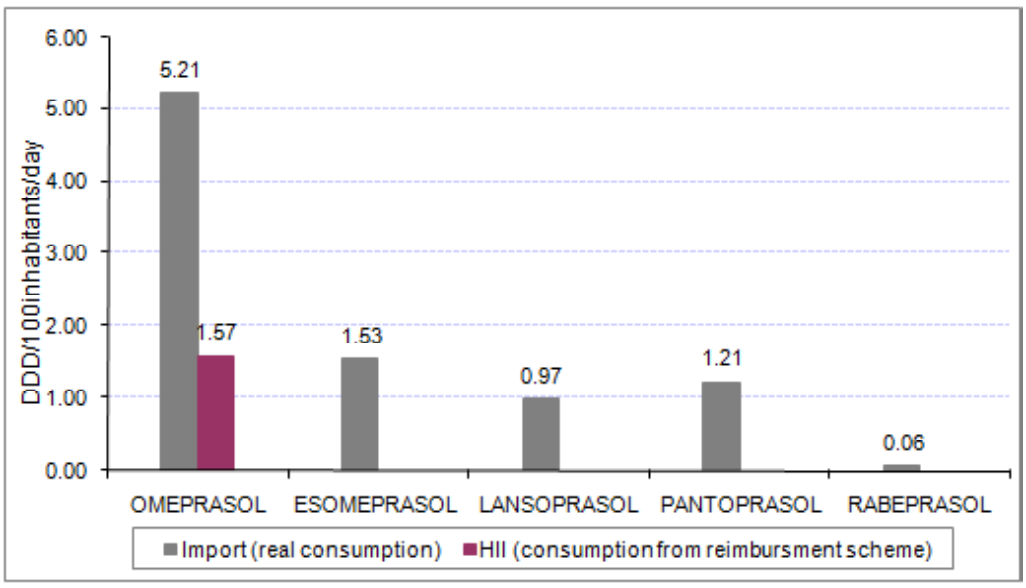


Figure 4. Annual average value of consumption of each PPI alternative: Consumption from import (real consumption) versus consumption from HII



Discussion

Figure 1 presents the consumption of anti-ulcer drugs (DDD/1000 inhabitants/day) versus ulcerous morbidity (cases/1000 inhabitants). There can be noted a visible discrepancy between the consumption of anti-ulcer drugs and ulcerous morbidity, indicating that only a minor part of the patients with

ulcerous disease actually benefit from the reimbursement scheme.

On the other hand, the trend of the ulcerous morbidity itself undergoes refraction, with the peak of increase in 2005, followed by a decrease in next years. Such decrease in the morbidity could partially reflect the healed cases, but this would be limited only to the years 2005-2008. In subsequent years, 2009-2014, the data on the morbidity show only a small increase.

Figure 2 shows that the consumption of anti-ulcer drugs varies visibly between the regions. Figure 3 presents a comparative consumption on the national level as well as in different regions, and clearly indicates an explicit variation in quantities consumed for each molecule. A common finding is that there is consumed a lot more ranitidine rather than omeprazole, while from the pharmacological perspective, PPI are superior compared to antiH_2 in the cure of ulcerous morbidity (4). Only during the last years (2008-2014), there can be noted a shift in consumption, with decrease antiH_2 receptors drugs and an increase of PPI, which is reasonable considering that PPI have the highest efficacy in the reduction of gastric hyperacidity.

The inhibition of hydrochloric acid secretion from PPI, although with a slow commencement of action, is more powerful and sustainable compared to H2 antagonists: they can be given once a day (4) considering the prolonged inhibition of the acid production. As a result, the cure of peptic esophagitis and of ulcerous disease with PPI is superior compared to with other anti-ulcer drugs.

The evident differences in the consumption of these drugs amongst different regions are difficult to explain with any significant changes in the ulcerous morbidity. This expressed lack of uniformity raises questions as to why this morbidity is so poorly cured e.g. in Kukës, Shkodër, Korçë, Durrës, compared to Tirana, Berat or Vlora, while instead the gastro-intestinal symptoms consist in the most common in the daily medical practice, with a prevalence of usually over 40% for persons over 75 years-old (5,6,7).

Figures 3 and 4 put emphasis on the poor coverage by the scheme of the necessary alternatives in the cure of peptic morbidity. The reimbursements scheme offers only omeprazole. Another issue which can be raised by analyzing these graphics is why the consumption of omeprazole under the scheme is such in lower values compared to the real consumption of omeprazole. This indicates that even this alternative, although covered by the scheme, is actually taken in large scale without prescription.

