Contents

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ORIGINAL ARTICLE

The First Evidence of Familial Lung Cancer in Latvia by Population Screening for Hereditary Cancer

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Summary
Introduction. Lung cancer is the major worldwide cause of tumour lethality. Recently, the role of heredity in lung carcinogenesis has become an important scientific target. While there is ongoing search for the respective gene mutations, it was suggested that presence of at least 2 first-degree relatives with lung cancer should reveal the familial lung cancer.
Aim of the study. We present the first documented evidence of familial lung cancer in Latvia by population screening data in order to improve the planning of early cancer diagnostics. Materials and methods. The investigation was designed as population screening for hereditary cancer. Family cancer history was obtained from 18642 adults in the Valka district (76.6% of the adult population). Data analysis and proband consultations were performed in Hereditary Cancer Institute. Hereditary cancer syndromes were diagnosed by clinical criteria.
Results. Familial lung cancer syndrome was diagnosed in 13 probands, but suspected familial lung cancer syndrome (sFLC) - in 93 probands, corresponding to the frequency 0.6% (95% confidence interval (CI) = 0.5-0.7%). The frequency of lung cancer among blood relatives was 25.5% (95% CI = 19.3-32.8%) in definitive and 17.2% (95% CI = 15.0-19.7%) in suspected syndrome groups exceeding significantly the cumulative risk for EU population. Number of affected generations was significant for sFLC. The age of cancer onset did not influence the frequency of cancer. The data about the cancer course are described. Conclusions. The high frequency of lung cancer in the identified pedigrees supports the role of familial factors in the lung carcinogenesis as well as the practical value of our diagnostic criteria helping to identify high-risk group for intervention measures to prevent lung cancer.
Key words: familial lung cancer, population screening.

INTRODUCTION

Lung cancer is the most common cancer in the world (Parkin *et al.*, 2005) and the leading cause of cancer death in Europe (Boyle and Ferlay, 2005) and the whole world (Nitadori *et al.*, 2006) due to its frequent occurrence and grave prognosis implying 5-year-survival of 15% in the case of best available treatment. Tobacco smoking is the main risk factor for lung cancer causing approximately 90% of the lung cancer cases. Lot of scientific efforts has been applied to the analysis of tobacco-initiated lung carcinogenesis. Diminishing of the smoking frequency in the population is an effective measure in lung cancer prevention causing concentration of organizational and informative efforts in this direction. Thus, studies of the hereditary factors in lung cancer run behind similar research concerning tumours in other locations although the first records suggesting the familial clustering of lung cancer date back to the middle of the previous century (Tokuhata *et al.*, 1963).

Up to 2005, 31 case-control studies and 17 cohort studies were published on this field (Matakidou, Eisen and Houlston, 2005). Multivariate analysis of the family history of women with lung cancer showed that presence of lung cancer in a first-degree relative significantly increases the frequency of lung cancer with odds ratio (OR) 1.61 (Rachtan *et al.*, 2008). Similar results were obtained in the United Kingdom by Matakidou *et al.*, 2005: the frequency of lung cancer in female was increased if a first-degree relative was affected by lung cancer (OR, 1.49; 95% CI = 1.13–1.96) and was further increased having higher numbers of affected relatives (OR, 2.68, 95% CI = 1.29–5.55). A large-scale population-based prospective cohort study, conducted in Japan over time period of 13 years, confirmed that frequency of lung cancer was higher in persons who had family history of lung cancer in first-degree relative (Nitadori *et al.*, 2006). In Iceland, increased relative risks for the kinship of lung cancer patients were demonstrated by Jonsson *et al.*, 2004.

Controversial data are published about the possible link between familial nature and histologic type of lung cancer. Squamous cell cancer had the strongest association with family history in several studies (Nitadori *et al.*, 2006; Ambrosone *et al.*, 1993). Statistically insignificant trend to the highest family-size-adjusted mean number of lung cancer cases per family was found for small cell cancer (Sellers *et al.*, 1992). The data from the Swedish Family – Cancer Database and Swedish Cancer Registry, used to compare the lung cancer frequency in persons with parental family history to those without positive family history, indicated that the frequency was highest for adenocarcinoma (Li and Hemminki, 2003). Adenocarcinoma showed stronger association with familial background among non-smoking USA females (Wu *et al.*, 1996). No association was found by Ganti *et al.*, 2009.

Although few data are available, positive family history has been associated with a trend towards slightly lower

survival in lung cancer patients (Ganti *et al*, 2009). The survival from lung cancer correlated between blood relatives but not in spouses (Lindström *et al*., 2009). The development of second lung cancer may be stronger influenced by genetic factors (Li and Hemminki, 2005).
There are different hypotheses about the mode of lung cancer inheritance. Highly penetrant recessive gene was suspected by Hemminki and Li, 2005 who found higher risk in siblings than in the offspring of lung cancer patients (2.15 and 1.77, respectively). The autosomal dominant inheritance implying rare gene was suggested by segregation analysis (Sellers *et al*., 1990). By finding no clinical evidence of link between Li–Fraumeni syndrome, familial retinoblastoma, familial melanoma and families with aggregation of lung cancer it was concluded that mutations of p53, Rb, p16 and mismatch repair genes although frequent in somatic lung cancer play no significant hereditary role in lung cancer (Tomizawa *et al*., 1998).
The possible genetic loci of familial lung cancer susceptibility are described in chromosome 6q23-25 (You *et al*., 2009; Bailey-Wilson *et al*., 2004), 15q24-25.1 (Liu *et al*., 2008) and chromosome 12 (Schwartz and Ruckdeschel, 2006). Significant association has been found between lung cancer susceptibility and 3 single nucleotide polymorphisms in the first intron of the RGS17 gene. Accumulation of the transcripts of this gene was shown in lung cancer tissues (You *et al*., 2009). A family with multiple cases of non-small cell lung cancer and germline mutation of the T790M is reported (Bell *et al*., 2005).
The initially mapped susceptibility locus 6q23-25 corresponds to genomic region that is deleted not only in lung cancers but in other malignancies (Bailey-Wilson *et al*., 2004). By family history analysis of Japanese lung cancer patients in the setting of multiple primary malignancies, it has been suggested that lung cancer can be associated with other tumours on genetic basis (Haraguchi *et al*., 2007). In Poland, a common variant of CDKN2A with alanine to threonine substitution (A148T) was found to be common in melanoma, lung cancer and colorectal cancer patients (Debniak *et al*., 2006). In Sweden, association between lung, rectal, cervical, kidney and urinary bladder cancers was found (Li and Hemminki, 2003). An increased risk of any cancer (relative risk, 1.25; 95% CI = 1.05-1.50) in the relatives of lung cancer patients as well as increased risk of breast cancer in female relatives of lung cancer patients was found by Gorlova *et al*., 2007.
In familial clustering of lung cancer, two main mechanisms could be proposed. The familial aggregation could be caused either by genetic factors or by heritability of lifestyle and passive smoking. The mapping of genetic susceptibility locus proves the presence of heritable genetic factor. Finally, epidemiologic analysis has demonstrated that heritability of smoking alone cannot explain the increased risk of lung cancer in the affected families (Bermejo and Hemminki, 2005). Indeed, synergistic influence of smoking and hereditary factors has been reported (Rachtan *et al*., 2008; Gorlova *et al*., 2007).
Although an expanding body of evidence substantiates the role of hereditary factors in the lung cancer development, the diagnostic criteria are not well-defined. It was suggested by Wood *et al*., 2000 that presence of at least 2 first-degree relatives with lung cancer in family, one of which is diagnosed before the age of 55, should reveal autosomal dominant hereditary lung cancer. However, these criteria were found to be too stringent by the same scientific group. There was an attempt to identify familial lung cancer by criteria that are analogous to Amsterdam criteria of hereditary non-polyposis colorectal cancer. However, in a cohort of 1068 families identified by a proband having lung cancer, no family corresponded to all three criteria (Tomizawa *et al*., 1998).
Earlier onset is a well-known trait of inherited tumour. Occasionally, lung cancer has been reported in very young patients with family history of lung cancer but absence of Li-Fraumeni syndrome (Tajiri *et al*., 1999). However, it is evident from the meta–analysis that age limit does not add prognostic information in the risk estimates. In contrast, higher number of affected relatives is associated with a trend towards higher frequency of lung cancer: relative risk is estimated as 1.57 (95% CI = 1.34 – 1.84) if 1 relative is affected and as 2.52 (95% CI = 1.72 – 3.70) if at least 2 relatives are affected (Matakidou, Eisen and Houlston, 2005).
In summary, hereditary factors are likely to act in the development of lung cancer. The risk of tumour determined by genetic factors increase the risk caused by smoking and retain importance upon smoking cessation programs. The exact genetic defect comprising the hereditary risk of lung cancer is under investigation, but the most probable candidate region is 6q23-25. Hypothetically, other regions and genes may be involved. It is likely that several genetic syndromes of familial lung cancer exist, probably one of these involving lungs and other – specifically involving several organs. The familial lung cancer in the scientific viewpoint offers also unique possibility to study interaction between genetic and environmental factors as the most frequent cause of lung cancer is well-described.
As the search for the exact genetic marker for familial lung cancer is still under way, the diagnosis depends on clinical criteria. There is no sufficient evidence to add age as a diagnostic criterion of familial lung cancer. The published data suggest that the number of affected relatives is associated with the risk of lung cancer and thus can be used as a criterion in risk estimates. Hypothetically, these data could be obtained by population screening approach. However, there are almost no similar studies with the remarkable exception of population screening for hereditary and familial cancer in West Pomerania (Gronwald *et al*., 2006).

AIM OF THE STUDY

Here we present the first data about familial lung cancer in Latvia by clinical diagnostic criteria in population screening approach in order to study the occurrence and biological properties of this syndrome as well as

the practical diagnostic possibilities. The data represent part of larger investigation, described in Materials and methods, aiming to analyse the role of population screening in the diagnostic pathway of hereditary cancer.

MATERIALS AND METHODS

The investigation was designed as population screening for hereditary cancer within the frames of the project "The development of hereditary cancer prophylaxis in Estonia and Latvia" co-financed by European Union Interreg IIIB Neighbourhood programme. The project was accepted by the Central Committee of Medical Ethics. The screening was carried out in the Valka district from 09/2005 to 06/2007, in collaboration with 22 family physicians. Family cancer histories in the form of questionnaires were voluntarily submitted by 18642 adults, representing 76.6% of the Valka district adult population. The questionnaire was designed to be simple. It contained questions if the blood relatives (siblings, children, parents and their siblings, grandparents) of the proband had had cancer, if yes – of what location, at what age, if the tumour has caused death, and at what age. Filled forms of family cancer history were sent to Hereditary Cancer Institute located at Paul Stradins Clinical University Hospital. The data about the presence and localisation of tumours in kinsmen, as well as about the age in the time of the diagnosis were analysed leading to clinical diagnoses of hereditary cancer syndromes by international criteria (Lynch *et al*., 2003; Gardovskis *et al*., 2005; Irmejs *et al*, 2007). If any hereditary cancer syndrome was suspected by the family cancer history, the corresponding persons were invited for consultation. During it, hereditary cancer syndrome entity was explained, written prophylactic recommendations were given and venous blood samples were proposed to take. Any person whose pedigree was consistent with any hereditary or familial cancer syndrome was considered and consulted as a proband giving him/her the widest opportunity to discuss the family cancer history with a specialist and to receive recommendations for follow-up if appropriate.

Additional data obtained during repeated consultations were applied in order to identify interrelated pedigrees. In this way, the possibility to include any person repeatedly in the analysis due to several kindred relationships was eliminated. Re-evaluation of the data characterising the affected persons was performed after detailed analysis of the blood relationships between different pedigrees. The frequency of lung cancer among blood relatives in the affected line was calculated. In order to characterise the cancer course within familial lung cancer syndromes, the data about tumour-affected persons were analysed, including the reported age of tumour diagnostics, tumour-related death and patient's survival. Descriptive statistical analysis was performed by CIA software.

Within the frames of the presented study, familial lung cancer syndrome was diagnosed clinically by presence of lung cancer in at least 3 persons who were mutually first-degree blood relatives. Suspected familial lung cancer syndrome was diagnosed, if 2 first-degree relatives had had lung cancer.

RESULTS

During the population screening, the first pedigrees with familial lung cancer in Latvia were identified. Several of these pedigrees demonstrated high number of the relevant tumours.

Familial lung cancer syndrome (FLC) was diagnosed in 13 probands (figure 1). Among them, there was oncologically healthy 44-year-old female, whose grandfather from paternal side as well as 3 brothers of father had had lung cancer. Another proband was 49-year-old female, whose kindred demonstrated 5 cases of lung cancer. Familial lung cancer syndrome was diagnosed also in 55-year-old male who's father and 3 brothers of the father had had lung cancer. Fifty two-year-old male presented family history of lung cancer in his mother, her father and brother; three other malignant tumours also were present in the kindred. The other FLC probands reported lung cancer in 3 mutually first-degree relatives. Suspected familial lung cancer syndrome was diagnosed in 93 probands. The population frequency of definitive and suspected familial lung cancer syndrome thus constituted 0.6% (95% confidence interval (CI) = 0.5–0.7%) of the studied Valka population. Nine of the probands diagnosed with FLC syndrome and
57 probands with sFLC syndrome were younger than
50 years (table 1). All probands were oncologically healthy themselves.

The 106 probands reported 232 blood relatives, affected by lung cancer. After re-evaluation as described in Materials and methods, 208 affected persons were recognised. Among them, there were 41 female (19.7%; 95% CI = 14.9–25.6%) and 167 males (80.3%; 95% CI = 74.4–85.1%). The lung cancer was diagnosed at the mean age of 57.9 years (interval, 18–90 years, standard deviation (SD) 12.3 years; 95% CI = 55.9–59.9 years). The affected persons whose exact death age was reported by the proband died at the mean age 60.3 years (interval, 13–90 years; SD 12.3 years; 95% CI = 58.5–62.1 years). The age of definite manifestation of the tumour was also evaluated including either data about the age of diagnostics if available or death age. The mean age of the definite manifestation of the tumour varied from 13 to 90 years, mean 58.8 years, SD 12.8 years, 95% CI = 57.0–60.6 years. Only 3 of the affected persons were alive at the time of population screening. The average survival (see also figure 2) after the establishment of lung cancer diagnosis was 2.0 years (interval 0–59 years; SD 5.5 years; 95% CI = 1.1–2.9 years). The first year lethality constituted 90/147 (61.2%; 95% CI = 53.2 - 68.7%) considering the persons about whom full data set is available.

The age of lung cancer diagnostics (figure 3) as well as the survival after the establishment of diagnosis showed differences between the pedigrees.

The number of affected persons and number of their blood relatives was determined for each kindred. The frequency of lung cancer was 40/157 (25.5%; 95% CI =

19.3–32.8%) in FLC pedigrees and 168/977 (17.2%; 95% CI = 15.0–19.7%) in sFLC pedigrees. Further analysis was devoted to the frequency of lung cancer in FLC and sFLC pedigrees affected in a single generation or in 2 generations. The pedigrees were also classified into early-onset and late-onset groups by occurrence or absence of at least 1 cancer case at or before the age of 50 years. The respective data are shown in table 2.

Among the early-onset FLC pedigrees, 4 of them were affected in 1 generation and 2 – in 2 generations. Among the late-onset FLC families, 1 of them was affected in 1 generation and 5 - in 2 generations. Analysing the early-onset sFLC pedigrees, 14 were affected in a single generation but 20 in 2 generations. Similarly, among late-onset sFLC families, 20 families were affected in a single generation and 39 – in 2 generations.

Possible association between familial lung cancer and occurrence of other malignancies was also studied. In general, no other tumours were found in 54 pedigrees. Among the other families, 11 cases of endometrial cancer, 10 cases of breast cancer, 8 cases of colorectal cancer, 8 cases of hematologic malignancies, 6 cases of stomach cancer, 1 case of duodenal malignancy, 4 cases of ovarian cancer, 3 cases of pancreatic cancer, 2 cases of renal cancer, 2 cases of head and neck cancer were observed. In 5 persons, bones were affected, invariably – spine. Liver was affected in 3 cases, brain - in 1 case. In 5 cases the location of cancer was unknown for the proband. The rate of lung cancer was 126/654 (19.3%; 95% CI = 16.4 – 22.5%) in the families without history of other tumours and 106/594 (17.8%; 95% CI = 15.0 – 21.0%) in families with mixed cancer history.

DISCUSSION

The published data suggest the existence of the hereditary background in the development in lung cancer. The epidemiological evidence of familial clustering is substantiated by mathematical models that help to evaluate the input of genetic and behavioural factors. The final proof will be brought by genetic research moving towards identification of genes and mutations responsible for lung cancer susceptibility.

In the result of population screening we have identified a group of familial lung cancer syndrome including 13 pedigrees of definitive and 93 – of suspected familial lung cancer syndrome with population frequency 0.6% (95% CI = 0.5-0.7%). The frequency of lung cancer cases among blood relatives in this group is high reaching 25.5% (95% CI = 19.3–32.8%) in FLC and 17.2% (95% CI = 15.0–19.7%) in sFLC. This exceeds significantly the EU cumulative risk of lung cancer (age 0-74 years) estimated as 6.5% in men and 1.6% in women (Boyle and Ferlay, 2005) taking into account the fact that lung cancer incidence in Latvia is close to the EU average (Kaiser and Gommer, 2007). Thus, our criteria are useful in order to identify high-risk group of practical size. It should be noted that at the present phase of medical science development the familial lung cancer concept is still under research. However, within the frames of our survey it has already shown its practical merit.

The identified probands were oncologically healthy. However, this cannot be interpreted as risk-lowering factor as, firstly, most probands are young; secondly, lung cancer once already developed would rapidly eliminate the affected person from screening. The last assumption is based on the survival data in our group showing first-year lethality 61.2% (95% CI = 53.2 – 68.7 %). Only 3 affected people were alive at the time of population screening.

The age of cancer onset varied widely. The youngest case in our group died at the age 13 years. Although this would be an unusually early onset of lung cancer in general, it is in accordance with literature data (Tajiri *et al.*, 1999). Further investigation of such cases would be necessary; however, it was beyond the scope of population screening. However, the mean age of cancer diagnostics was 57.6 years (95% CI = 55.7–59.5 years) and of definite tumour manifestation – 58.8 years (95% CI = 57.0–60.6%). Thus, very early onset is not a rule. Adding the age of tumour onset to the analysis, no significant differences in cancer frequency were observed. Thus, our findings are in agreement with the published evidence that familial cancer has a trend towards early occurrence but the age of cancer diagnostics still is not ready for implementation as a strict diagnostic criterion (Matakidou, Eisen and Houlston, 2005).

We have detected trend towards higher rate of lung cancer in definitive familial lung cancer families (95% CI = 19.3–32.8%) than in suspected cases (95% CI = 15.0–19.7%). This is in agreement with the published data suggesting increased frequency with higher number of affected relatives. Although the trend did not reach 95% probability level, it initiated the search for additional factors that might help to evaluate the risk more exactly. The number of affected generations was found to be important. The frequency of lung cancer was significantly lower in sFLC families affected in single generation (95% CI = 12.3–19.1%) in comparison to the FLC group (95% CI = 19.3–32.8%). Complexity was added by the observation that FLC families that are affected in single generation show trend to even higher cancer frequency than FLC families affected in 2 generations. Further analysis in larger group would be necessary. If the trend would be confirmed, complex genetic background could be suspected. This would be in accordance with the medical literature (Li and Hemminki, 2005; Sellers *et al.*, 1990). Genetic heterogeneity could also be further suspected on the basis of our data, namely, the clinical differences between families.

In our group, similarly to the published findings (Nitadori *et al.*, 2006), the frequency of lung cancer in the familial lung cancer families was not influenced by general family cancer history.