According to HII, omeprazole is reimbursed only for ulcerous disease and gastroesophageal reflux disease certified through endoscopic examination and the duration of the treatment is 4-6 weeks. After 4-6 weeks, the patient should re-perform the endoscopy in order for the family doctor to have the right to repeat the prescription.

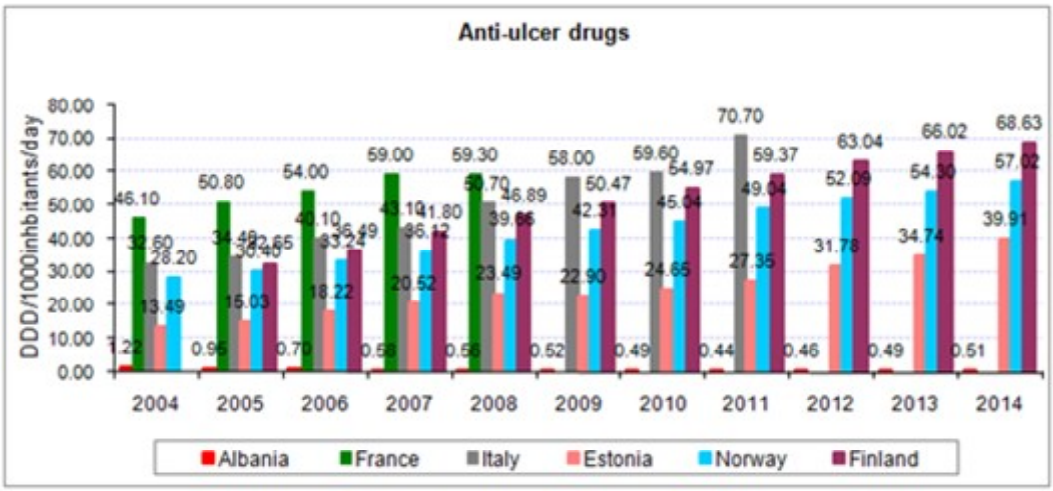
It is comprehensible that in a similar situation, the patient is almost conditioned to obtain the drug directly in the pharmacy by avoiding the consultation with the family doctor.

International comparison of consumption

As shown in Figure 5, the consumption of anti-ulcerous drugs in Albania, as compared to other countries, is very low (consumption values presented for all countries, including Albania, are the official values as referred by the respective reimbursement systems) (8-21). Consumption values which are the highest and show an increase year after year in all these countries are those of PPI. For example, in Italy, PPI during 2014-2009 is reported at 25.9-52.2 DDD/1000 inhabitants/day, whereas anti-H2 drugs at 2.8-1.8 DDD/1000 inhabitants/day (7). In 2004, in Italy (22), the consumption of omeprazole was 10 DDD/1000 inhabitants/day, whereas that of ranitidine 2.6- DDD/1000 inhabitants/day. There is a noticeable progressive growth of omeprazole consumption. In France, PPI: 39-52.1 DDD/1000 inhabitants/day, anti-H2: 2.4-1.7 DDD/1000 inhabitants/day (2004-2008) (6). A significant growth in PPI-consumption is noticed, especially in recent years. In Australia, the consumption of omeprazole indicates values of 278.9-220.8 DDD/1000 inha-

bitants/day (2002-2006); the decrease of omeprazole consumption is explained by the launch of other alternatives of the PPI class in the market and by the increase in their prescription, while the total consumption of the class grows (23).

Figure 5. International comparison in the consumption of anti-ulcer drugs class: Albania, France[8,9], Italy[10-12], Estonia[13-15], Norway[16-18], Finland [19-21]



Conclusion

There exists a significant decrease in anti-ulcer drugs use from HII covering, while there is a significant increase in their use from out-of-pocket expenditure during 2004-2014.

However the pattern of ulcerous morbidity corresponds with the pattern of anti-ulcer drugs use. The highest decrease was seen with the ranitidine use, while there is an increase in omeprazole use. The highest values of ulcerous morbidity were seen in 2005, which corresponds with the highest prescription of omeprazole in national level. The total consumption of these drugs remains very low in comparison with other European countries which probably reflect a significant part of consumption out of the reimbursement system.