The presented study was devoted to the analysis of family cancer history. It was limited by several factors. First, the influence of environmental etiological factors was not considered at the present stage. The interplay between smoking and positive family history has been checked by Bermejo and Hemminki, 2005 providing evidence that familial aggregation of lung cancer cases cannot

be explained by shared etiologic factors or heritability of lifestyle. The role of hereditary factors and smoking in lung cancerogenesis is not mutually exclusive as synergistic influence of smoking and hereditary factors has been reported (Rachtan *et al.*, 2008; Gorlova *et al.*, 2007). Second, the family history was reported by proband only. The published studies describe sufficient accuracy of reporting a severe disease in a close relative by non-medical person (Love *et al.*, 1985). Besides that, the ancient medical documentation in Latvia also can be subjected to various bias.

The results of Valka population screening should be considered as a pilot study. In the whole Latvia, distinctive results could be expected due to different ethnic composition, levels of environmental factors or other factors. However, the population screening has demonstrated the possibility to identify risk persons by completing an easy questionnaire. While the scientists in the whole world are still searching for the underlying genetic defect and thus diagnostics of mutations is not accessible, there is no possibility to consider the completeness of such approach.

As shown by our results, the population screening identifies the persons-at-risk at sufficiently early age to provide prophylaxis programs. The literature data mostly suggest development of non-small cell lung cancer in familial lung cancer families. Thus, the population screening as a cancer prevention tool and the biology of familial lung cancer fits together in order to provide the best assistance for the risk group. Both FLC and sFLC should be included in the risk group and undergo regular surveillance for timely diagnostics and surgery. Additionally, the identified group could be a target of educational efforts aiming at smoking cessation. It could be reasonable to propose that these people after receiving adequate information may develop high motivation for healthy life style.

The familial lung cancer concept thus is promising in the practical field as well as in further scientific studies including the final solution of genetic workup.

CONCLUSIONS

1. The population screening has brought the first evidence of familial lung cancer in Latvia with the frequency 0.6% of population.
2. The diagnostic criteria based on the presence of at least 2 lung cancer cases among blood relatives allow identifying oncologically healthy persons from high-risk families at early age. Thus, population screening is an effective tool in identification of risk group and initiation of protective measures.
3. Familial lung cancer syndrome can be diagnosed by use of simple questionnaire that was applied in the presented study.
4. Number of lung cancer cases in the pedigree is the most important diagnostic criterion allowing discriminating between groups of pedigrees with different although high frequencies of lung cancer. The diagnostic role of early cancer development and higher number of affected generations should be further investigated.

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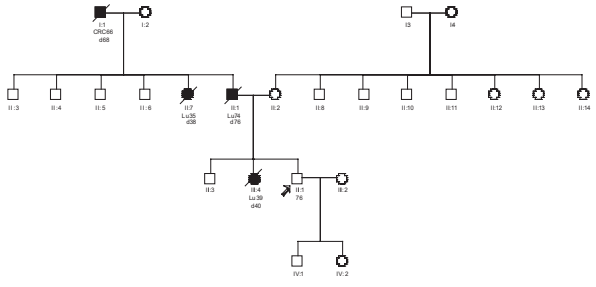

Fig. 1. Pedigree showing familial lung cancer

Table 1. Age distribution of probands with familial lung cancer syndromes

Diagnosis	Total number	Age, years						
		18–29	30–39	40–49	50–59	60–69	70–79	≥ 80
FLC	13	4	1	4	2	1	1	0
sFLC	93	15	18	24	13	13	5	5

Abbreviations in table: FLC, familial lung cancer; sFLC susp., suspected familial lung cancer; ≥, greater than or equal to.

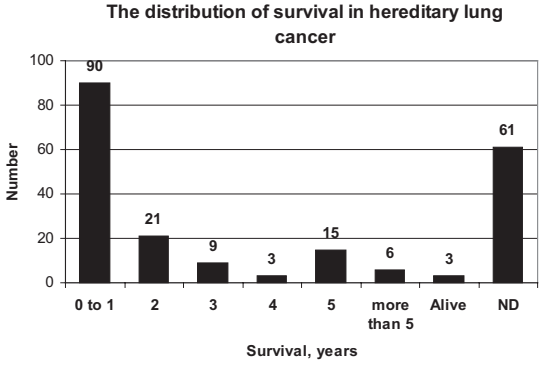

Fig. 2. Survival of lung cancer patients from familial lung cancer families. Abbreviation in figure: ND, no data available

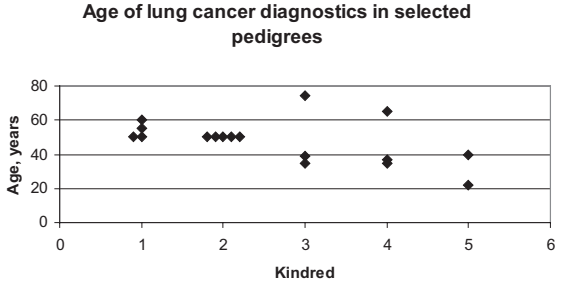

Fig. 3. Age of lung cancer diagnostics in the affected persons from definitive and suspected familial lung cancer pedigrees

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ORIGINAL ARTICLE

Initial Neoplastic Proliferations and Background Pathologies of Kidneys with Clear Cell Renal Carcinoma

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Summary.
Introduction. Nephrosclerosis and development of renal tumours is desribed in literature. But there is a few dates about correlation between grades of nephrosclerosis and renal tumours.
Aim of the study. Aim of our study is to find out and to analyze initial neoplastic proliferation around renal cell carcinoma in case of nephrosclerosis of different degrees. The hypothesis: the initial neoplastic proliferation in renal cell carcinoma is related to the degree of nephrosclerosis.
Materials and methods. We investigated 206 surgical removed kidneys with clear cell renal carcinoma (RCCC) cases. To evaluate the correlation between grading of RCCC and its stages with nephrosclerosis we framed 5 macroscopic degrees of its severity. According the expression of nephrosclerosis we analyzed microscopically renal tissue to diagnose initial neoplastic proliferations.
Results. In our research we have got weak positive correlation of R = 0.2 (p = 0.01) between nephrosclerosis and grading of renal clear cell carcinoma. Practically the same correlation exists between renal tumor TNM stage and nephrosclerosis degrees. In 62 cases from all 206 examined surgically removed kidneys microscopically and grossly we have diagnosed initial neoplastic proliferations. Histological variations of them were: papillary adenoma, oncocytoma, nephrogenic adenoma and clear cell proliferations. Accordingly the localization of these neoplastic proliferations we can divide them into subcapsular, cortical and medullar both in the tumour capsule and outside it. Around the neoplastic proliferations we have evaluated the intensity of inflammation, arteriosclerosis, glomerulosclerosis, cystic transformations of renal tubules and stromal fibrosis.
Conclusions. We have proved that the most marked correlation was between the background pathologies and papillary adenoma (n = 29). More common the diameter of fibrosis was several times larger than the size of adenoma. There was also strong correlation with cystic changes of renal tubules. There is a correlation R = 0.305 (p = 0.01) between the frequency of papillary adenoma findings and of nephrosclerosis stages. The second most common initial neoplastic proliferation was clear cell proliferations (n = 26). But it has less pronounced association with the background changes. We have not proved the correlation between the frequency of renal clear cell carcinoma and severity of nephrosclerosis.
Key words: renal clear cell carcinoma; background pathologies of kidneys; initial proliferations of tubular epithelium.

INTRODUCTION

The pathogenesis of renal epithelial tumours has provided one of the most enduring controversies of modern pathology. The renal epithelial tumours are very heterogenous group of neoplasms according to their morphological types, genetic changes, aetiological factors and prognosis. One of the problems is the association of these tumours with background renal tissue changes caused by hypertension, chronic pyelonephritis, hydronephrosis etc.

In our population there is increase of morbidity with these diseases and their complications and therefore more actual is question about the development of renal tumours. The investigations of renal tumours which have arisen in changed kidneys gives us information about monitoring and treatment opportunities.

Correlations between scar formations in organs and the development of malignancies were already mentioned by R. Virchow who wrote about chronic irritation and the development of cancer (18). In literature mainly are described renal tumours associations with terminal changes of kidneys. More common it is described in patients with papillary adenoma (1, 3). At the terminal renal failure the risk for the renal tumour development is maximal but histological variations and frequency of appearance are various in different districts of world. Patients with haemodialysis have also higher chance for the appearance of tumours in prostate, urinary and haematopoietic system, liver, oral cavity but there are lack of connections with risk of development of breast, gastric, rectal and lung cancer (6). Renal tumours have the characteristic feature of the development of pseudocapsule. In this zone sometimes are described tiny neoplastic and displastic changes of renal tubular epithelium (7). In literature there are few dates about correlations between the degree of nephrosclerosis and the development of renal tumours.

Kidneys rather common are involved in a lot of congenital tumours' syndromes. Mutations occur in the germinative cells and therefore there is possible to diagnose syndrome and to detect asymptomatic gene carrier (16).There are described some syndromes (von Hippel- Lindau, Birt-Hogg- Dube, Tuberous sclerosis) with typical renal tumours and their interdependences

(5).The term "initial neoplastic proliferations" is used accordingly to microscopic findings of very small lesions starting of 5 mm in diameter.

AIM OF THE STUDY

Aim of our study is to find out and to analyze initial neoplastic proliferations around clear cell renal carcinoma in case of nephrosclerosis of different degrees. The hypothesis: the initial neoplastic proliferation in renal cell carcinoma is related to the degree of nephrosclerosis.

MATERIALS AND METHODS

We have analyzed grossly and microscopically changes of 304 surgically removed kidneys in the years of 2004 – 2007 with renal cell carcinoma. 151(49.7%) were male and 153(50.3%) – female. Ratio male:female was 0,85:1. The age of patients varied from 22 till 81 year, average age was 61.5 ± 11,8. Division of patients according their age is showed in the 1.figure. Accordingly the frequency or renal cell tumours we have found such neoplasms: clear cell carcinoma (RCCC), oncocytoma, papillary renal cell carcinoma, chromophobe renal cell carcinoma. Distribution of renal tumours is showed in table No.1.
From all renal tumours RCCC was found in 67,8% (n=206) of cases. The average age of these patients was 61.96 ± 0,741. Ratio male to female was 1:0,86. Grading of RCCC was done accordingly Thones at al., 1986. It is shown in table No.2.
RCCC were subdivided into groups according to TNM classification (5). It is shown in table No.3.
For the grossly evaluation of nephrosclerosis we have created some criteria for the degrees of nephroscerosis. We noted normal anatomical kidney size and terminal pyelonephritic scrinkage as values of ends of ranges. Other degree scores in literature are not described.
0 degree of nephroscerosis: kidney is normal in size (11–12 cm long, 5–7 cm wide and 2,5–3 cm thick), weight: 115–170 g (11).
1. degree: Kidney is not smaller in size but there are some small areas of nephrosclerosis 1 till 5% from total surface, weight – 115–170 g
2. degree:Weight of kidney is around 80% from normal weight. Renal surface is with scars or diffuse granularity for 10-20 % from total surface of kidney.
3. degree: Weight of kidney is 70–80 %, but fibrosis occupies 20–30% of renal surface
4. degree: Weight of kidney is 50–60% of normal weight of organ and fibrosis takes 30–40% of renal surface or there is diffuse granularity.
5. degree: Weight is till 50% of normal renal weight with wide scars of kidneys what is described as terminal pyelonephritic scrinkage (17).
For the microscopic examination tissues from nephrectomies were fixed in 10% neutral buffered formalin, after dehidratation and embedding in paraffin 4–5 microns thick specimens were stained with hematoxylin eosin. In case of initial neoplastic proliferations we evaluated intensity of inflammation, microcystic lesions, three stages of glomerulosclerosis and arteriosclerosis.
Evaluation of inflammatory processes around tumours was expressed into 3 degrees accordingly the amount of inflammatory cells in 1 field of vision 200x (in 1,26mm^2): weak (degree 1)- till 50 cells, mild (degree 2)- 50–200 cells and severe (degree 3) – more than 200 cells.
Microcystic lesions were evaluated accordingly such stages:
Stage 1: one cystic structure in two 100x magnification fields of vision.
Stage 2: one cystic structure in one of 100x magnification field.
Stage 3: three or more cystic structures in one 100x magnification field.
The expression of glomerulosclerosis we divided in such stages:
Stage 1: 0 – 10 % of glomeruli are affected by fibrosis near the neoplastic proliferation;
Stage 2: 10 – 40 % affected glomeruli; Stage 3: 40% and more affected glomeruli. But arteriosclerosis was noted as presence or lack of it.
We estimated ratio between diameter of tumour and surrounding fibrosis. Measurements were provided in millimeters. Results were divided into scales of nominals, rangs and intervals. For the comparison of two independent numbers we have used t-test. We detected also Spearman' s and Kendel tests (p= 0, 01). All calculations were done by SPSS 13 program in Windows XP.

RESULTS

Correlations between tumour grading and severity of nephrosclerosis are shown in table No.4.
Statistical reliable correlation was found between the grading of clear cell carcinoma and the degree of nephrosclerosis. Spearman correlation R=0. 2 (p=0.01).
During investigations of the pseudocapsule of tumours and nearby areas we often found small neoplastic proliferations of clear cells – accumulations of eosinophylic cells and dysplastic changes of tubular epithelium. Such changes were proved in 38.8% (n=87) of clear cell renal carcinomas cases.
Microscopic investigations of the pseudocapsule of RCCC and tissues nearby it have shown papillary adenoma in 29 cases of totaly examined 206 kidneys (Figure No.2). Accordingly the location of papillary adenoma in 10 cases they were detected in the pseudocapsule of tumour (figure No.2) but in 6 cases a little bit further in cortical part. Papillary adenomas elsewhere in the parenchyma outside the tumor area were found in 13 cases: 11 in subcapsular and 2 in cortical part. Of 29 papillary adenoma cases, multiple growth character was found in 12 cases with more than 1 papillary adenoma. In 8 of these cases multiple growth character was detected at the capsule of tumour.
Papillarity histologically is characterized by tubulary-papillar and tubular formations in diameter from 0.01 mm to 3 mm. The arithmetical average of diameter of these changes was 1.3 ± 1.2 mm. The ratio of diameter of the adenoma and surrounding fibrosis was between 0,03–0,5 in 81% cases but ratio of 0.6–1.0 was found in 19% of tumours.

Cystic transformation in the adenomas was detected in 85% of cases. In 10 cases it was of 3rd stage and in 7 cases of 2nd stage. Glomerulosclerosis at adenomas was fixed in 57.1%. In 7 cases as 3rd stage and in 6 cases as 2nd stage.
Vascular fibrosis at the adenoma was observed in 77.2% of cases. Intensity of inflammation was as follows: 1. degree was found in 20% of cases, 2.-in 76% and 3. degree in 14% of cases. Papillary adenomas was in 36.3% cases with G1, 40.9% of G2 and 22.8% in G3 of RCCC tumors.
The adenomas were found in 45.4% of clear cell carcinoma of stage T1a, 27.2%-stage T1b, 4.5%-T2– stage and 22.9% – T3 stage.
There is a correlation between the macroscopic degrees of severity of nephrosclerosis and the frequency of papillary adenoma findings (Kendall correlation R = 0.305, p=0.01).
Proliferations of clear cells in kidneys with the clear cell renal carcinomas were detected in 26 cases. (12.6%); size of them was from 0.1 mm to 4.0 mm. The average number of diameter was 1.2 mm ± 1.23 mm. Multiple proliferations were diagnosed in 12 cases. In six cases was combination with other tumour - papillary adenoma. At the vicinity of RCCC there were found clear cell proliferations in 11 cases. 3 of them were situated in subcapsular area and 8 – in cortical part. Mainly we have found compact design of tumors with tubular structures. Proliferations of clear cells in 60% of cases were detected in the adjacent renal tubular epithelial dysplastic changes (Figure No.3). Ratio of the diameter of proliferations and fibrosis was from 0.01-0,5 in 65,3% but from 0.6-1.0. in 34.7% of the cases. Cystic changes were detected in 15.3% of cases. In all cases these lesions were of 1st degree. Glomerulosclerosis was detected in 53.8% of analyzed tumours. In 7 cases it was of 2nd degree but in 3 cases it was glomerulosclerosis of 3rd degree. Vascular fibrosis we have found in 57.6% of cases. Weak inflammatory reaction of first degree was seen in 26.9% of cases, but mild (2nd degree) in 23.1%. Macroscopically the expression of nephrosclerotic was of 2. and 3. degree. There was no correlation between the degrees of nephrosclerosis and frequency of clear cells proliferations. Foci of clear cell proliferations were in 38.4% of G1, 38.4% of G2, 23.2% of G3 RCCC. Renal cancers with clear cell proliferations were at such stages – 46.3% – T1a, 30.7% -T1b, 11.5% – T2 un 11.5% at T3.
The investigation of changes nearby the clear cell renal carcinoma and other parenchymal zones identified 3 cases with oncocytic adenoma. In one case, oncocytic adenoma was found in the tumour capsule of cortical part (Figure No. 4) but others – in cortical areas of the kidney. Microscopically they consist of small cysts covered by middle sized eosinophylic cells.
Histologically oncocytic proliferates ranged from 0.5–1.0 mm in diameter. In two cases the background of fibrosis was not observed at all. But in one case in the background was a massive fibrosis (Figure No.4). Cystic lesions of renal tubules were observed in one case. Glomerulosclerosis was not observed. In two cases, inflammatory changes were weak – of first degree and in one case – of 3rd degree. Arteriosclerosis was not found. There is lack of correlations between the expression of nephrosclerosis and frequency of oncocytic adenomas. In one case oncocytic adenoma was found in kidney with G 1 clear cell carcinoma and in two patients with G2 tumour. Stages of neoplasms were at T 1a and one in T 1b. Microscopical research of kidneys with RCCC proved 3 angiomyolipomas too. All these satellite tumors were found in subcapsular zone away from RCCC, their diameter was 0.3-0.8 cm. Angiomyolipoma is characterized by the proliferation of smooth muscle cells who was strongly shielded from the surrounding stroma without capsule around them (Figure No 5). In the wall of this tumour were multiple muscular type arteries with radially arranged perivascular epitheloid cells. In two cases were few fat tissues but in one fat tissues occupied 40 % of stroma. Cystical changes of renal tubules nearby angiomyolipoma were observed in non of cases. The ratio of diameter of neoplasm and surrounding fibrosis was 1.0. Glomerulosclerosis and arteriosclerosis was not found in these cases. Inflammatory reaction was of 1st degree in all cases. Macroscopical stage of nephrosclerosis was of 3rd degree. In one case angiomyolipoma was in patient with G 1 RCCC and in two cases with G2 cancer. In 2 kidneys angiomyolipoma was parallel with carcinoma of T1b stage and in 1 kidney with T2.During careful examination of the renal CCC background we have found also nephrogenic adenoma in 63 year old female with 6cm large RCCC. It was situated nearby primary renal CCC in medullary part of kidney (figure No.6). Histologically tumour was built up from tubular elements and structures were covered by urothelium 1,0 mm diameter. Epithelium formed tubules like shapes (figure No.7). Nephrogenic adenoma was found in scarified tissues at the renal clear cell carcinoma of G2 stage. Besides there were arteriosclerosis, but inflammatory reaction was of third degree.

DISCUSSION

The most common form of renal cancers in adults was renal clear cell carcinoma. In our study it was 67.8% of all kidney tumors. Renal cell carcinomas have a high ability to induce inflammatory changes in the same tumor and adjacent tissue, with marked angiomatosis (8, 13). Therefore around renal cell carcinoma changes are mostly expressed. Some researchers indicate increased neoplastic processes in the areas of nephrosclerosis (12). Finding of neoplastic proliferations in these places, raise several problems in the diagnosis of such changes in routine hematoxylin eosin specomens. Several authors as a precancerous condition note intratubular and intracystic epithelial neoplasia. This problem is still not solved (7). When Yorukoglu K et al. (1999) analyzed the renal tubular epithelium in 5% of the cases they have found "carcinoma in situ" changes (19). In this investigation they proved that these lesions of tubular epithelium are phenotypicaly correspondent to dysplasia. The authors consider that ischemic situation in the tissues cause metabolic disturbances in tubular

epithelium. Changes were characterized by clarifying of epithelium and imitation of clear cell proliferation (13, 4.) In case of tubular disturbances may occur also mitosis (2). In these structures are also marked immunohistochemical changes (15). On the other hand, each tumour begins as a single cell proliferation areas with features of dysplasia. Such changes were often found in microscopic tumors in the immediate context of with previous tubular epithelial structures. When we analyzed the type of neoplasia and their correlation with changes in the background, in the areas of nephrosclerosis the most common satellite tumour was papillary adenoma. It correlates with dates of other authors but they discuss these problems in polycystic disease of kidneys and in cases of hemodialysis (3). We have proved that renal CCC mainly has developed in the areas of nephrosclerosis. But in case of oncocytic adenomas such correlation was not found. Nephrogenic adenoma is rare pathology and pathologists consider this tumour as metaplastic process of urothelium without real neoplastic transformation (10). But in renal transplants is proved that nephrogenic adenoma is of tubular epithelium origin (9).

CONCLUSIONS

1. Background pathologies around tumour are typical for clear cell renal carcinoma in different stages and with various grading of it opposite to other neoplasms of kidney.
2. In association with severe nephrosclerosis with terminal shrinkage of kidney we have detected only G1 clear cell renal carcinoma and neoplastic proliferations in the zones of nephrosclerosis were also of G1 grade.
3. Our research has proved positive correlation between the type of tumour and cystic changes in tubules and arteriosclerosis but we found weak correlation with glomerulosclerosis and inflammation.
4. The most frequent type of tumor which was in association with nephrosclerosis and clear cell renal carcinoma was papillary adenoma.

Conflict of interest: None

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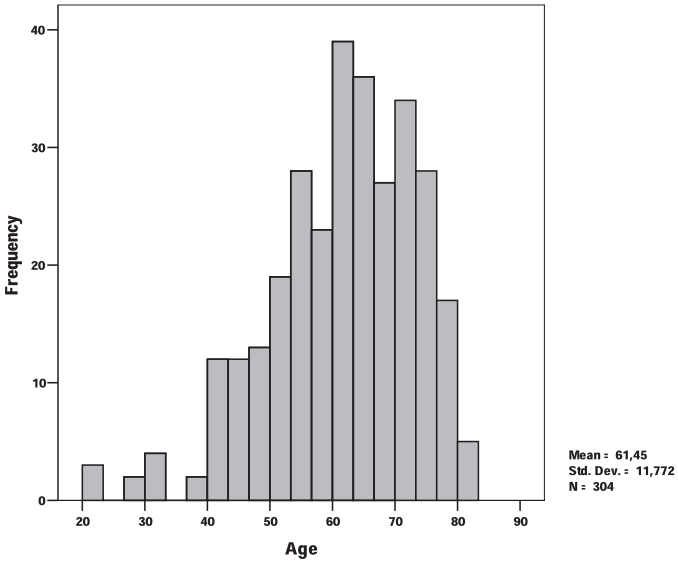

Fig. 1. The histogram of distribution of patients with renal tumours according the age

Tab. 1. Distribution of renal tumours accordingly the frequency

Diagnoses	Number	%
Clear cell carcinoma	206	67,8
Oncocytoma	30	9,9
Papillary renal cell carcinoma	22	7,2
Chromophobe renal cell carcinoma	23	7,6
Other tumours	23	7,5
Total	304	100,0

Table 2. Grading of RCCC cases according Thoenes

Grade of tumours (Thoenes)	Frequency	Percent %
1	71	34,5
2	89	43,2
3	46	22,3
Total	206	100

Tab. 3. TNM stage frequencies of RCCC

TNM stage of RCCC	Frequency	Percent %
T1a	81	39,3
T1b	62	30,9
T2	42	20,3
T3a	12	5,8
T3b	7	3,4
T4	2	0,9
Total	206	100

Table 4. Correlations of tumour grading and severity of nephrosclerosis

Degree of nephrosclerosis	Grading (Thoenes)		
	G1	G2	G3
0		1	
1	31	47	29
2	19	26	25
3	16	14	2
4	4	1	
5	1		

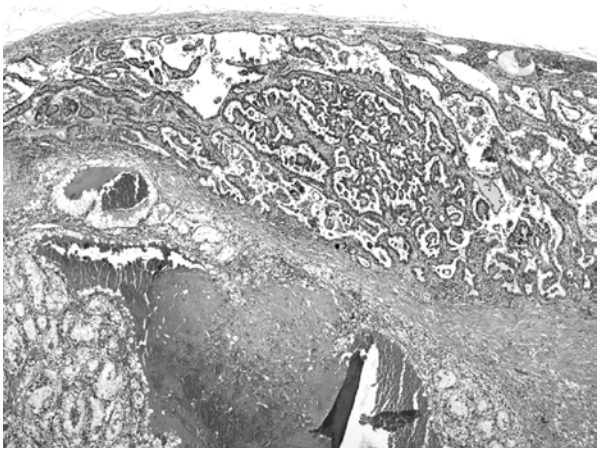

Fig. 2. Papillary adenoma (above) in the pseudocapsule of renal clear cell carcinoma (beneath). Hemotoxilin-eosin. x 40

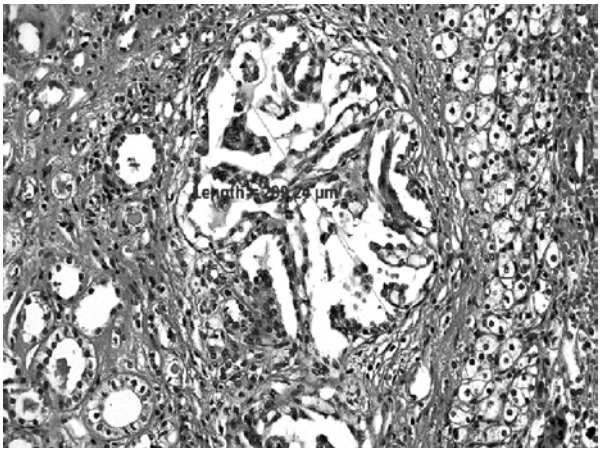

Fig. 3. Clear cell proliferations foci in pseidocapsula of RCCC. There is visible displastic changes of tubular epithelium with early clear cell differentiation. Hematoxilin-eosin. x100

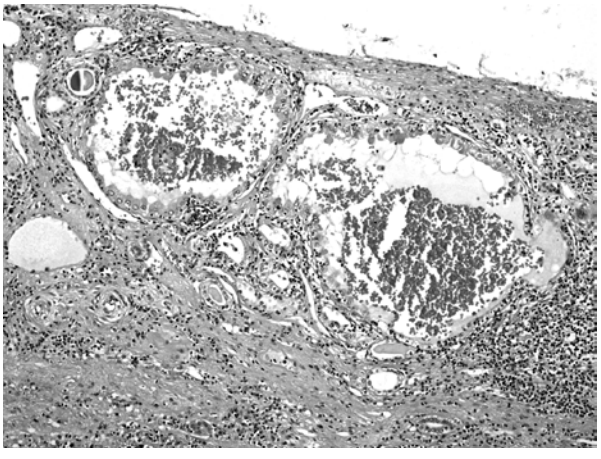

Fig. 4. Oncocytic adenoma in the area of severe nephrosclerosis nearby the clear cell carcinoma. H/E X100

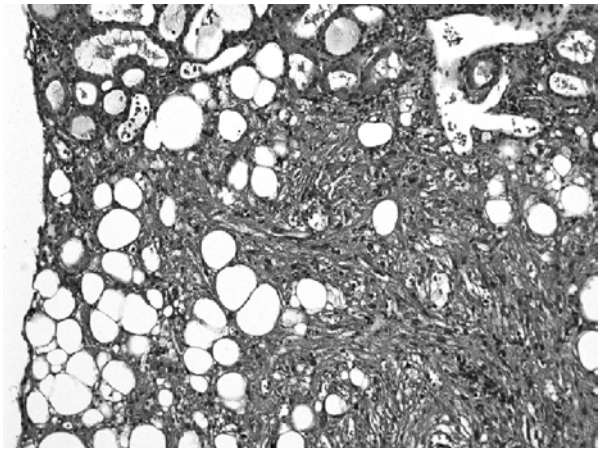

Fig. 5. Angiomyolipoma with smooth muscle cells and fat tissue. Hemotoxilin-eosin. x100

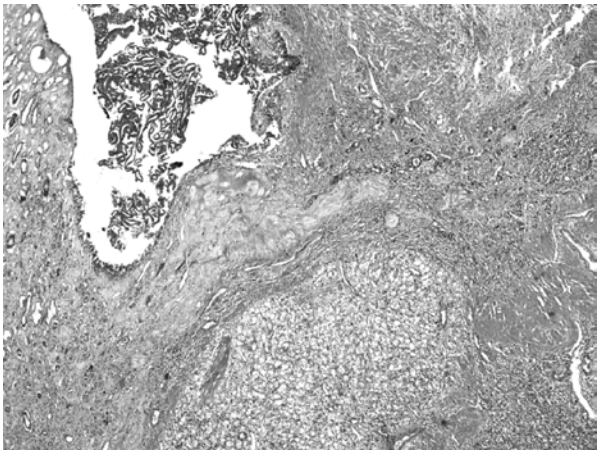

Fig. 6. Nephrogenic adenoma (above) situated at the renal CC carcinoma (beneath). Hemotoxilin-eosin x40

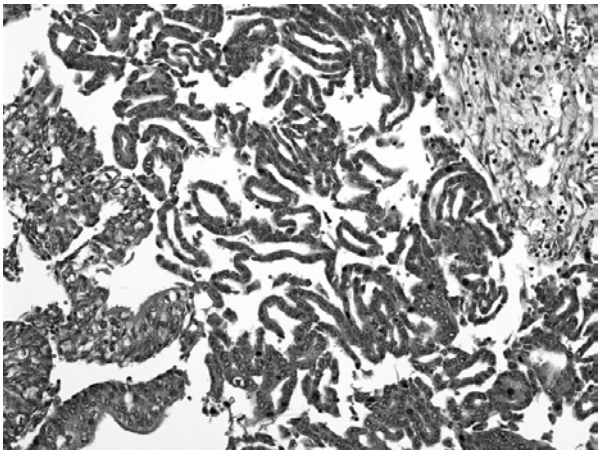

Fig. 7. Nephrogenic adenoma consists of solid tubular structures with eosinofilic epithelium. There are seen connection with urothelium. Lesion from fig. No. 6. X 200

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ORIGINAL ARTICLE

First Experience in Quality of Life Analysis After Bariatric Surgery

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Summary
Introduction. The prevalence of obesity has increased markedly in the past 20 years therefore, bariatric surgery plays an important role in obesity treatment.
Aim of the study. The aim of this study was to evaluate the efficacy of Laparoscopic adjustable gastric banding (LAGB) and it influence on patients' quality of life (QOL) in the management of morbid obesity.
Materials and methods. 18 patients with morbid obesity underwent LAGB procedure at the Paul Stradins Clinical University Hospital, Riga, Latvia. Data collected were: age, sex, weight, height and operation time, body mass index (BMI), excessive body weight (EBW) and comorbidities. Patients fulfilled general Short Form Health Survey with 36 questions before operation (a), 6 month (b) and 12 month (c) after operation. License was acquired for SF-36v2® use in the study.
Results. The mean ± SD age of patients was 42.5 ± 11.8 years (25-63) with 83.3% of them female. The mean ± SD weight was 133.7 ± 20.3 kg (104-176), mean ± SD height 168.7 ± 9.4 cm (155-189) and mean BMI 46.2 ± 6.6 kg/m2 (36.8-58.8).
The mean ± SD weight at 6 month follow up was 113.5 ± 17.8 kg (p=0.001). The postoperative mean BMI at 6 month follow up was 39.32 ± 6.1 kg/m2 (p=0.001). The mean percentage of excessive body weight (EBW) loss at 6 months was 30.4%.
QOL life was higher after operation (b, c) comparing to preoperative data (a), but there were no statistically significant difference in any of the scales
Conclusions. The efficacy of LAGB surgery in long-term is still uncertain, but our early results are comparable to other series. To determine the true efficacy of LAGB in weight control and impact on QOL, a longer follow-up period is necessary, more cases to analyze and data from control group of general population.
Our findings suggest that life quality questionnaire SF-36v2® is good tool to measure patient's quality of life before and after operation, to discuss effectiveness of operations techniques and to observe quality of life dynamics.
Key words: laparoscopic adjustable gastric banding, LAGB, QOL, SF-36, obesity.

INTRODUCTION

The prevalence of obesity has increased markedly in the past 20 years, becoming a major public health issue (18, 19, 26). According to the World Health Organization, 'overweight' is defined as a body mass index (BMI), ie weight (kg)/[height (m)]^2, of greater than 25 kg/m^2, whereas 'obese' is defined as a BMI of greater than 30 kg/m^2 (29). Obesity surgery is currently one of the most frequently performed surgical procedures in the USA and Europe with steadily increasing numbers (15). It is well-known that the higher one's BMI value, the higher his or her mortality wherewith many obesity-related comorbidities have been documented (2, 8). The health risks of obesity include coronary artery disease, hypertension, diabetes, gallstones, cancer, orthopaedic problems, sleep apnoea and general debility (4).
Morbid obesity, defined as a BMI of greater than 35 kg/m^2, occurs in 2% to 5% of the population of Europe, Australia, and the USA, and it is becoming increasingly common (1, 24). Despite nonsurgical treatment of obesity such as behavioral and pharmacologic methods have been proved to have an effect on short-term weight loss of approximately 5 to 10% of body weight (7), these methods are not very effective on long-term resolution of excesive body weight and its related comorbidities.
Therefore, bariatric surgery plays an important role in obesity treatment (6, 12). Nowadays many surgeons have learned to perform this type of surgery laparoscopically rather than using an open approach (22). Currently LAGB and the laparoscopic Roux-en-Y gastric bypass are the most widely used methods for obesity treatment (16). LAGB which was introduced in early 1990s offers the advantages of minimally invasive surgery, adjustability, and reversibility. This purely gastric restrictive procedure involves the use of an adjustable silicone band placed around the gastric cardia, which creates a small gastric pouch (15 mL) with a narrow outlet. We used Ethicon Endo-Surgery LGB12™ Adjustable Gastric Band and Gastric Calibration Tube GCT-360.
To date, comparative studies are mainly looking at clinical data of weight loss, change of comorbidities and morbidity (27), but only few comparative studies on health-related quality of life exist (10, 28).
QOL is relevant parameter in evaluation of surgical treatment because it perfectly shows physical as well as mental health of the patient and it is one of the most important criteria to judge about patient's well being after operation.

AIM OF THE STUDY

The aim of this study was to evaluate the efficacy of laparoscopic adjustable gastric banding and it influence on patients QOL in the management of morbid obesity.

MATERIALS AND METHODS

Between 1 January 2008 and 1 May 2009, a total of 18 patients with morbid obesity underwent LAGB procedure at the Paul Stradins Clinical University Hospital, Riga, Latvia. Patients were considered as candidates for operation if the following conditions were met: aged between 18 and 65 years, BMI of greater than 40 kg/m^2 or greater than 35 kg/m^2 with serious comorbidities, no excessive intake of sweets or alcohol. All patients had history of obesity for more than 5 years and had failed conservative management such as diet, behavioral therapy, and pharmacotherapy in the past. Obesity-related comorbidities was present in 55.5% of patients, which included 3 patients with diabetes mellitus (DM), 6 patients with hypertension, 6 patients with joint and spine pain, and 1 patient with dyspnoea. Exclusion criteria included previous gastric surgery, a large hiatal hernia, and a history of alcohol addiction and poorly controlled non-obesity-related medical diseases.

This study was approved by the Ethics Committee of Paul Stradins Clinical University Hospital. Written consent was given by all patients.

Operative procedure. A high-dose low-molecular-weight heparin was given in the morning of operation, and an antibiotic prophylaxis was given during induction. The operation was performed under general anaesthesia. The patient was in the reverse Trendelenburg position with the legs extended in stirrup, and the operating surgeon was standing between the patient's legs. A five-trocar technique was used with a 15-mm port to allow insertion of the gastric band into the peritoneal cavity. After the liver was retracted with laparoscopic retractor, the pars flaccida technique was performed (30). The dissection began at the base of the left crus well above the first short gastric artery to free the angle of the His of the stomach. Following the opening of the left phrenoesophageal ligament, the gastro-hepatic ligament (pars flaccida) was divided, and blunt dissection was performed to create a passage between the diaphragmatic pillars and the posterior aspect of the gastro-oesophageal junction. The empty band was then inserted through the 15-mm trocar and pulled through the retrogastric tunnel with the help of the Goldfinger dissector. Once the band was locked in place, the device was anchored anteriorly by three gastrogastric stitches with non-absorbable sutures. The fundus of the stomach below the band was stitched to the gastroesophageal junction completely covering the anterior aspect of the band to prevent herniation of the fundus. After the band was secured, a separate incision was made just above the xiphoid, and the port was connected and sutured subcutaneously to the presternal fascia (29). In the postoperative course, all the patients received a contrast study of the esophagus and stomach 1 day after procedure. The patients were discharged as soon as sufficient oral fluid and soft food intake was possible.

For all patients, the data collected were age, sex, weight, height and operation time, BMI, excessive body weight (EBW) and comorbidities.

At two points in time, patients were weighed, and BMI was calculated. The first measurement was taken before surgery and the second 6 month after operation.

Patients fulfilled general SF-36v2® Short Form Health Survey with 36 questions before operation (a), 6 month (b) and 12 month (c) after operation. License was acquired for SF-36v2® health survey questionnaire use in the study.

Questionnaire consists of internationally accepted SF-36v2® health research protocol which set so that minimum psychometrical standard parameters which are not specific neither to age nor illness or treatment method where included (9). Acquired data entered into the data base and statistical processing was made. After data processing eight health concepts is possible to measure: physical functioning – PF, role physical - RP, bodily pain - BP, general health – GH, vitality – VT, social functioning – SF, role emotional – RE, mental health – MH. For scales the highest possible score of 100 is achieved when no limitations or disabilities are observed. Missing values in the SF-36v2® were replaced by mean individual scores, as recommended in the manual.

3 health scales (PF, RP, and BP) with statistical significance measure physical health but each of them describes different aspects: physical functioning (PF) measure limitation in physical activities; role physical (RP) - inability to do everyday activities due to physical problems; bodily pain (BP) focuses on physical pain.

Mental component consist of MH, RE and SF parameters: social functioning (SF) and role emotional (RE) shows limitations due to personal or emotional problems; mental health (MH) indicates psychological stress symptoms.

General health (GH) and vitality (VT) scales are sensitive on physical and mental health. Vitality (VT) characterises tiredness or joy of living.

Twelve month after operation patients answered also to specific questions related to operation result.

For acquiring data and further processing ABBYY FormReader 6.5, QualityMetric Health Outcomes™ Scoring Software 2.0, SPSS 16.0 and Microsoft Excel programs were used. For analysis, the presence of the questionnaires at the three points in time was required. For analysis, the nonparametric Friedman K-related samples test was used, as variables were not normally distributed. Statistical significance was defined as $p<0.05$. To analyze QOL between 2 surveys we used Wilcoxon Signed Ranks test.