Conflicts of interest: None declared.

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Comparison of two different methods used in the assay of Her 2/Neu (C-erb B2) in breast cancer

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Abstract

Aim: To investigate the comparison of two different methods used in the assay of Her 2/Neu (C-erb B2) in patients with breast cancer.
Methods: Hospital records of 150 cases diagnosed with breast cancer between 2010-2012 at the Oncology Clinic of Sakarya Hospital, Turkey, were retrospectively evaluated. The paraffin blocks obtained from the pathology laboratory were examined. In these patients, Fluorescence in situ hybridization (IHC) and Fluorescence in situ hybridization **(**FISH) methods were compared in the assay of Her 2/Neu. The number of the patients in whom IHC and FISH were negative and IHC and FISH were positive were identified.
Results: FISH was determined to be negative in all of the 112 patients in whom Her 2/Neu was determined to be negative through the IHC method. FISH was determined to be positive in 19 (95%) out of 20 patients in whom Her 2/Neu 2+ and 3+ were detected through the IHC method. FISH was determined to be positive in 11 (61.1%) out of 18 patients in whom Her 2/Neu 1+ was detected through the IHC method.
Conclusion: The results of this present study suggest that FISH method seems to be superior to IHC in determining the overexpression of Her 2/Neu. Besides, determining the overexpression of Her 2/Neu is of great importance in terms of the transtuzumab treatment in the patients with advanced-stage breast cancer who are not recommended to have a surgical resection.

Keywords: breast cancer, Her 2/Neu, fluorescence in situ hybridization, immunohistochemistry, trastuzumab.

Introduction

Breast cancer, which is the most prevalent malignancy throughout the world, is the second leading cause of death in women. HER-2/neu oncogene located in the chromosome 17q is the proto-oncogene which encodes the receptor of epidermal growth factor or the transmembrane tyrosine kinase growth factor receptor belonging to HER family (1,2). Due to the gene amplification 95% of the time (3,4), HER2/neu overexpression is observed in 20-30% of the cases suffering from breast cancer. Slamon DJ et al. were the first authors to discover a statistically strong and significant correlation between gene amplification and the periods of disease relapse and survival (5). Transtuzumab, as a monoclonal antibody, was powerfully positive in HER-2/neu and is effective in the patients with metastatic disease who don't respond to the chemotherapy treatment (1,6). There are a number of methods in determining HER-2/neu gene status in the tumour tissues. Immunohistochemistry (IHC) is the method used most frequently in the evaluation of gene overexpression. Fluorescence in situ hybridization (FISH), on the other hand, is a more recent technique which detects gene amplification in tumour tissues. Both techniques can be applied to formalin-fixed and paraffin-embedded tissues. The evaluation of HER-2/neu status through IHC is a more simple and practical method easily performed with lower costs in all the pathology laboratories. Yet, IHC testing may lead to several problems resulting from diverse antibodies, tissue processing and the interpretation of the results in various ways. FISH is quite an accurate method with a perfect sensitivity and specificity in determining HER-2/neu gene amplification. It has also a low interlaboratory variability thanks to a standardized threshold used for determining HER-2/neu gene amplification (7-9). Each detection performed on HER-2/neu gene amplification by using the FISH method is considered to be the best indicator to begin the transtuzumab treatment in the patients with invasive breast carcinoma (10). The FISH method is not preferred as a technique in many pathology laboratories due to its more technical and costly qualities (1). Our primary objective in this study was to compare the expression and amplification values of HER-2/neu gene between IHC and FISH methods.

Methods

In this present study, the hospital records of 150 cases diagnosed with breast cancer between 2010-2012 at the Oncology Clinic of Sakarya Hospital, Turkey, were retrospectively scanned. The paraffin blocks obtained from the pathology laboratory were examined.

In these patients, IHC and FISH methods were compared in the assay of Her 2/Neu. The number of the patients in whom IHC and FISH were negative and IHC and FISH were positive were identified.

All the analyses were performed using the SPSS for Windows (version 21,0; SPSS/IBM).