RESULTS

Eighteen patients were enrolled in the study. The mean ± SD age of patient was 42.5 ± 11.8 years (25-63) with 83.3% of them female. The mean ± SD weight was 133.7 ± 20.3 kg (104-176), mean ± SD height 168.7 ± 9.4 cm (155-189) and mean BMI 46.2 ± 6.6 kg/m^2 (36.8-

58.8). The demographic and clinical characteristics of 18 patients with morbid obesity are summarized in Table 1.

Median operating time was 100 minutes (80-120 minutes) and all cases were successfully performed without open conversion. The median hospital stays 3 days (2–4 days).

The mean ± SD weight at 6 month follow up was 113.5 ± 17.8 kg (p=0.001). The postoperative mean BMI at 6 month follow up was 39.32 ± 6.1 kg/m^2 (p=0.001). The mean percentage of excessive body weight (EBW) loss [% EBW loss=weight loss/(initial body weight–ideal body weight at BMI 25) x 100%] at 6 months was 30.4%. The results are summarized in Table 2.

There were no intraoperative complications and no blood transfusion was needed. We analyzed quality of life using SF-36v2® Short Form Health Survey before operation (a), 6 weeks (b) and 6 month after operation (c).

To analyze QOL dynamics we used Friedman test and evidently QOL life was higher after operation (b, c) comparing to preoperative data (a), but there were no statistically significant difference in any of the scales (Figure 1).

Using Wilcoxon Signed Ranks test we analyzed QOL between 2 surveys: c-a, c-b, b-a, and there were no statistically significant improvement in any of the scales.

With the center-specific questionnaire 12 month after operation, the patients were asked how satisfied they were with results of the operation. Of the patients, 100% stated to be satisfied with the operation. However two from five patients wish to have greater weight reduction. All patients reported to have increase in their QOL and could recommend this type of operation to others.

DISCUSSION

Obesity is becoming a worldwide phenomenon. Since the first jejuno-ileal bypass performed by Kremer and Linner in 1960s, various approaches and procedures have been developed to deal with obesity. Surgical treatment has had an established role in the management of morbid obesity. The criteria for selecting patients for bariatric procedures are based mainly on the BMI definition of obesity as recommended by the World Health Organization.

Numerous comparative studies for the treatment of morbid obesity have been done, but the bariatric procedure of choice is still under debate.

Malabsorptive procedures, such as jejuno-ileal, biliopancreatic, or gastric bypass allow the patient to 'lose weight while eating' (20). The Roux-en-Y gastric bypass has recently been adapted for use with laparoscopy, achieving an acceptably low rate of complications (3.3% major and 27% minor) and an excellent weight loss of at least 60% EBW (21). However, this procedure is both difficult and lengthy (2 to 3 times longer than our procedure), and complications, such as anastomotic failure and intestinal obstruction, are potentially lethal.

Restrictive procedures like LAGB induce slow gastric emptying via a small pouch and create early satiation allowing a 40% to 50% reduction in the excess weight in 1 to 2 years (17).

An adjustable gastric banding (AGB) device which was introduced by Kuzmak (11) has been adopted and nowadays is widely used in Europe, Australia, and the US. Main advantages of AGB include its simplicity, reversibility, the adjustability of the band's stoma size.

In the medical literature, the SF-36 questionnaire is widely used and well validated in all types of studies as well as in the obesity literature (3). Several studies suggest that quality of life is improved by bariatric procedures in general and that bypass affects the clinical aspects of weight loss and comorbidities more positively than the banding procedure (13, 25, and 27).

In comparative study of laparoscopic banding vs Roux-en-Y gastric bypass *Muller et al.* assessed the QOL using SF-36 and Moorehead-Ardelt II (MA II) questionnaire and found that patients after laparoscopic gastric bypass and laparoscopic gastric banding have a "good" quality of life, as measured by the MA II. This finding was reinforced by the SF 36 that also demonstrated postoperative quality of life scoring similar to normal values. Quality of life indexes were not different between the two operation procedures, as long as the banding procedure was successful without conversion to another procedure in the long term (15). *Sears et al.* in his study has shown that significant weight loss is achieved after gastric bypass surgery and that there is a significant improvement in both quality of life scores and obesity-related conditions (23). *Mui et al.* in prospective study evaluated the outcome of intragastric balloon (IGB) on weight loss and the impact of it on obesity-related illnesses and quality of life. He was able to show that IGB improves seven out of eight domains and both physical and mental component of quality of life of SF-36. It is a very valid option for those who do not opt for or not suitable for bariatric surgery (14). *Chang et al.* in his study included healthy control subjects and found that QOL for patients with morbid obesity in Taiwan is worse than for those of general population and if BMI level is above 32 then lower are the domain scores (4).

In the past decade, the LAGB technique has improved with an estimated 100 000 patients having had this procedure (5). The LAGB procedure coupled with the increased experience of the surgeon seems to minimize the chances of complication.

In our study of the LAGB for the treatment of morbid obesity we analyzed QOL outcomes as well as weight reduction. In our series, we achieved a mean excessive weight loss of 30.4% in the 6 month period which is comparable to results published in literature. 6 month and 12 month after operation compared to preoperative data, QOL improved in all the scales of SF-36v2® but it was not statistically significant due to small number of cases.

To determine the true efficacy of LAGB in weight control and impact on QOL, a longer follow-up period is necessary, more cases to analyze and data from control group of general population.

CONCLUSIONS

For morbidly obese patients weight reduction surgery is the only proven method for long-term weight control. The efficacy of LAGB surgery in long-term is still uncertain, but our early results are comparable to other series. The laparoscopic approach allows early mobilisation, short hospital stay, early return to work, and fewer wound complications. This reversible and adaptable procedure promises to be a good alternative to other bariatric surgeries, provided that the morbidity rate remains low.

Multidisciplinary team consisting of physician, nutritionist, surgeon, together with the psychologist is essential in treatment of obese patients.

Our findings suggest that life quality questionnaire SF-36v2® is good tool to measure patient's quality of life before and after operation, to discuss effectiveness of operations techniques and to observe quality of life dynamics.

Conflict of interest: None

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Table 1. Demographic and clinical characteristics of patients

Total no. of patients	18
Age[a] (year)	42.5 ± 11.8
Gender (F:M)	15/3
Weight[a] (kg)	133.7 ± 20.3
Height[a] (cm)	168.7 ± 9.4
BMI[a] (kg/m^2)	46.2 ± 6.6
% with comorbidities	55.5

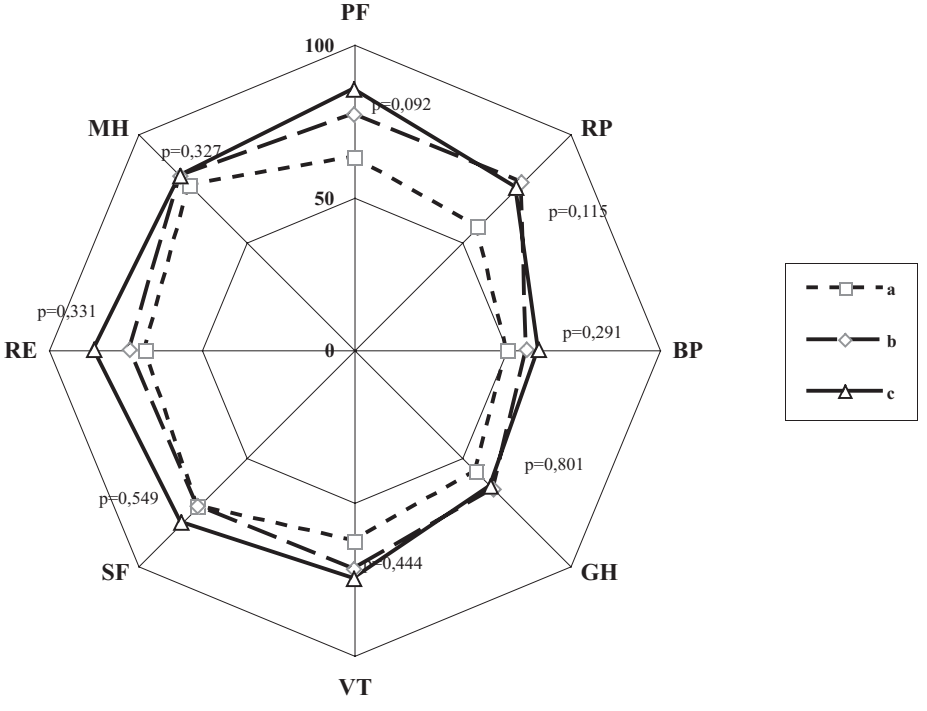
a Mean ± SD

Table 2. Weight reduction results

	Before LAGB n=18	After LAGB (6 month), n=18	*p* value
Weight[a] (kg)	133.7 ± 20.3	113.5 ± 17.8	0.001[b]
BMI[a] (kg/m^2)	46.2 ± 6.6	39.32 ± 6.1	0.001[b]
EBW loss (%)		30.4	

[a] Mean ± SD

[b] Statistically significant



	PF	RP	BP	GH	VT	SF	RE	MH
a	63.00	57.08	50.00	56.40	62.50	72.50	68.33	76.00
b	77.00	77.50	56.60	64.55	71.87	72.50	73.33	80.50
c	85.14	75.00	60.40	63.15	75.00	80.00	85.00	80.25
X^2	4.778	4.333	2.471	0.444	1.625	1.200	2.211	2.235
p[a]	**0.092**	**0.115**	**0.291**	**0.801**	**0.444**	**0.549**	**0.331**	**0.327**

[a] **Friedman test**

Fig 1. Preoperative, 6 month and 12 month postoperative SF-36v2® QOL dynamics

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ORIGINAL ARTICLE

The role of biopsy in differential diagnostics of kidney graft pathology

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Summary
Introduction. Different pathological changes of kidney transplants have similar symptoms, thus differential diagnostic is sometime difficult. The important information that may help to set correct diagnose can be obtained from the kidney transplant biopsy followed by pathohistological investigation.
Aim of the study. The aim of this study is to demonstrate the role of biopsy in differential diagnostics of kidney graft pathology.
Materials and methods. 109 kidney graft biopsies were performed at the Latvian Transplantation Centre in 2007: 20 were protocol biopsies and 89 were performed according to indications (graft dysfunction). All biopsies were performed under USS control followed by pathohistological investigations of the material obtained. Morphological changes were evaluated accordingly to the Banff 97 classification.
Results. The morphological findings were as follows: borderline changes – 8 cases (7.34%); acute cellular rejection – 80 cases (73.4%); acute humoral rejection – 1 case (0,92%); hronic graft nephropathy – 27 cases (24.8%); tubolointersticial nephritis – 36 cases (33%); apostematous nephritis – 1 case (0.92%). We observed mild hematuria in only 2 patients after biopsy which stopped spontaneously in a few days.
Conclusions. Kidney graft biopsy followed by pathohistological investigation of the material obtained is a precise and sensitive method in the diagnostic process of pathological changes of the graft with a small rate of complication.
Key words: kidney transplantation; kidney graft biopsy; kidney graft pathology.

INTRODUCTION

Common symptoms – an increase in the creatinine level in the blood, a rise in body temperature, a decrease in diuresis etc. - characterize different pathological changes in kidney transplants, and differential diagnostic based merely on the clinical symptoms and laboratory tests sometimes is difficult. In these cases a kidney transplant biopsy followed by pathohistological investigation of the material obtained is a very important procedure that can help to set the correct diagnosis, choose the correct therapy and to begin the therapy in time. The important information can be obtained from the so called 0-biopsy, a donor kidney biopsy made before transplantation because the post-transplantation problems could be associated to donor kidney pathology (7).

AIM OF THE STUDY

The aim of this study is to demonstrate the role of biopsy in kidney graft pathology, differential diagnostics and the safety of the method. In order to achieve this aim:

1. All kidney graft biopsies performed from 01.01.2007 to 31.12.2007 were analyzed.
2. All biopsy-related complications were analyzed (number, nature, outcomes).

MATERIALS AND METHODS

109 kidney grafts biopsies were performed at the Latvian Transplantation Centre in 2007. One patient from this group received the kidney from a living donor, all others from deceased donors (Fig. 1). 20 biopsies (18.34%) were analysed - to kidney grafts with stabile function with aim to diagnose subclinical rejection - and 89 (81.66%) were performed according to the indications (graft dysfunction or delayed graft function) (Fig. 2).
Morphological changes were evaluated accordingly to Banff 97 classification (5).
All biopsies were performed under USS control by means of *Vitesse* biopsy set and 18 G *Vitesse* needle twice for each kidney (Fig. 3, Fig. 4).

RESULTS

The morphological findings were as follows:

1. Borderline changes– 8 cases (7.34%);
2. Acute cellular rejection – 80 cases (73.4%);
3. Acute humoral rejection – 1 case (0,92%);
4. Chronic graft nephropathy – 27 cases (24.8%);
5. Tubolointersticial nephritis – 36 cases (33%);
6. Apostematous nephritis – 1 case (0.92%).

(Fig. 5).
We observed mild hematuria in only 2 patients (1.84%) after biopsy, which stopped spontaneously in a few days (Fig. 6).

DISCUSSION

Kidney graft biopsy is one of the main differential diagnostic method of graft pathologies. Normally, the kidney transplant biopsy is done under USS control, in order to avoid surrounding structure damage (kidney vascular system, urethra and intestine). Method precision correlates with the amount of obtained material. An

adequate biopsy is the one where 10 or more glomeruli and at least 2 arteries present. Ideally, the material should be obtained from two separate cortex parts, thus the biopsy is usually done twice, in order to acquire two samples (5). For instance, two samples ensure 99% of method sensitivity in cases of acute rejection whereas one sample ensures 90% (2,6).

The location of the place from where the material is taken is of high importance as well. For instance, in order to diagnose acute rejection reaction, material from the cortex is necessary. If the bioptate generally contains medullar tissues a serious rejection reaction could be missed or underestimated (50% of cases) (1). On the other hand, medullar tissue study can suffice to diagnose other pathologies, for instance, acute pyelonephritis (3), polyomavirus nephropathy (4). For an adequate interpretation of obtained data, it is necessary to provide the pathologist with adequate clinical information. A pathologist should normally know:

- Time posttransplant;
- Donor type (deceased or living);
- Perioperational surgical complications;
- Indications for the biopsy;
- Rapid or slow creatinine level growth;
- Type of immunosuppression;
- Changes in the immunosuppression protocol;
- Use of nephrotoxic drugs;
- Proteinuria;
- Infection;
- Renal artery stenosis;
- US data (hydronephrosis, oedema etc.).

If a pathologist has the above mentioned information he can interpret the pathohistological investigation data more precisely.

Kidney graft biopsy can help to diagnose such pathology as acute humoral and cellular rejection, acute pyelonephritis, polyomavirus nephropathy, CMV infection, calcineurin inhibitor toxicity, *de novo* or recurrent glomerular disease, donor related issues.

CONCLUSIONS

Kidney graft biopsy followed by pathohistological investigation of the obtained material is a precise and sensitive method in diagnostics of pathological changes of the graft with small rate of complications. However, correct interpretation of its data isolated from the clinical picture and laboratory research is impossible. Only a cooperation of physicians and pathologists can ensure correct and timely diagnostics of pathological conditions of a kidney transplant.

Conflict of interest: None

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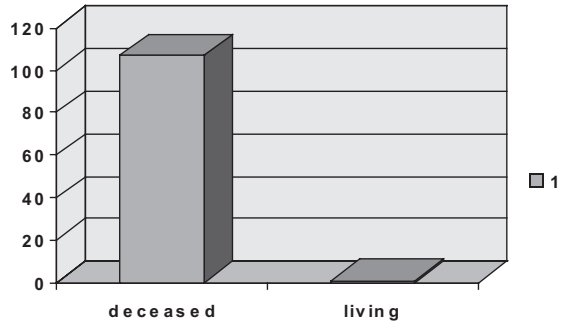

Fig 1. Donor's type

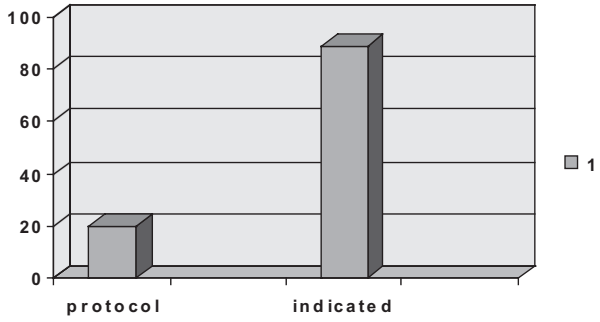

Fig. 2. Aim of biopsy

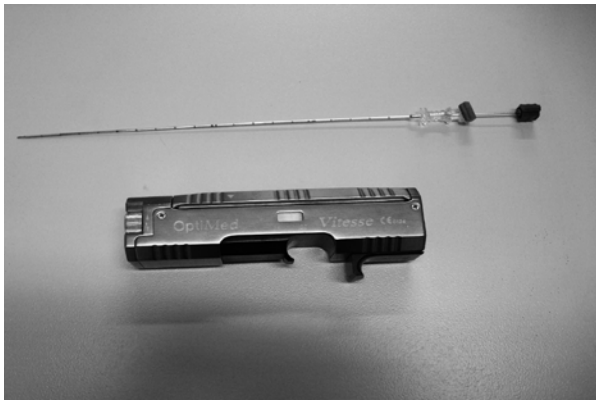

Fig. 3. Biopsy set Vitesse

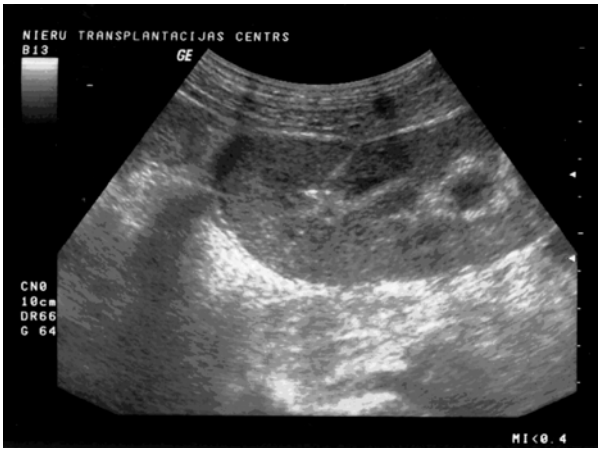

Fig. 4. Kidney graft biopsy under USS control
ACR-acute cellular rejection; IN – intersticial nephritis; HGN – hronic grafēt nephropathy; BC – boderline changes; AN – apostematous nephritis; AHR – acute humoral rejection

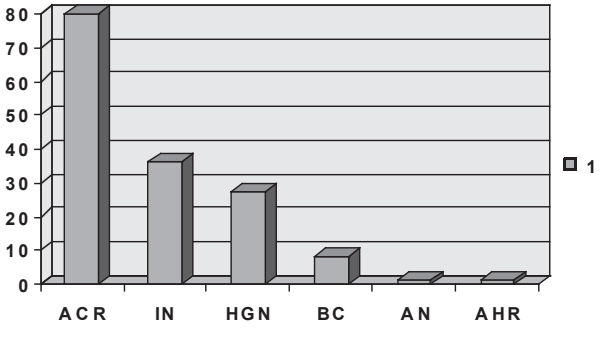

Fig. 5. Morphological findings in the obtained material

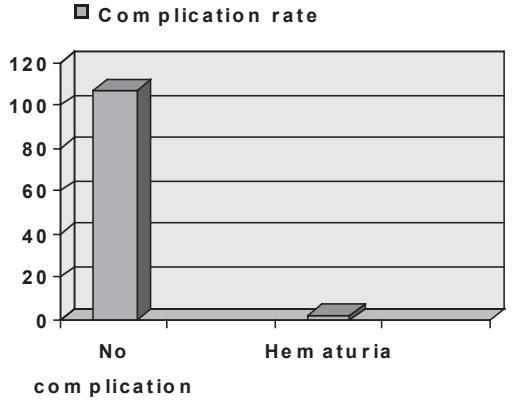

Fig. 6. Complications rate

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ORIGINAL ARTICLE

The Metabolic Changes in Fresh Versus Old Stored Blood Used in Priming of Extracorporeal Circuit in Cardiopulmonary Bypass for Pediatric Patients – First Results

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Summary

Introduction. One of the main goal in modern pediatric bypass equipment is to reduce the size of the extracorporeal circuit in order to minimize the prime volume. High priming volume can produce a low hematocrit on CPB in small infants, which results in electrolyte imbalance and decreased tissue oxygenation, therefore the donor blood should be used in the prime.