Results

The hospital records of 150 patients with breast cancer were documented retrospectively. 68 (45.3%) of the patients were premenopausal and 82 (54.7) of them were postmenopausal. The complete instruments for operation consisted of the Modified Radical Mastectomy (MRM) materials. 19 of the patients (12.7%) used OCPs (oral contraceptives), 6 (4%) of them had HRT (hormon replacement therapy), 13 of them (8.7%) had smoking habits and 1 of them (0.7%) consumed alcohol. The number of patients with medical record of breast cancer in their families was 13 (8.7%). The number of patients with the estrogen receptor positivity was determined to be 100 (66.7), whereas the number of those with the progesterone receptor positivity was 87 (58%), the number of patients with HER 2/Neu positivity was 30 (20%) (Table 1). A significant difference was found when the results of FISH and immunohistochemical Her 2/Neu were compared in the cases within our study group. FISH was found to

be negative in 112 cases in whom the immunohistochemical Her 2/Neu was evaluated as negative. FISH was positive in 11 out of 18 IHC positive patients, whereas FISH was positive in 3 out of 4 IHC positive patients, and FISH was evaluated as positive in all of the 16 patients in IHC positive patients (Table 2).

Table 1. Demographic, clinical and laboratory findings

Parameters	%
Premenopausal Patient	45.3
Postmenopausal Patient	54.7
Use of OCP	12.7
Use of HRT	4
Smoking	8.7
Alcohol Consumption	0.7
Breast Cancer within the family	8.7
Estrogen Receptor Positivity	66.7
Progesterone Receptor Positivity	58
Her 2 /Neu positivity	20

Table 2. Comparison of HIC and FISH Methods

IHC method	FISH method	Similarity %
Her 2/Neu negative patients number: 112	112 FISH -	100%
Her 2/Neu 1 + patients number: 18	11 FISH +	61%
Her 2/Neu 2 + patients number: 4	3 FISH +	75%
Her 2/Neu 3 + patients number: 16	16 FISH +	100%

Discussion

The results of this present study suggest that FISH method seems to be superior to IHC in determining the overexpression of Her 2/Neu. Besides, determining the overexpression of Her 2/Neu is of great importance in terms of the transtuzumab treatment in the patients with advanced-stage breast cancer who are not recommended to have a surgical resection. Transtuzumab (monoklonal anti-HER2 antibody) has been used in the treatment of breast cancers where HER2 receptor is over-expressed (11). There were numerous patients with the overexpression of HER2 protein and/or HER/2neu gene amplification who had benefited from the Transtuzumab treatment (12). In our study, FISH was determined as negative in all of the 112 patients in whom Her 2/Neu was negative by means of the IHC method. FISH was determined to be positive in 19 (95%) out of 20 patients in whom Her 2/Neu 2+ and 3+ were detected by means of the IHC method. FISH was determined to be positive in 11 out of 18 patients (61.1%) in whom Her 2/Neu 1+ was detected through the IHC method. In a number of studies in the literature, it is stated that with IHC there is no need

for 0 and +1 cases to be verified by means of the FISH method. However in this present study, it was concluded that +1 and +2 cases in particular required to be verified through the FISH method. In a study conducted in this matter, the FISH positivity rates in the cases immunohistochemically scored as 0,+1,+2 and +3 were found as 3.5%, 6.4%, 25.7%, and 81.5%, respectively. According to these results, considering the transtuzumab treatment, it was concluded that the FISH method could be aplied to 0 and +1 cases. The fact that the amplification rate determined in +3 cases was 81.5% suggested that the FISH method in this group of cases does not provide further information. It could be inferred from the literature that the FISH was a much more reliable method than the IHC method in determining the HER2/neu status and that more sophisticated measurements for quality control were required for more accurate immunohistochemical results (13,14). In the literature, the major reason for the discordance between FISH and IHC is considered in those +2 cases. In our study, an amplification by FISH was determined in 75% of the cases in whom +2 was found through the IHC. The concordance between FISH and IHC in +2 cases have been reported as 20-30% in the literature. Our findings in +2 cases were the same to the literature, in addition we could say that the cases in this group required to be confirmed through the FISH method. The amplification by FISH is positive in 100% of +3 cases by IHC. In the literature, the amplification by means of FISH is reported to be at a rate of 79-100% in +3 cases. According to the literature there is no need for +3 cases to be confirmed through the FISH method. In conclusion, it was inferred from this present study that FISH method should be aplied in +1 and +2 cases detected by the IHC method. However, FISH method isn't necessary to be aplied in +3 cases detected by the IHC method.

Conflicts of interest: None declared.