The use of donor blood itself has several disadvantages, including complement activation, induction of a transfusion reaction, infusion of lactate, glucose and potassium, therefore this should be avoided as much as possible. When blood is stored, many alterations occur in its constinuents, in particular an increase in potassium and lactate levels, and decrease in pH, which have been associated with severe complications.

Aim of the study. To evaluate the effect of length of storage of packed red blood cells (PRBC) on the concentration of potassium, sodium, lactate, glucose and pH in PRBC used in pediatric cardiac surgery.

Materials and methods. From October,2006 until June,2009 blood samples were drawn from 78 PRBC used in cardiopulmonary bypass before they were added to the priming solution. All PRBC were splited in two groups depending on the age of blood: 5 days≥ (Group 1), n=44 and 5 days< (Group 2), n=34. Blood samples were analyzed by GEM PREMIER 3000.

Results. Seventy eight PRBC used in pediatric cardiac surgery were included in the study. In 44 blood samples the age of PRBC was under 5 days (Group 1) and in 34 blood samples the age of PRBC was over 5 days (Group 2). The mean storage time of PRBC in Group 1 was 4 +/- 1 days and in Group 2 - 8 +/- 3. The pH value in Group 1 was higher than in Group 2 (6.7 +/- 0.1 vs 6.5 +/- 0.2, P=0.000859). There was a significant difference between both groups in terms of potassium level (6.1 +/- 1.8 vs 9.4 +/- 2.6, P <0.0001) and lactate level (7.2 +/- 1.4 vs 10.1 +/- 1.7, P <0.0001), but no significant difference in terms of sodium level (138.3 +/- 3.9 vs 135.6 +/- 4.7, P=0.056688) and glucose level (346.5 +/- 36.1 vs 336.3 +/- 50.7, P=0.509321). There was an intermediate correlation in both groups in terms of storage time and level of potassium, storage time and level of lactate, pH and level of potassium, pH and level of lactate, and the levels of potassium and lactate. There was a linear increase in the levels of potassium and lactate depending on the storage age of PRBC. Conclusions.It is absolutely crucial to use as fresh packed red blood cells as possible in pediatric cardiac surgery to avoid such unexpected complications as transfusion-induced cardiac arrest due to hyperkalaemia. There are also other metabolic changes such as hyperlactemia and low pH, should be avoided, when performing pediatric cardiopulmonary bypass. The research is going to be continued, possibly starting to evaluate the levels of potassium and acid-base balance, when circulating the priming solution for some time before the initiation of cardiopulmonary bypass.

Key words: packed red blood cells; cardiopulmonary bypass.

INTRODUCTION

One of the main goal in modern pediatric bypass equipment is to reduce the size of the extracorporeal circuit in order to minimize the prime volume, which can actually exceed the blood volume of a neonate by as much as 200% to 300%, whereas in an adult patient, the priming volume accounts for only 25% to 30% of the blood volume. Even though nowadays there has been developed smaller tubing and oxygenators, which reduces priming volume significantly, it is still an issue in pediatric cardiopulmonary bypass (CPB). High priming volume can produce a low hematocrit on CPB in small infants, which results in reduction of plasma proteins and clotting factors, decrease of the colloid osmotic pressure (interstitial edema), electrolyte imbalance, exaggerated release of stress hormones with activation of complement, white blood cells and platelets, therefore it is compulsory to use the donor blood in the prime. The use of donor blood itself has several disadvantages, including transmission of viral particles, complement activation, induction of a transfusion reaction, infusion of lactate, glucose and potassium, and citrate-phosphate-dextrose infusion, therefore this should be avoided as much as possible. However, for the reason of hemodilution most institutions use packed red blood cells in their priming solutions (4).

Although packed red blood cells (PRBC) are essential to maintain appropriate hematocrit level and adequate oxygen delivery in children, they are associated with significant metabolic imbalances (acid-base, glucose,

electrolyte), leading to a number of severe complications. When blood is stored, many alterations occur in its constinuents, in particular an increase in potassium and lactate levels, and decrease in pH. The most important changes from the physiological range pointed out by the Ratcliffe JM et al in their research are in sodium, potassium, glucose and lactate concentration. They experienced increase in the level of potassium and lactate, and decrease in the level of sodium and glucose. Besides they pointed out a strong correlation between the duration of storage and the level of sodium, potassium and lactate (7). The biggest problem appears to be hyperkalaemia. Because red blood cell membranes are only slightly permeable to potassium, their movement is largely dependent on energy-dependent transport mechanisms (glycolytically derived adenosine triphosphate). During storage, red blood cell membranes age, adenosine triphosphate synthesis and potassium pumping decrease, and intracellular potassium leaks into the supernatant, which leads to hyperkalaemia (9). There has been reported cases of mortality in neonates during transfusion of stored PRBC, mainly because of hyperkalaemia-induced cardiac arrest, even of a relatively fresh 6-day old blood unit (1,3). Hyperkalaemia related to transfusion depends also on volume and rate of PRBC administration. It was shown that transfusion-associated cardiac arrest may develop with rapid administration of PRBC even with modest transfusion volume (9). Besides, as there is no oxygen in PRBC, the energy is mainly produced in the way of anaerobic glycolysis, therefore there is an increase in the level of lactate and decrease in the level of glucose in the supernatant leading to acidosis (4,5,8).

Hyperkalaemia and hyperlactatemia related to transfusion itself is not a big problem. What makes it an important issue in pediatric cardiac surgery are many other mechanisms contributing to the risks of PRBC transfusion-induced hyperkalaemia or increased potassium cardiotoxicity include hyperglycemia, hypocalcemia, hypothermia and acidosis. First, surgical stress and shock are associated with hyperglycemia, which induces an increase in serum osmolality causing potassium to exit cells. Second, massive transfusion of citrated blood is associated with hypocalcemia, which predisposes to cardiac membrane instability at lower potassium levels. Hypothermia also slows the metabolism of citrate, which exacerbates hypocalcemic states. Third, in hypothermia heart becomes more sensitive to the toxic effects of potassium (9). Therefore many institutional protocols advocate limiting the use of PRBC in priming solution for the pediatric CPB to relatively fresh stored PRBC to avoid such complications.

AIM OF THE STUDY

To evaluate the effect of length of storage of PRBC on the concentration of potassium, sodium, lactate, glucose and pH in PRBC used in pediatric cardiac surgery needed for cardiopulmonary bypass in Children's University Hospital, Clinic for Pediatric Cardiology and Cardiac Surgery.

MATERIALS AND METHODS

From October, 2006 until June,2009 blood samples were drawn from 78 PRBC used in cardiopulmonary bypass before they were added to the priming solution. Fresh PRBCs were preferred and patients would receive old ones only if no fresh PRBC was available. All PRBC were splited in two groups depending on the age of blood: 5 days≥ (Group 1), n=44 and 5 days< (Group 2), n=34. The groups were made according to the mean age of PRBCs available in the blood bank of our hospital for pediatric cardiac surgery. Blood samples were drawn from the PRBC, stored at 4°C in preservative solution, consisting of citrate, dextrose, phosphate and adenine (CP2 and SAGM), and were analyzed by GEM PREMIER 3000. Comparisons between corresponding variables at different times in each group were carried out by using the Student t test. There were calculated correlation index between different levels of electrolytes and the age of PRBC.

RESULTS

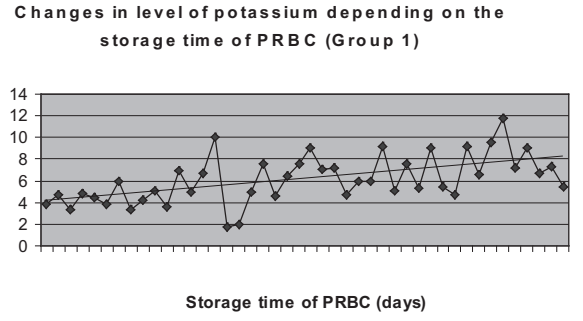
Seventy eight PRBC used in pediatric cardiac surgery were included in the study. In 44 blood samples the age of PRBC was under 5 days (Group 1) and in 34 blood samples the age of PRBC was over 5 days (Group 2). The mean storage time of PRBC in Group 1 was 4 +/- 1 days and was significantly shorter compared with the storage time in Group 2 (8 +/- 3 days, P=0.000264). The pH value in Group 1 was higher than in Group 2 (6.7 +/- 0.1 vs 6.5 +/- 0.2, P=0.000859). There was a significant difference between both groups in terms of potassium level (6.1 +/- 1.8 vs 9.4 +/- 2.6, P <0.0001) and lactate level (7.2 +/- 1.4 vs 10.1 +/- 1.7, P <0.0001), but no significant difference in terms of sodium level (138.3 +/- 3.9 vs 135.6 +/- 4.7, P=0.056688) and glucose level (346.5 +/- 36.1 vs 336.3 +/- 50.7, P=0.509321) (*TABLE 1*).

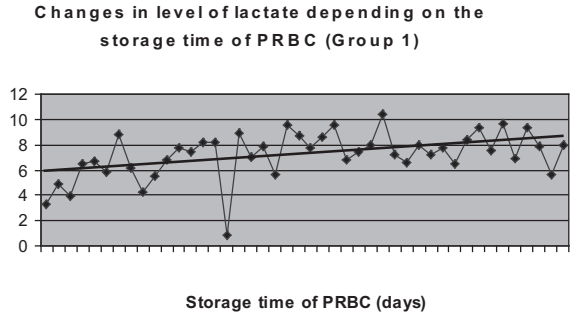
Table 1. Mean values +/- SD of different parameters in both groups

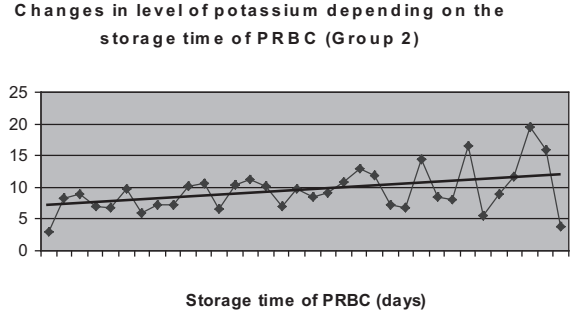
	Goup 1 (n=44)	Group 2 (n=34)	P value
Storage time	4 +/- 1	8 +/- 3	0.000264
pH	6.7 +/- 0.1	6.5 +/- 0.2	0.000859
Potassium	6.1 +/- 1.8	9.4 +/- 2.6	<0.0001
Sodium	138.3 +/- 3.9	135.6 +/- 4.7	0.056688
Glucose	346.5 +/- 36.1	336.3 +/- 50.7	0.509321
Lactate	7.2 +/- 1.4	10.1 +/- 1.7	<0.0001

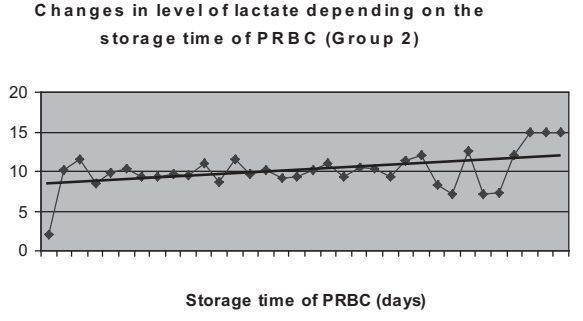
There was an intermediate correlation in both groups in terms of storage time and level of potassium (correlation coefficient 0.42 vs 0.24) (*Figure 1* and *Figure 2*), storage time and level of lactate (correlation coefficient 0.45 vs

0.6) *(Figure 3* and *Figure 4*), pH and level of potassium (correlation coefficient 0.49 vs 0.77), pH and level of lactate (correlation coefficient 0.61 vs 0.55), and the levels of potassium and lactate (correlation coefficient 0.61 vs 0.6). There was a linear increase in the levels of potassium and lactate depending on the storage age of PRBC (*Figure 1, Figure 2, Figure 3, Figure 4*).









DISCUSSION

As this is compulsory to use RPBC for priming the extracorporeal circuit in pediatric cardiac surgery to maintain the adequate hematocrit and oxygenation, it is very important to have as fresh PRBC as possible, because fresh ones are presumably more balanced metabolically than stored PRBCs. They contain less potassium and lactate, more glucose, and higher pH. It is an important issue in pediatric cardiac surgery due to many other mechanisms contributing to the risks of PRBC, such as surgical stress, shock, hypothermia.

One of our major concerns with prolonged storage was excess potassium in the red cell supernatant, which could cause cardiac problems. There has been reported cases of mortality in neonates during transfusion of stored PRBC, mainly because of hyperkalaemia-induced cardiac arrest, even of a relatively fresh 6-day old blood unit (1,3). What is more, in neonatal surgery, the stored blood may be irradiated, if there is a possibility of impaired imunity, which may cause a further increase in potassium levels (5,10,11). There have been a number of researches showing a significant increase in potassium in old PRBCs (5,7,9,10,11). Our results clearly shows that potassium level was significantly higher in old stored PRBCs, and there was an intermediate correlation in both groups in terms of storage time and level of potassium. The other papers shows that there is an increase in the level of lactate and decrease in the level of glucose in the supernatant leading to acidosis due to the energy mainly being produced in the way of anaerobic glycolysis (4,5,8). We experienced the same increase in concentrations of lactate and decrease of pH, and an intermediate correlation in both groups in terms of storage time and level of lactate. Besides there was also an intermediate correlation between the levels of potassium and lactate, which made us think that all metabolical processes taking place in PRBCs during the storage could be conjoint. What is the most important thing what we can see in our results is a linear increase in the levels of potassium and lactate depending on the storage age of PRBC, therefore it is compulsory to use as fresh PRBCs as possible in pediatric cardiac surgery.

Our other results shows only a slight decrease in the level of sodium and glucosis during storage, similarly stated by Ratcliffe JM et al in their research (7).

CONCLUSIONS

In conclusion, our findings shows that it is absolutely crucial to use as fresh packed red blood cells as possible in pediatric cardiac surgery to avoid such unexpected complications as transfusion-induced cardiac arrest due to hyperkalaemia. Besides there are other metabolic changes such as hyperlactemia and low pH, should be avoided, when performing pediatric cardiopulmonary bypass. The research is going to be continued, possibly starting to evaluate the levels of potassium and acid-base balance, when circulating the priming solution for some time before the initiation of cardiopulmonary bypass, or the change of quality of PRBCs according to the age.

Conflict of interest: None

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ORIGINAL ARTICLE

Intraabdominal Pressure in Children After Cardiothoracic Surgery

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Summary

Introduction. Intraabdominal pressure (IAP) now is widely recognized as an important variable and its monitoring is used in a variety of critically ill patients.

Aim of the study. The aim of this study was to measure and to recognize the influence of various factors on IAP in children after surgical correction of congenital heart disease.

Materials and methods. We conducted non-randomized, prospective observational study in Pediatric intensive care unitat a University Children's hospital. Study protocol was approved by Hospital Ethics commision. Measurements of IAP were performed in 15 children with mean body weight 8±5,83 kg, (Range 3,1-28 kg), mean age of 18,01 months (range 8 days-8 years) after cardiothoracic surgery. Cardiopulmonary bypass (CPB) was used in 12 patients. IAP was measured during first 24 hours postoperatively at 12 hour intervals via indwelling urinary catheter with bladder volumes of 1 ml/kg of normal saline. Of the 15 patients, 12 were mechanically ventilated at the time of the IAP measurements. Ventilation pressures: PIP (peak inspiratory pressure), MAP (mean airway pressure), PEEP (positive end expiratory pressure) and central venous pressure (CVP) via femoral vein were recorded. In some patients (6 from 15) amount of fluid evacuated via intraperitoneal drain from peritoneal cavity in first 24 hours was measured.

Results. IAP was 12,24±3,54 mm Hg (Range 5,44-20,4 mm Hg), CVP 13±2,19, PIP 20±2,48 cm H2O, MAP 9±2,3 cm H2O, PEEP 5±1,35 cm H2O. Amount of fluid removed from peritoneal cavity during first 24 hours was 0,8±0,54 ml/kg/h (Range 0,04-1,7 ml/kg/h).

Conclusions. We find elevated intraabdominal pressure (IAP>12 mm Hg) in 10 from 15 (66,67%) pediatric patients in the first 24 hours after cardiothoracic surgery.

There was a difference in IAP in patients with abdominal paracenthesis versus patients without the drain. The difference between groups was not statistically significant (P=0,4). We did not find a correlation between IAP, MAP and CVP. We, however, did not observe development of abdominal compartment syndrome (ACS).

Key words: intraabdominal pressure; intraabdominal hypertension; abdominal compartment syndrome; cardiothoracic surgery.

INTRODUCTION

The impact of elevated IAP were described in the 19th century but were only recognized as a significant problem in surgical adults in the 1980s. However, since the 1940s pediatric surgeons have observed multisystem organ failure associated with increased IAP following primary closure of congenital abdominal wall defects and were the first to use prosthetic materials for abdominal decompression (1). In 1989, Fietsam et al. (2) introduced the term "abdominal compartment syndrome" (ACS) to describe the pathophysiological effects of increased IAP. The authors reported four patients who developed oliguria, hypoxia, hypercapnia, increased PIP, and increased CVP associated with massive abdominal distension in the postoperative period of ruptured abdominal aortic aneurysms. Over the last decade, ACS has been increasingly diagnosed in critically ill patients, which has coincided with a significant increase in the number of publications related to this topic. Recently, a group of critical care specialists convened at the second World Congress on ACS and developed consensus definitions and guidelines for the diagnosis and treatment of IAH and ACS. In healthy individuals, a normal IAPis 5 to 7 mm Hg according to the consensus definition of the World Society of Abdominal Compartment Syndrome (WSACS, http://www.wsacs.org/). The upper limit of IAP is generally accepted to be 12 mm Hg by the World Society (3). Clinical conditions that can lead to IAH are well known in pediatrics. Life-threatening IAH has been well described in neonates born with gastroschisis and omphalocele, when primary closure of the abdominal defect was attempted, resulting in decreased thoracic compliance and in hemodynamic compromise (4). Surgical techniques currently used to treat ACS, like temporary abdominal closure with synthetic materials and staged abdominal repair, were pioneered in these patients (5). IAH and its effects also were described in children with major burns (6), resulting in poorer prognosis. Clinical presentation of ACS is similar to adults, but children may develop ACS at a lower IAP (as low as 16 mm Hg) (7). Direct IAP measurement is impractical in most situations and the most common method is an indirect measurement via the bladder, a technique that has been validated (8) and which correlates well with IAP (9).

Number of studies are devoted to describe cardiovascular effects of elevated IAP. IAH leads to a reduction in cardiac output (CO). Although this effect may be seen with IAP as low as 10–15 mmHg, it is most consistently seen at an IAP greater than 20 mmHg (10,11). The decrease in CO is related to diminished venous return, increased peripheral resistance, or increased intrathoracic pressure. Venous return is reduced by a number of mechanisms (12). Increased IAP leads to reduction in caval and retroperitoneal venous flow. Venous flow is also reduced by functional narrowing of the inferior vena cava at the suprahepatic, subdiaphragmatic level, where the high pressure zone of the abdomen meets the lower pressure zone of the thorax. Elevation of peripheral vascular resistance is likely to be related to mechanical compression of capillary beds. IAH increases intrathoracic pressure by elevating the diaphragm. As a result, ventricular filling pressure increases while ventricular compliance decreases. All these factors (diminished venous return, increased peripheral resistance, and increased intrathoracic pressure) lead to a reduced stroke volume with compensatory increase in heart rate. The blood pressure usually remains unchanged (13).

AIM OF THE STUDY

The aim of this study was to measure IAP and to recognize relationship of various factors (CVP, variables during mechanical ventilation, amount of fluid accumulated in the abdominal cavity) on the IAP in pediatric patients after surgical correction of congenital heart disease. We hypothesized that IAP in patients after cardiothoracic surgery is close to normal range.

MATERIALS AND METHODS

We conducted non-randomized, prospective observational study in Pediatric Intensive care unit at the University Children's Hospital. Study protocol was approved by Hospital Ethics commision. Measurements of IAP were performed in 15 children, with mean body weight of 8±5,83 kg, (Range 3,1-28 kg), mean age of 18,01 months (range 8 days-8 years) after the surgical correction of congenital heart disease. (Fig. 1). Surgical interventions, performed on patients, are summarized in Table 2. Cardiopulmonary bypass (CPB) during operative procedure was used in 12 patients. IAP was measured during first 24 hours postoperatively at 12 hour intervals via indwelling urinary catheter in supine position with bladder volumes of 1 ml/kg of normal saline. The end of the catheter was connected to transparent, open ended plastic tubing, and the level of the water column above the midaxillary line reflects IAP. Of the 15 patients, 12 were mechanically ventilated with Avea (Viasys Respiratory Care Corp., USA) and Evita 4 (Dräger Medical, Lübeck Germany) ventilators in a pressure control mode to keep patients blood gases within normal range. Ventilation pressures: PIP (Peak inspiratory pressure), MAP (Mean airway pressure), PEEP (Positive end expiratory pressure) and central venous pressure via femoral vein (Fig.2) simultaneously with IAP were recorded. To avoid ventilation induced lung injury and to ensure normal venous blood return to right heart PIP was limited to 25 cm H_2O and PEEP to 5-7 cm H_2O. In some patients (6 from 15) to prevent fluid accumulation in abdominal cavity peritoneal paracenthesis was performed introperatively and catheter was connected to reservoir. Amount of fluid evacuated via catheter from the abdominal cavity in the first 24 hours was measured. Descriptive statistics for mean, standard deviation and range were used to describe the sample. Coeficient of determination (r^2) was used to determine the relationship between two variables. Significance level $P<0,05$ was considered statistically significant. Statistical analyses were performed using Microsoft Excell Data analysis tool.

RESULTS

In our study group IAP was 12,24±3,54 mm Hg (Range 5,44-20,4 mm Hg). Majority of patients (60%) had IAP in the range of 12-20 mmHg, one third of children (5-33%) had normal IAP (<12 mmHg), only one patient had abdominal hypertension, sligtly exceeding 20 mmHg (Fig.6.). CVP was measured via femoral vein catheter with its tip in the inferior v. cava (IVC), mean CVP was 13±2,19 mmHg, PIP 20±2,48 cm H_2O, MAP 9±2,3 cm H_2O, PEEP 5±1,35 cm H_2O. Amount of fluid removed from peritoneal cavity during first 24 hours was 0,8±0,54 ml/kg/h (Range 0,04-1,7 ml/kg/h). (Table 1). The difference in IAP between children without catheter inserted in abdominal cavity and those who had peritoneal drainage was not statistically significant (P=0,4, Fig. 5). Our study demonstrates a weak inverse correlation between IAP and MAP (Fig.3) and weak positive correlation between IAP and CVP, measured in the IVC via femoral vein (Fig.4).

DISCUSSION

We did not expect such a large number of patients (66,67%) with elevated (>12 mmHg) IAP and that finding did not confirm our initial hypothesis**.** It is difficult to determine precisely the direct cause of IAP elevation. The complicated flow pattern during CPB as well as intra- and postoperative therapy may result in such pathology. Nevertheless, hemodilution and inflammatory response, which induce tissue oedema, seem to be the most important factors. Recently, the effect of normovolemic blood dilution during CPB was documented (14). Moreover, aggressive fluid resuscitation was associated with an increase in gut permeability, which leads to intestinal edema (15). Interestingly, the beginning of CPB results in decreased colloid osmotic pressure, increased microvascular permeability and increased capillary pressure (16). Several authors demonstrated that CPB induced the inflammatory reaction, which led to tissue oedema. According to Tassani et al. (17), who analyzed the microvascular protein escape before and after newborn cardiac procedures, CPB resulted in increased levels of inflammatory cytokines, such as IL- 6 and IL-10, and decreased plasma colloid pressure. Furthermore, the radiologic oedema was observed

in the children examined. Likewise, Seghaye and colleagues (18) reported the inflammatory response at the beginning of CPB. According to them, this reaction indicated microvascular permeability, which led to total body water accumulation. Importantly, this pathology is observed in each cardiac surgery patient, yet predominantly in paediatric patients (17,18).

There is also some controversy with regard to the effect of mechanical ventilation and the use of PEEP on IAP. Expansion of the abdominal cavity from elevated IAP results in a cephalad displacement of the diaphragm with reduction in dynamic pulmonary compliance and a requirement for increasing PEEP to deliver the same tidal volume. Sussman (19) was the first to look at the effects of PEEP on IAP and showed in their experiment that increasing PEEP to 15 cm of H_2O did not affect the IAP. This was confirmed by Guimaraes and animal data (20). However, on increasing PEEP to 15 cm of H_2O, others have found only a mild increase in IAP in patients with a baseline IAP below 12 mmHg (21). Further, in patients with a baseline IAP above 12 mmHg, the effect of PEEP seems to be more pronounced (22). Our patients were ventilated in "protective mode" with limited PIP to avoid lung overdistension, volutrauma and ventilation-induced lung injury, PEEP was limited too, in order to minimize effect of increased intrathoracic pressure to right atrial filling (23). Therefore our study did not show any influence of ventilation variables on IAP.

In 6 patients (from 15) to prevent fluid accumulation in abdominal cavity peritoneal paracenthesis was performed intraoperatively. Percutaneous catheter drainage of free intraabdominal fluid, air, abscess, or blood is an effective technique for reducing IAP and potentially correcting IAH-induced organ dysfunction (24, 25). Percutaneous decompression can significantly reduce IAP and decrease morbidity of surgical decompression. Removal of even small volumes of fluid can significantly lower IAP (26). This minimally invasive approach to IAH/ACS management is most effective in patients with secondary ACS due to excessive fluid resuscitation, burns, acute pancreatitis, or ascites (27).

CONCLUSIONS

We find elevated intraabdominal pressure (IAP>12 mm Hg) in 10 from 15 (66,67%) pediatric patients in the first 24 hours after cardiothoracic surgery.

There was a difference in IAP in patients with abdominal paracenthesis versus patients without the peritoneal drainage**.** The difference between groups was not statistically significant (P=0,4).

We did not find correlation between IAP, MAP and CVP, measured in the IVC. We, however, did not observe development of abdominal compartment syndrome.

Preemtive abdominal paracenthesis in children after cardiac surgery prevents fluid accumulation and uncontrolled rise in IAP, leading to ACS.

Conflict of interest: None

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Table 1.

Summary of results

Variables	Mean	Range	SD
Age, months	18,01	14 days-8 years	±24,08
Weight, kg	8,93	3,1–28	±5,83
IAP mm Hg	12,3	5,44–20,1	±3,54
CVP (via femoral vein), mm Hg	12,68	10–19	±2,19
PIP cm H_2O	19,17	15–23	±2,48
MAP cm H_2O	9,28	6–15	±2,30
PEEP cm H_2O	5,06	4–6	±1,35
Fluid removal, ml/kg/h	0,88	0,04–1,7	±0,63
IAP (mm Hg) in non- ventilated pts	13,87	9,52–20,4	±4,13
IAP (mm Hg) in ventilated pts	11,86	5,44–17,68	±3,36
IAP (mm Hg) in pts with drain	11,79	5,44–20,4	±4,08
IAP (mm Hg) in pts w/o drain	13,6	6,8–17,68	±3,85

Abbreviations: IAP-intraabdominal pressure, CVP-central venous pressure, PIP-peak inspiratory pressure, MAP-mean airway pressure, PEEP-positive end expiratory pressure

Table 2.

Operative procedures

Type of surgical intervention	No of pts.
Coarctation of the aorta repair	2
Patient ductus arteriosus ligation	1
Tetralogy of Fallot repair	2
Atrial septal defect closure	2
Ventricular septal defect closure	6
Atrioventricular septal defect closure	2
Total:	15

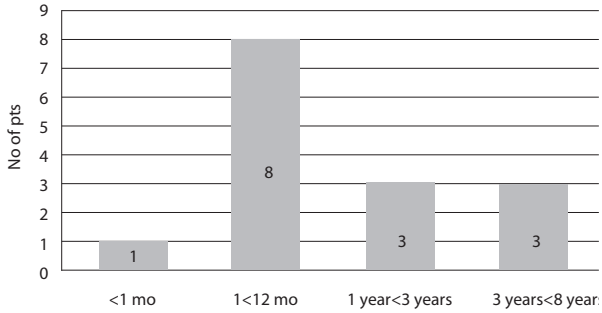

Fig. 1. Age of patients

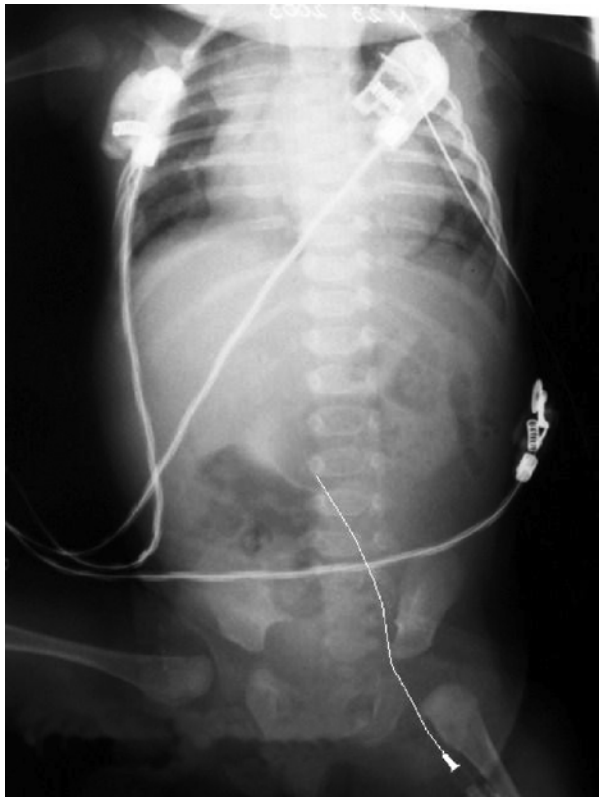

Fig. 2. Catheter passed through femoral vein with its tip in the inferior v. cava

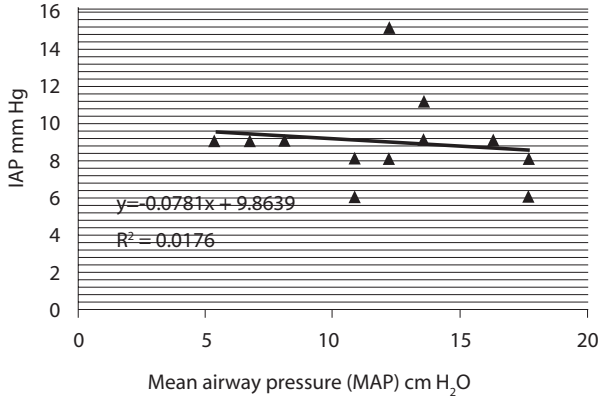

Fig. 3. MAP versus IAP

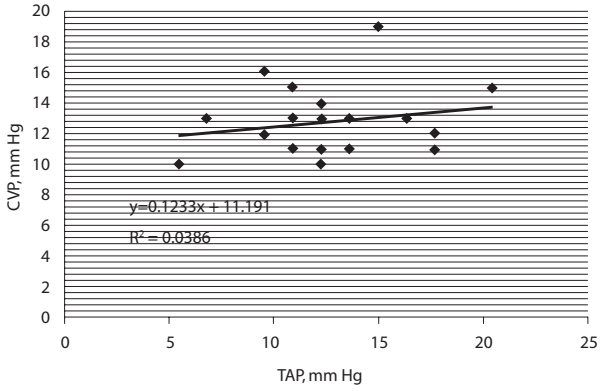

Fig. 4. CVP versus IAP

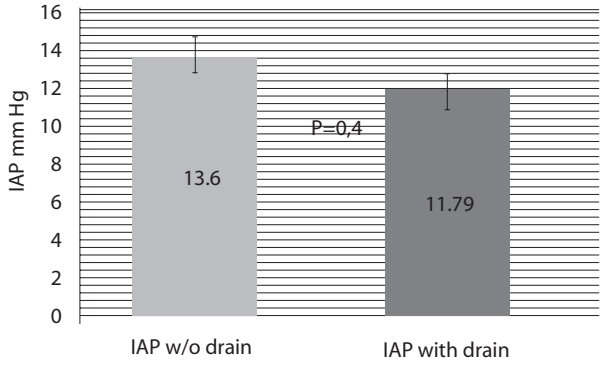

Fig. 5. Abdominal decompression and IAP

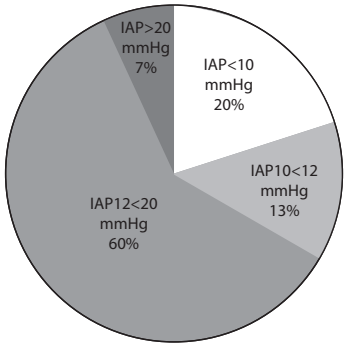

Fig. 6. Patients grouped by IAP level

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ORIGINAL ARTICLE

Carotid Endarterectomies in Latvia

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Summary

Introduction. Carotid endarterectomy (CEA) is a treatment of choice for significant carotid stenosis, and it has an important role in stroke prophylaxis, that is proved in many trials all over the world.

Aim of the study. To evaluate preoperative diagnostic methods, risk factors and co-morbidities, operation results for patients undergoing CEA in Latvia.

To analyze used anaesthasia and operation technique in CEA in Latvia.

To reflect carotid endarterecomy results that depends from chosen operation technique.

Materials and methods. 317 patients were included in the retrospective study from January 1st till December 31st, 2008. Patient charts were evaluated for information about history of disease and other comorbidities, preoperative investigation, operation and early postoperative period followed by protocol. All patients were divided into three groups depending on type of endarterectomy (CEA with primary suture, with patch or eversion CEA).

Results. In 59% cases carotid disease were asymptomatic. Computer tomography angiography (CTA) and duplex ultrasound were the most common diagnostic methods to evaluate carotid stenosis. 339 carotid endarterectomies were performed, where CEA with primary suture was used in 56.2% of cases (group1). There were no significant diferences in patient atherosclerotic risks factors, comorbidities, preoperative investigation methods, time of surgery and complications rate between the groups.

Conclusions. In Latvia carotid endarterectomy is carried out mostly in classical conventional tecniques and arteriotomy is being closed with a primary suture.

Key words: carotid endarterectomy (CEA).

INTRODUCTION

Ishaemic stroke is one of the main causes of death in the world, and the leading cause of morbidity in many countries. Mortality rate from stroke is 10-30% and there is a high risk for recurrent stroke or trancient ischaemic attack, and coronary syndrome as well for survivors (1, 2).

In 2007 there were 222 cases of death on 100 000 persons from cerebrovascular events in Latvia (3).

Atherosclerosis of brachiocephalic arteries, especially internal carotid artery is the main cause of ischaemic stroke.

Carotid endarterectomy is a conventional surgical method, which was established in 1954, and widely used at present. CAE is evacuation of atherosclerotic plaque with intima of vessel in carotid bifurcation and in segmental proximal part of internal carotid artery (4).

There were two large clinical trials in Europe and North America, which approved, that CAE has a significant benefit in ishaemic stroke prophylaxis. In 1991 published NASCET (North American Symptomatic Carotid Trial) and ESCT (European Carotid Surgery Trial) results showed, that CAE is more effective in reducing recurrent cerebrovascular events than conservative treatment for symptomatic carotid patients. NASCET trial results reflected, that after 2 year follow-up relative risk reduction was 81% in carotid endarterectomy group in comparison with medical therapy. Very similar results were found in European ESCT trial. After 3 year follow-up risk for stroke was 2.8% in carotid endarterectomy group vs 16.8% in medical treatment group, respectively there were sixfol reduction of risk. Comparing to previously mentioned trials, Veterans Affairs Symptomatic Trial (VAST) showed a significant reduction of risk in CAE group in symptomatic patients as well. After one year follow-up risk for cerebrovascular events was 7.7% in endarterectomy group vs. 19.4 in conservative treated pacients (4, 5, 6).

Similar trials were done for asymptomatic carotid artery disease as well. In return Veterans Affairs Asymtomatic Trial (VAAT) was one of the first trials that demonstrated stroke reduction for patients, who received prophylactic carotid endarterectomy. ACAS (Asymptomatic Carotid Atherosclerosis Study) trial showed, that perioperative risk for stroke was 5.1% in cases of surgery group vs. 11% in non-surgical group, that means operation reduces relative stroke risk for 53%. In other asymptomatic carotid trial - Asymtomatic Carotid Surgery Trial (ACST) risk of fatal stroke, fatal and disabling stroke and all strokes were assesed. The study reflected in 5 year follow-up, that risk for fatal stroke were 2.1% in carotid endarterectomy group vs 4.2% in conservative

group, for fatal and disabling stroke 3.5% vs 6.1% and all strokes were 6.4% vs 11.8%. ACST demonstrated that stroke risk for women reduces after longer time after CAE, while in men population a significant benefit from surgery is observedfaster . In ACAS trial there were no significant benefit from CAE for women proven (7, 8, 9, 10).

Two invasive treatment options for severe carotid artery stenosis are moust used in medical community today – carotid endarterectomy and carotid stenting.

Indications for invasive treatment are based on five aspects: neurologic symptoms, degree of carotid stenosis, comorbidities, local and vascular anatomy features, morphology of the atheroma. The choice of invasive or conservative treatment is determined by neurologic symptoms of patients and degree of carotid stenosis. Comorbidities, local and vascular anatomy features and morphology of the atheroma are important aspects of the selection of the type of invasive treatment. The choice of the treatment is determined also by complication severity and incidence of treatment (11).

According to European Society of Vascular Society guidelines for invasive treatment of carotid stenosis indications for carotid endarterectomy is for symptomatic patients with stenosis 70-99% and contraindicated for symptomatic patients with less than 50% stenosis. For symptomatic patients perioperative stroke or death rate should be less than 6%.

For asymptomatic patients CAE is recommended for men with carotid stenosis 70-99%, if surgical risk is less than 3%. For women with asymtomatic carotid stenosis benefit from carotid endarterectomy is significantly less than for men. Therefore there are still many discussions about the choice of the method of treatment (11).

Recently the carotid stenting is becoming as a promising method to treat carotid stenosis. However European Society of Vascular Society collaborator group for treatment of carotid stenosis, concluded, that there is a need for more trials to find suitable treatment for carotid stenosis in special patient groups. Therefore today carotid stenting is used for patients with high surgical risk, but carotid endarterectomy is still a treatment of choice (11).