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Helicobacter pylori eradication experience with sequential treatment consisting of antibiotics which are used in conventional triple therapy

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Abstract

Aim: We aimed to investigate the eradication rate of the sequential treatment using the antibiotics which are used in classic triple therapy.
Methods: Ninety-two non-ulcer dyspepsia patients with positive Helicobacter pylori were included in this study. The presence of Helicobacter pylori was detected by histopathological examination of the biopsy specimen taken from the antrum and corpus. Our Helicobacter pylori eradication treatment protocol was 30 mg lansoprazole twice daily and 1g amoxicillin three times daily were given to the patients in the first 7 days, in the second 7 days 30 mg lansoprazole twice daily, 500 mg clarithromycin twice daily and 1 g amoxicillin twice daily. Six weeks after the end of the treatment, all of the patients were re-evaluated for Helicobacter pylori eradication by the urea breath test. Eradication was considered to be successful in patients with negative urea breath test.
Results: Thirty-four patients (37%) were male and fifty-eight (63%) were female. Mean age of the patients was 44±14 years. H pylori was eradicated in 89.1% of the cases.
Conclusion: Our study demonstrated that the method of nitro-imidazole-free sequential treatment was found to be a more successful method than the classic triple therapy. Given the success rate of eradication, we suggest a sequential treatment that consists of the antibiotics used in classic therapy which may be an important option in the first-line treatment.

Keywords: conventional triple therapy, Helicobacter Pylori eradication, sequential therapy.

Introduction

H pylori infection is one of the most significant morbidity and mortality reasons worldwide. Non-ulcer dyspepsia, peptic ulcer and gastric MALT-lymphoma are the diseases associated with H pylori, and eradication is recommended in these cases according to the recent guidelines published (1). In the literature, it is also reported to be associated with idiopathic thrombocytopenia and idiopathic iron deficiency (2,3). H pylori infection is decreasing in developed countries, whereas it is detected at very high rates in developing countries and the disease is acquired through oral-fecal route. Low socio-economic status and education level, living in a crowded house-hold, drinking infected water are among the important risk factors that cause infection. Although H pylori eradication is not a fully resolved issue, many studies provided regimens with higher rates of eradication success. Antibiotic resistance seems to be an important problem in the H pylori eradication, and updating the treatment regimens with new studies constantly becomes a necessity. Serious decline in the rate of success with the classic triple therapy, in parallel with the increasing clarithromycin resistance all over the world, gave birth to other treatment regimens (4). The success rate of these have risen to >90% by the use of nitro-imidazole with the sequential treatment rather than Clarithromycin (5).

In this present study, we aimed to investigate the eradication rate of the sequential treatment using the antibiotics which are used in classic triple therapy.

Methods

Ninety-two non-ulcer dyspepsia patients with positive Helicobacter pylori were included in the study. The presence of Helicobacter pylori was detected by histopathological examination of the biopsy specimen taken from the antrum and corpus. Our Helicobacter pylori eradication treatment protocol was 30 mg lansoprazole twice daily and 1 g amoxicillin three times daily were given to the patients in the first 7 days, in the second 7 days 30 mg lansoprazole twice daily, 500 mg clarithromycin twice daily and 1 g amoxicillin twice daily. Six weeks after the end of the treatment, all of the patients were re-evaluated for Helicobacter pylori eradication by the urea breath test. Eradication was considered to be successful in patients with negative urea breath test.

Results and Discussion

The demographic data of the patients and the eradication success rates are shown in Table 1. Thirty-four patients (37%) were male and fifty-eight (63%) were female. Mean age of the patients was 44±14 years. H pylori was eradicated in 89.1% of the cases.

Table 1. The demographic features of the patients and the H. pylori eradication success rate

Variable (n=92)	
Age (mean ± standard deviation)	44 ± 14 years
Gender (Male/Female)	34/58
H. pylori Eradication rate (n %)	89.1%

There are studies indicating that the H pylori eradication success may vary among ethnic groups (6). Although the eradication success rates with the classic triple therapy were reported as 80% approximately in the literature, this ratio is found even lower in Turkey in recent years (7,8).

In conclusion, our study demonstrated that the method of nitro-imidazole-free sequential treatment was found to be a more successful method than the classic triple therapy. Given the success rate

of eradication, we suggest a sequential treatment that consists of the antibiotics used in classic therapy, which may be an important option in the first-line treatment.

Conflicts of interest: None declared.

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Albanian Medical Journal – Instructions to authors

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References

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