Carotid endarterectomy can be performed under local or general anaesthesia, both methods are safe (11). This been confirmed by GALA (General Anaesthesia vs. Local Anaesthesia) trial recently (12).

GALA Trial was the largest trial where the methods of anaesthesia were compared for 3526 patients undergoing CAE. Results showed, that there is no significant diferences in operation results in general and local anaesthesia groups. GALA trial proved that surgical complications are less than in NASCET and ECST trials. It might be a sign that the results of carotid endarerectomy are improving. Vascular Surgery centers of Latvia took part in GALA trial as well (12).

Carotid endarterectomy can be performed in several tehniques: carotid endartectomy with primary suture, with synthetic or autologous patch or eversion endarterectomy.

There are some trials comparing carotid endarterectomy with primary suture and with patch. In groups of carotid endaterectomy with a patch there were significantly less perioperative stroke and death rates in early postoperative period, as well less restenosis risk in late postoperative period, but quality of those trials were poor, and results are not considered consistant. Studies, where eversion endarterectomy vs conventional endarterectomy with patch were compared showed no significant diferences in perioperative stroke, death rates and in risk of restenosis. Today there are no recomendations in which technique carotid endarterectomy should be performed. Worldwide all techniques are used by the decision of the surgeon (13, 14).

Cerebral blood supply through carotid artery are temporaly interrupted on time of CAE. Intraluminal shunts can be used to decrease the time of temporal occlusion. In trials, that compared operation results depending on the use of intraluminal shunts, there were no significant diferences in both groups. There is no evidence of the benefit of routine use of intraluminal shunts (15).

Stroke and death are the most serious CAE complications. The number of cerebrovascular events was 6,5% in NASCET,of, 5,8% in ECST and 5,5% in VAST trial. In asymptomatic carotid trials cerebrovascular events are more rare, for example, in ACAS trial only 1,1 %. Deathmakes 0,6% in NASCET and ECST trials in perioperative and early postoperative period,but 0,1% in ACAS trial (4, 5, 6, 8, 11).

Some cranial nerve disturbances have been reported after CAE. Temporal or permanent nerve disturbances develop in 2-15% of cases. *n. hypoglossus* and *n. facialis* are more often involved in damages (4).

Wound haematomas are rear and been reported in 1-3% of cases. Cardiovascular events like myocardial infarction develops around 1% for patients after CAE, in NASCET trial non-fatal myocardial infarctions were observed in 0,8% of cases and 0,4 % in ACAS trial (4, 5, 16).

In the same time perioperative CAE results have not been reported so far.

AIM OF THE STUDY

To evaluate preoperative diagnostic methods, risk factors and co-morbidities, operation results for patients undergoing CAE in Latvia.

To reflect carotid endarterecomy results depending from the chosen operation technique and anesthesia.

MATERIALS AND METHODS

The retrospective analysis of all patients operated in vascular surgery centers in Latvia from January1st till December 31st ,2008 was performed. 317 consecutive patients undergoing 339 CAE at Pauls Stradins Clinical University Hospital and Riga Eastern Clinical University Hospital were included into study.

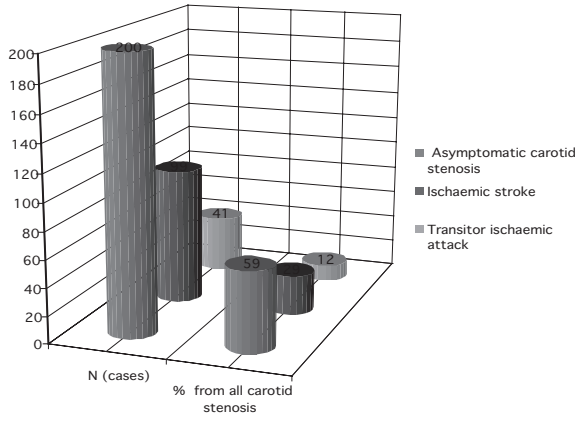
Severity of carotic stenosis was evaluated with duplex ultrasound (US) examination, computer tomography angiography (CTA), or digital subtraction angiography

(DSA), or magnetic resonance angiography (MRA).
Following atherosclerosis risk factors were evaluated: arterial hypertension, diabetes mellitus and dyslipidemia. 80% of all patients were on statin therapy, 95% were on aspirin. Significant comorbidities were analyzed: coronary hart disease, chronic heart failure, peripheral artery occlusive disease and abdominal aortic aneurysm.
The following information about the operation was collected: duration of surgery, operation technique, type of anaesthesia, use of intraluminal shunt and active aspiration dranagee.
Following postoperative complications were evaluated such as: stroke or transitor ishaemic attack, cranial nerve injuries (temporal and permanent), myocardial infarction, pulmonal thrombembolism, wound complications (haematoma and infection).
First 24 postoperative hours all patients were in intensive care unit, where they received prophylactic antibacterial therapy and correction of arterial hypertension, if indicated.
Chi-Square test was used to analyze the results, and p-values were evaluated.

RESULTS

The average age for patients was 67 years (45 - 85 years). From all patients 112 were women (35%) and 205 men (65%). All carotid artery stenosis were significant (70-99%) by international guidelines. 200 (59%) of patients were asymptomatic. Distribution of neurological symptomology is depicted in Table 1.

Table 1. Distribution of neurological symptomology for patients with symptomatic carotid stenosis



Distribution of asymptomatic carotid artery stenosis in all study groups is shown in Table 2.

Table 2. Asymptomatic carotid artery stenosis

Group	Asymptomatic carotid stenosis (cases; n)	% from all carotid stenosis in each group
1. group (CAE with primary suture)	93	59
2. group (CAE with a patch)	27	48
3. group (eversion CAE)	79	65

Arterial hypertension in 70% of cases was as a dominant atherosclerosis risk factor. In 32% of cases patients surferred from coronary artery disease and severe heart failure (NYHA class III and IV).
Atherosclerosis risk factors and comorbidities are summarized in Table 3 and 4.

Table 3. Atherosclerosis risk factors for the patients undergoing CAE

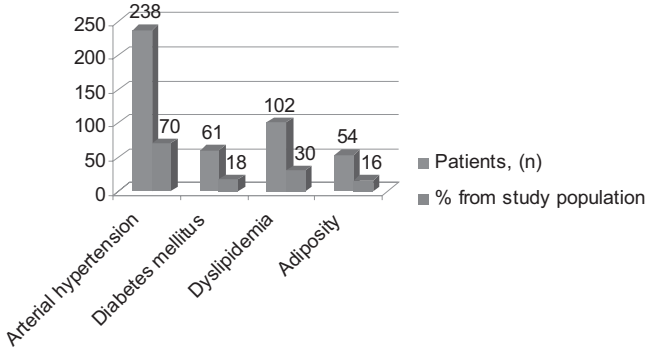
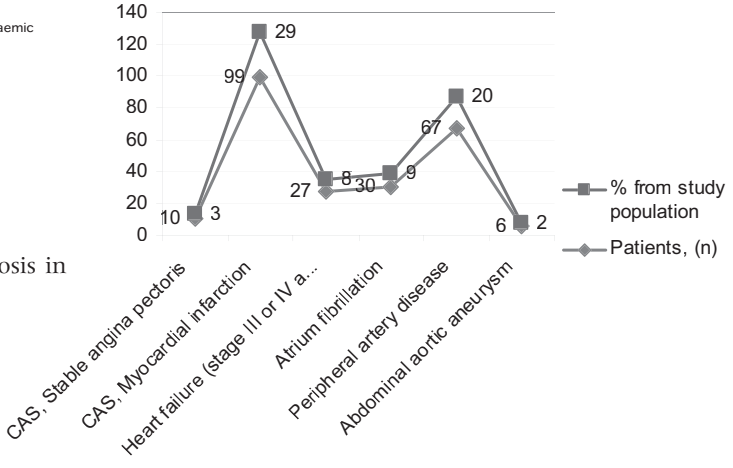


Table 4. Main comorbidities for study population undergoing CAE



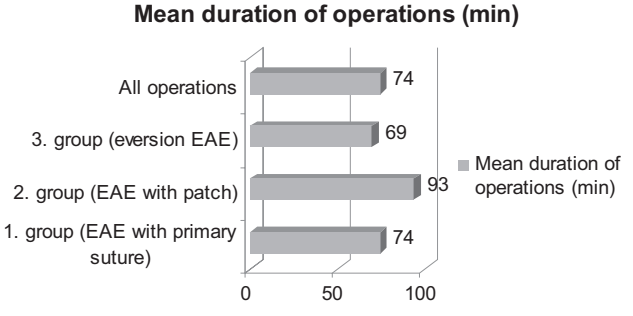
CTA was more often used investigation method for evaluation of carotid artery disease. In 43.6% of cases carotid arteries were investigated with CTA only. Significant role in carotid evaluation belonged to US, which in combination with CTA was used in 33,9% of cases, but it was seldom used as a single diagnostic tool (13,5%). DSA and MRA were rarely used as diagnostic methods for carotid arteries (Table 5).

Table 5 Choice of methods in preoperative investigation of carotid artery

Method	Cases (n)	%
US	46	13.5
CTA	148	43.6
US + CTA	115	33.9
US + DSA	11	3.2
CTA + DSA	6	1.7
US + MRA	7	2.0
Only DSA	4	1.1
US + CTA + DSA	2	1.0

In total 339 carotid endarterectomies were made in one year period in Latvia. 158 (46.3 %) of cases underwent conventional endarterectomy with primary suture (Group 1), 56 (16,5%) of cases were made in conventional endarterectomy with synthetic patch (Group 2) and 122 (36.9%) of cases received eversion endarterectomy (Group 3). Five carotid endarerterectomies were made in conventional open endarterectomy with Y-type reconstruction of internal and external carotid artery.
334 surgeries were done under general anaesthesia and only 5 operations were performed under local anaesthesia (1.5%). The average duration of operation was 74 minutes (40 – 135 minutes). Operations were significantly longer in Group 2. Results of duration of operation are showen in Table 6.

Table 6. Mean duration of CAE surgery



Intraluminal shunts were used in more than half of operations (55 %), comparatively rarely intraluminal shunts were used in eversion CAE group (Table 7.).
Active aspiration drainage up to 88% in Group 1 and 59% in group 2 was used after wound closure.

Table 7. The use of intraluminal shunts and active wound drainage in operations (p<0.05%)

Groups	The use of intraluminal shunts (i/a) (n)	The use of intraluminal shunts (% from the number of operations in the group)	Active wound drainages (n)	Active wound drainages (% from the number of operations in the group)
1. group (EAE with a primary stich)	106	67	139	88
2. group (EAE with a patch)	43	77	33	59
3. group (eversions EAE)	35	29	96	79
All operations	187	55	268	79

Early postoperation complications, that are linked with new cerebrovascular events were observed in 4.0% from all operations. Ischaemic stroke was the most often cerebrovascular complication. There were no death in perioperative period. Postoperative cerebral complications are summerised in Table 8 and 9.

Table 8. Early postoperative complications due to new cerebrovascular events for all patients (p<0, 05%)

Group	Ischaemic stroke (%)	Haemorrhagic stroke, (%)	Transient ischaemic attack, (%)
1. group (CAE with a primary stich)	3 (1.9)	0	2 (1.3)
2. group (CAE with a patc)	2 (3.6)	0	1 (1.8)
3. group (eversion CAE)	2 (1.6)	1 (0.81)	1 (0.8)
All operations	7 (2.0)	1 (0.29)	4 (1.8)

Table 9. Early post-operative complications due to new cerebrovascular events for symptomatic and asymptomatic carotid stenosis (p<0,05%)

Group	Ischaemic stroke (%)	Haemorrhagic stroke, (%)	Transient ischaemic attack, (%)
Asymptomatic carotid stenosis	1 (0.29)	1 (0.29)	2 (0.6)
Symtomatic carotid stenosis	6 (1.8)	0	2 (0.6)

There were no wound infection or pulmonary artery embolism been observed after CAE . Wound haematoma was the most reported from the all non-neurological complications (2.9%). Postoperative complications that are not connected with cerebrovascular events are summarized in table 10.

Table 10. Non-cerebral post-operation complications after CAE (p<0, 05%)

Group	Wound haematoma (%)	Miocardial infarction (%)	Cranial nerve injury (%)
1. group (EAE with a primary stich)	8	3	1
2. group (EAE with a patch)	1	1	0
3. group (eversion EAE)	1	1	1
All operations	10 (2,9)	5 (1.5)	2 (0.6)

DISCUSSION

Results of carotid endarterectomy in Latvia are similar to results of large randomized trials published recently. 59% of Latvian patients undergoing CAE are asymptomatic, that is similar to North American practice of treatment carotid stenosis, higher than reported in European countries CTA is more often used as a only diagnostic tool compare with ultrasound evaluation in Latvia, while this is opposite with North American and most of the European reports. Perioperative complication rate in Latvia is well below the international guidelines targeted. Cerebrovascular complication rate is 4.09% (both symptomatic and asymptomatic patients), that is due to satisfied carotid treatment practice, however. there is space for improvement.

There were no statisticaly significant diferences in atherosclerotic risks factors, comorbidities, preoperative investigation methods, operation time, complication rate in all groups. Our results do not show any benefit between groups and current patient selection seems proper.

Unfortunately this report do not includes carotid artery PTA and stenting procedures becoming more frequent last years. To get more objective data about carotid invasive treatment in Latvia prospective comparison would be very helpful.

CONCLUSIONS

CTA prevales as a preoperative diagnostic method to evaluate carotid artery stenosis in Latvia.

Carotid endarterectomy is carried out mostly in classical conventional tecniques and arteriotomy is being closed with a primary suture in most of the cases.

We conclude that vascular surgeons of Latvia are performing carotid surgery safely with internationally recognizable results within the complication limits.

Conflict of interest: None

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ORIGINAL ARTICLE

The Significance of the Ultrasound Parameters of Carotid Artery Atherosclerosis in Neuroangiosurgical Practice

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Summary

Introduction. Atherosclerosis of the extracranial part of the cerebral arteries is a major pathogenetic risk factor of cerebral infarction (CI) and transitory ischemic attacks (TIA). An ischemic stroke is one of the leading causes of death and long-term disability in many developed countries and is a condition that becomes more prevalent with age.

Aim of the study. To define the diagnostic implications of the US examinations of the extracranial part of the carotid arteries in respect of neurological practice and taking into consideration the age, gender and the localisation of the ischemic damage of patients with atherothrombotic MCA infarction.

Materials and methods. 540 patients treated in the Stroke Unit of Gailezers University Clinic who were diagnosed as having a first MCA infarction of atherothrombotic genesis in the acute phase were examined. The patients were 36 to 91 and were divided into 6 age groups. The group of patients examined comprised of 267 people – 49.4%– females and 273 – 50.6% - males. US examinations of the carotid arteries were performed with a high resolution Philips iU22 ultrasonographic device with a multifrequency linear probe of 3.0 to 9.0 MHz.

Results. The ultrasonologic parameters of the carotid artery atherosclerosis in patients with first atherothrombotic infarction of MCA revealed that 70.8% were aged between 60 to 79. This indicated that the risk of atherotrombotic MCA infarction was highest in this age group and this needs to be taken into account in angiosurgical practice. The largest number of women – 50.6% – with an MCA infarction were in the 70 to 79 age group but the largest number of men – 41.0% were found in the 60 to 69 age group. In patients with a first MCA infarction stable carotid artery plaques with lumen stenosis of less than 50% in the age group below 59 were found in 47.2% of cases among other parameters, i.e. 7.4% more frequently than in the age group from 60 to 79 ($p<0.1$) and 11.8% more than in the age group above 79 ($p<0.05$). The frequency of carotid artery extracranial segment stenosis of less than 50% did not differ significantly in different age groups and was 12.6% on average. Occlusion as a delayed pathogenetic risk factor of atherotrombotic infarction was observed in 62 – 11.5% – of patients. In a large number of patients, 34.3% on average, unstable plaques with an uneven surface, ruptures, craters, wall thrombosis and with hemorrhages were found less frequently. This frequency increased significantly with age. There was frequent evidence of the localization of carotid artery atherosclerotic lesions in relation to the locality of CI to be bilateral as well as heterolateral.

Conclusion. To diagnose atherosclerotic lesions of the carotid artery and to confirm indications of surgical and pharmaceutical therapy more statistically significant information needs to be obtained through specialized neuroangio-US examinations.

Key words: atherosclerosis; carotid artery; cerebral infarction; neurovascular ultrasound; carotid endarterectomy; endovascular intervention.

INTRODUCTION

Atherosclerosis of the extracranial part of the cerebral arteries is one of the most common causes of cerebral infraction (CI) and transitory ischemic attacks (TIA). An ischemic stroke is one of the leading causes of death and long-term disability in many developed countries and is a condition that becomes more prevalent with age(1). Atherosclerosis causes approximately one third of all strokes, including repeated strokes and accounts for approximately 20% of all strokes (2). Carotid artery stenosis is an unpredictable disease and it can progress slowly or quickly or maybe not change over significant periods of time(2, 3).

The aim of modern pharmaceutical therapy is to reduce the development of atherothrombosis and enable the prevention of ischemic strokes. It has been proved that antiagregants can reduce the incidence of CI and TIA and that statins have an atherothrombotic plaque stabilizing effect (4, 5, 6, 7, 8).

The surgical correction of an atherosclerotic lesion of the carotid arteries can significantly reduce the risk of an ischemic stroke. Several large random studies, for example the North American Symptomatic Carotid Endarterectomy Trial (NASCET), The European Carotid Surgery Trial (ECST) and The Asymptomatic Carotid Artery Atherosclerosis Study (ACAS) have proved the efficacy of surgical therapy and a reduction in mortality (9, 10, 11, 12).

Decisions in clinical practice concerning carotid endarterectomy or endovascular therapy are still mainly based on the degree of artery lumen stenosis and the age of the patient. The endarterectomy of the carotid artery is considered as a standard revascularization therapy. The indications for surgical therapy of the

carotid artery disease can be viewed from five different aspects: neurologic symptoms, the degree of stenosis, concomitant diseases, local changes in the blood vessel as well as plaque morphology. In clinical practice, the choice of indications for the invasive method is based on the first two points, while the choice between both methods – endarterectomy or endovascular therapy – is based mainly on points 3, 4 and 5 (12, 13).

Nowadays in clinical practice, lumen stenosis of the carotid artery extracranial part is diagnosed by noninvasive methods at first and most commonly with the duplex-dopplerographic US method, particularly for express diagnostics, with computer tomography angiography (CTA) or by the use of magnetic resonance angiography (MRA). While digital subtraction angiography (DSA) is still an historical gold standard, it is most commonly substituted by noninvasive examination methods, particularly in cases of indications of endarterectomy (14).

The use of modern high quality devices for US examination of cerebral blood vessels enables a neurosonologist specialist to verify and visualize atherosclerotic changes of the extracranial part of the carotid artery wall, the detection of which is limited by noninvasive angiography methods. Consequently, the thickness of the intima-media and the whole artery wall can be measured. The stability of atherosclerotic plaque and wall thrombotic changes that are often sources of cerebral arteries emboli can be determined in detail. The US parameters can give additional information to an angiosurgeon when considering indications and choosing a method of surgical correction. The US method enables the width of localisation, the degree, the structural characteristics of the stenosis of the extracranial part of the carotid artery to be established and monitored in dynamics with no limitations. This can be done before and after surgical and endovasal manipulations and also while monitoring the frequency of microemboli.

It should be noted that in order to consider the options for the angiosurgical correction of an atherosclerotic lesion of the carotid artery an evaluation of the general health of the patient and age and gender are very important. The specifics of the extracranial US parts of carotid artery parameter deviations in different age groups taking into account gender have not been described well in research, particularly for patients clinically to have confirmed atherothrombotic MCA infarction. The most frequent localization of different degree atherosclerosis US parameters of the carotid artery in relation to cerebral infarction localization needs to be evaluated too.

AIM OF THE STUDY

To define the diagnostic implications of the US examinations of the extracranial part of the carotid arteries in respect of neurological practice and taking into consideration the age, gender and the localisation of the ischemic damage of patients with atherothrombotic MCA infarction.

MATERIALS AND METHODS

Examinations were performed on 540 patients treated in the Stroke Unit of the Gailezers Clinic who were diagnosed as having a first MCA infarction of atherothrombotic genesis in the acute phase. Patients were aged from 36 to 91 and were divided into 6 age groups. There were 267 – 49.4% – females and 273 – 50.6% – males.

Repeated computer tomography (CT) of the head, ultrasound examinations of extra and intracranial arteries were performed and blood coagulation, lipid and glucose biochemical values were measured of all patients. Transthoracic echocardiography, magnetic resonance (MR) of the brain, CT angiography (CTA), MR angiography (MRA) or digital subtraction angiography (DSA) and other examinations necessary for the evaluation of the state of patients in the Stroke Unit were carried out.

Duplex ultrasonography was the first noninvasive diagnostic method of choice for examination of atherosclerotic lesions of the carotid artery in this study. This is because it is easily available and usable, as well as being relatively low-cost compared to other methods. As is commonly known, this method depends on the type of the ultrasound device, the experience of the operator and his/her skills. When the data of different writers is compared, the sensitivity of the duplex dopplerographic method for the establishment of hemodynamically significant carotid artery stenosis is 86 to 90%, but specificity 87 to 96%.(15–17).

CTA or DSA was used more frequently than MRA in our practice as a confirming test following duplex ultrasonography if the hemodynamic parameters of significant carotid artery stenosis were doubtful. This choice was made by the angiosurgeon.

Ultrasound examinations of the carotid arteries were performed with a high resolution Philips iU22 ultrasound device with a multifrequency linear probe of 3.0 to 9.0 MHz. The common carotid artery and the extracranial part of the internal carotid artery were measured using B-mode ultrasonography or the visualization of blood vessel in gray scale, colour-coded duplex dopplerography and dopplerography with a flow spectral analysis.

The thickness of the intima-media (N<1.0mm), thickening 1.0 to 1.5mm was established (18). Atherosclerotic plaques were defined as local wall thickening by less than 1.5 mm and absolute plaque size, localization, surface evaluation, plaque structure, echogenicity and stability. These were analysed. The plaques were classified as stable – homogenous with a smooth surface with or without calcinates, or unstable – heterogenous with uneven surface, niches, ulceration, hemorrhages and thrombosis.(19-20). The degrees of carotid artery stenosis were classified as the reduction of lumen by < 50%, > 50% and lumen occlusion. The ECST method was used to establish the degree of artery stenosis.

Transcranial duplex dopplerografy was also carried out for all patients to define the genesis of atherothrombotic cerebral infarction more precisely, evaluating the

segmental flow values of cerebral blood vessels, verified stenoses and occlusions of intracranial arteries, the diagnostics of embolic signals and the compensatory mechanisms of cerebral blood flow were also measured.

The statistical processing and analysis of data was performed with SPSS 16.0 using the χ^2 test.

RESULTS

When the US parameters of the carotid artery atherosclerosis of patients with first atherothrombotic infarction of MCA were evaluated, the division by age groups was taken into account (See Table 1). In the examined patient group, 70.8% of patients were 60 to 79. Only 20.3% were below 60 and 8.9% older than 80.

Table 1. The number (n) of patients with atherothrombotic stroke of MCA in female, male and combined group in different age groups

Age group	<40	40-49	50-59	60-69	70-79	≥80	Total
Female	-	9	23	59	135	41	267
Male	2	24	52	112	76	7	273
Total	2	33	75	171	211	48	540

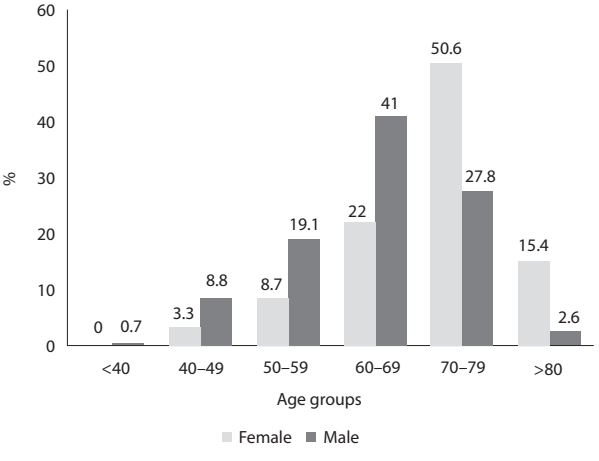


Fig. 1. The number of female and male patients by percentage in different age groups

This table shows that the risk of atherothrombotic MCA infarction was highest in the 60 to 79 age group and this needs to be taken into account in angiosurgical practice. When the number of patients in the female and male groups were analysed, the largest number - 50.6% of women with MCA infarction - were aged between 70 to 79 but 60 to 69 in males (41.0%) ($p<0.01$). There were only 2 men in the age group below 40. There were 16.7% more men than women in the age group from 40 to 59. However there were 12.8% ($p<0.05$) more women then men in the age group ≥ 80.

The incidence of different carotid artery atherosclerosis US parameters was different in patients with MCA infarction, and in 222 - 41.1% – of 540 patients stable plaques with lumen stenosis < 50% were observed. The stenosis of less then 50% of the extracranial segment of the carotid artery as a major risk factor of CI was established in 71 - 13.1% – of patients. Occlusion as a risk of delayed atherothrombotic infarction pathogenesis was noted in 62 – 11.5% – of patients.

Unstable plaques of the carotid artery extracranial segment with uneven surfaces, ruptures, craters, wall thrombi and, less frequently, with hemorrhages were identified in a large number of patients – 18 % up to 34.3%.

Anamnesis data showed that none of the patients observed had undergone any examination of the cerebral blood vessels, including US, until the development of a cerebral infarction. It should be noted that none of the patients observed had undergone carotid artery endarterectomy or angioplasty until they had suffered a cerebral infarction.

When researchers analysed the frequency of the US parameters of carotid artery atherosclerosis in the six different age groups, it was noticed that stable plaques with lumen stenosis in the carotid artery of less then 50% was found only in 2 patients below 40. In other age groups all analysed atherosclerosis US parameters were present in different ratios (see Figure 2).

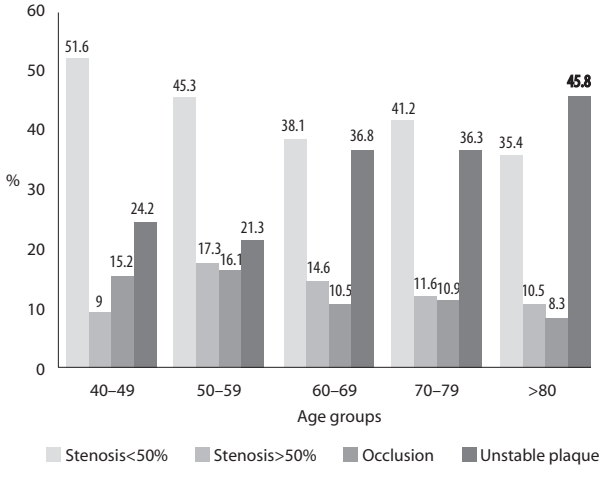


Fig. 2. The frequency (%) of carotid artery atherosclerosis US parameters in patients with an MCA infarction by different age groups

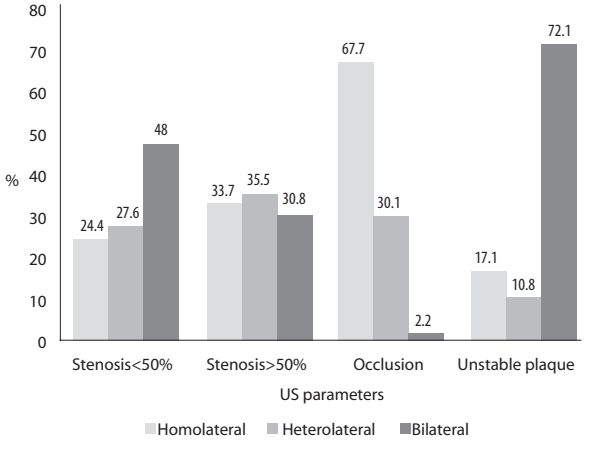
The frequency of observed carotid artery atherosclerosis US parameters differed depending on the patient age in the age groups between 40 to 59 and 60 to 79 and over 80. In patients with a first MCA infraction carotid artery stable plaques with lumen stenosis of less then 50% in the age group below 59 were 47.2% among other parameters, 7.4% more than in the age group of 60 to 79 ($p<0.1$) and 11.8% more than in the group above

79 (p<0.05). It should be noted that the frequency of carotid artery extracranial segment stenosis of less then 50% did not differ significantly in different age groups and was approximately 12.6%. The frequency of carotid artery occlusion in the age group from 40 to 59 was 15.7%, from 60 to 79 – 10.7%, but above 80 it was 8.3%. The incidence of occlusion in different age groups did not differ significantly. In contrast to other carotid artery atherosclerosis US parameters the number of unstable plaques in patients with an MCA infarction in relation to other parameters increased significantly with patient age (p<0.01). In the 40 to 59 age group the frequency of unstable plaques was 22.3%, but it was 36.4% in the 60 to 79 age group and 45.8% in those over 80. Consequently, the frequency of unstable plaques of the carotid artery – which is a significant factor of risk of cerebral infarction and has a negative impact on the results of neurosurgical treatment - increased significantly with the age of CI patients.

When the frequency of particular carotid artery atherosclerosis US parameters in female and male groups was compared, it was established that the number of unstable plaques in female group was 7.1% bigger, but in male group stenosis of > 50% of carotid artery extracranial segment and occlusion were observed 8.5% more frequently. (p<0.1) The frequency of carotid artery atherosclerosis US parameters in female and male groups of different ages were compared and some differences were observed. The largest number of women with MCA atherothrombotic infarction was from 70 to 79 (135 of 267 patients), but from 60 to 69 (112 of 273 patients) in men. Carotid artery stenosis of > 50% was not observed in the female group from 40 to 49.

The localisation frequency of carotid artery extracranial part US parameters in relation to MCA infarction localization was also different (see Figure 3).

Fig. 3. The localisation frequency (%) of US parameters of carotid artery extracranial part atherosclerosis in relation to the localization of MCA infarction



The most commonly noted stable plaques with artery lumen stenosis of < 50% were bilateral and 23.6%; they were less frequently homolateral and 20.4% heterolateral. Stenoses of > 50% with the same frequency were present in the infarction localization or the opposite side, as well as bilateral. When the bilateral localization of stenoses in relation to MCA infarction localization was compared, it was that the stenoses of < 50% occurred more frequently – 17.2% - than that of > 50% (p<0.05). Occlusion of the carotid artery was most commonly homolateral in 42 of 62 patients; in 18 of these patients 29.0% stenosis was of > 50% in the second carotid artery.

The frequency of the localisation of unstable plaques differed significantly; it was noted that in 72.1% of cases they were bilateral and in other cases 6.3% more frequently ipsilateral than heterolateral.

It was also observed that the localization of frequency of the US parameters for patients with MCA infarction of the extracranial part of carotid artery atherosclerosis was bilateral in 45.1% of cases, 28.5% of cases were homolateral and 26.4% heterolateral. The established frequent bilateral and heterolateral localization of the atherosclerotic process is also the significant criteria in choosing indications and methods for surgical treatment of damage to the carotid artery.

DISCUSSION

The first noninvasive diagnostic method for examination of carotid artery atherosclerotic lesion is doppler ultrasonography; this is because it is easy available and usable.(14) It should be noted that the use of this method is significantly influenced by the experience of the user and the skills using and interpreting data. In comparison to catheter angiography, the sensitivity of the Duplex Dopplerography method is 86%, specificity – 87% for the determination of hemodynamically significant carotid artery stenosis (14-16).

This examination method for screening needs to be used for all patients with a history of TIA or CI, as well as in patients with risk factors of cardiovascular disease, diabetes mellitus, arterial hypertension, established atherosclerotic damage to the coronary and peripheral blood vessels, smokers and in particular for all people with a history of cerebrovascular incidents after the age of 50. This would enable a timely start for preventive measures, both pharmaceutical and surgical. The American Society of Echocardiography (ASE) has published a report on the clinical use of carotid US to prove subclinical vascular disease and analyse cardiovascular risk. However, this report has not been sufficiently explicit in evaluation the criteria of the US parameters in the examination of the carotid artery, specifically the morphologic parameters of walls of artery. These parameters have a significant role in neurovascular and neuroangiosurgical practice(21).

The degree of carotid artery stenosis is a parameter of the risk of a cerebral infarction in patients with severe stenosis, but morphological characteristics of the atherosclerotic plate are important for slight

and moderate stenosis; however plaque instability significantly increases the risk of a stroke.

According to data from a number of different researchers and also from our data, approximately 15 to 70% of cerebral infarctions result from thromboembolism as a result of unstable plaques in the carotid bifurcation region (22–24).

In experimental and clinical studies it has been shown that plaques with ulcerations have been observed with the same frequency in patients with both homolateral and heterolateral cerebral dyscirculation symptoms and that atherosclerotic plaques frequently facilitate the formation of wall thrombi and carry the risk of thromboembolism. In patients with symptomatic carotid artery disease the risk of plaque ulceration is higher, causing lesion-independent ischemia and may be associated with the occurrence of thromboemboli or hemodynamic disorders (25).

We have established conclusively in our study that in the pathogenesis of atherothrombotic MCA infarction the major role is most frequently played by unstable plaques of the extracranial part of the carotid artery with wall thrombi and possible thromboembolism can increase significantly with age.

Our study found that acute cerebral ischemia is based on a relatively frequent extracranial part of carotid artery stenosis <50% which is bilaterally located and spread equally across different age and gender groups. Carotid artery stenosis> 50% was observed less than 18 % of cases and the stenosis did not depend on the age of CI patients.

A particularly important risk factor in the development of MCA infarction proved to be unstable plaque in the carotid arteries, regardless of the degree of stenosis, and which may be a source of arterio-arterial embolism and the main pathogenic factor for CI. The most important role in the determining of these embolus is transcranial duplex dopplerografy in recent years and this has also been confirmed by the data of other writers (25-27).

The study demonstrated the significance of ageing in relation to the continuing instability of plaque in the extracranical part in the carotid artery.

The neuroangio-US method gives sufficient clinical information for the diagnosis of carotid artery plaque instability, and also for the evaluation of thromboembolism. Consequently it is important to perform transcranial dopplerography, particularly monitoring.

CONCLUSION

In the examination of the extracranial part of the carotid artery in patients with atherothrombotic MCA infarction stable plaques with stenosis of < 50% was observed that this is the most common cause that underlays the ischemic process however unstable plaques are a common source of thromboembolism. These US parameters of carotid artery atherosclerosis were common in all age groups however the frequency of unstable plaques increased significantly with age.

The frequency of artery stenosis of > 50% and occlusion in different age groups was not significant in 9.0 to 17.3% of cases.

The largest number of patients with MCA infarction in the female group was in the age group from 70 to 79 and in the male group from 60 to 69. However, the characteristics of the changes of carotid artery atherosclerosis US parameters did not differ substantially in either groups.

The relationship between the localization of the US parameter of the carotid artery and the localization of MCA infarction varied. Unstable plaques and stable plaques with a stenosis by < 50% were bilateral most commonly, stenoses by > 50% with the equal frequency were homo-, hetero- and bilateral, but occlusion was homolateral most commonly.

Significant additional information for the diagnosis of carotid artery atherosclerotic lesion and for the more precise establishment of surgical and pharmaceutical therapy indications can be obtained with specific neuroangio-US examinations.

Conflict of interest: None

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ORIGINAL ARTICLE

Histomorphology of Salivary Glands after Ligature and Reconstruction of Common Carotid Artery in Rabbits

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Summary
Introduction. In occlusive diseases of carotid arteries may be involved salivary glands which play important role in oral and general homeostasis.
Aim of the study. The purpose of this study was histomorphological evaluation of rabbit's salivary glands after regional reduction af arterial circulation and reconstruction of ligated common carotid arteries comparing with normal histological sight.
Materials and methods. The experiments were authorized by the Animal Ethics Committee of the Latvian Food and Veterinary Service. 20 male Californian rabbits weighing approximately 3 kg were used. Under intravenous general anesthesia and local anesthesia the ligature of a. *carotis communis dextra* was done. After 28 days 10 rabbits were sacrificed and samples were taken from all major salivary glands and histological measured acinar dimensions and acinar epithelial cell.
For rest 10 rabbits under the general anesthesia were performed revascularization of a. *carotis communis dextra*. After 28 days the rabbits were sacrificed with intrapulmonal injection of T61. The acini dimensions and acinar epithelial cell diameter in microns in all glands were measured except buccal glands were measured.
Results. In ischemic conditions all glands react with decrease of acinar epithelial cell diameter and also diametr of functional unit of salivary gland.After revascularisation reestablishment of acini and secretory epithelial cells was observed.
Conclusions. Rabbit's salivary glands have potential to recovery in diminished after ligation of ipsilateral common carotid artery size of acini and secretory epithelium after 4 week ischemia if the blood supply is reestabilished.
Key words: salivary glands; histology; carotid artery ligature; reconstruction.

INTRODUCTION

Salivary secretion is crucial in maintenance of oral health and play important role in prevention of dental caries also in healing of extraction wounds (6,10).
Age related alterations of normal structure of salivary glands includes decrease of parenchimatous structures and increase of stromal, ductal part and fat tissue content (16). On data from human necropsies it was supposed that increasing level of degenerative vascular disease through ischaemia has some causal relation to the aging atrophy of salivary gland parenchyma.Significant sex diferences in aging changes of human salivary glands were not present (20). Different results were obtained on secretory function in young adult and aged male and female rats. No significant alterations in submandibular gland salivary flow rate or the concentrations of total protein, Na^+, K^+ and neutral sugar in the secreted saliva were observed between different aged animals. Significant sex diferences in salivary flow rate and total protein content were found in rats(22). Necrotic laesions of salivary glands as necrotizing sialometaplasia is regarded as result of compromised blood supply to the salivary gland usually by local trauma, some role has smoking (1, 3).
In experiment lobular necrosis of salivary glands was observed after ligating of vesels close to gland (22). The lobular configuration of necrosis is explained by the separate blood flow to each lobule. In experimental studies there are more data on histomorphological response of salivary glands to ligation of salivary ducts as vesels feeding salivary glands. We can't find any publication to compare histomorphology of salivary glands after ligation and reconstruction of feeding artery.

AIM OF THE STUDY

The aim of the study is to determain the influence of ischemia to salivary gland tissue and ability to recover after reestablishment of regional blood supply.

MATERIALS AND METHODS

The experiments were authorized by the Animal Ethics Committee of the Latvian Food and Veterinary Administration. Twenty male Californian rabbits weighing approximately 3 kg were used. Under intravenous general anesthesia with Diazepam 2mg/kg and 5% Ketamin hydrochloride 15 mg/kg and local infiltration with 0.5% Lidocain solution 5 ml, the ligature of *a. carotis communis dextra* was performed with 4-0 silk and the wound was closed with Vicryl 5-0. After 28 days under general anesthesia 10 rabbits were sacrificed and samples were taken from all major salivary glands. Samples were fixated in 10% neutral

formalin solution, embedded in paraffin and prepared for staining with hematoxilin – eosin. In light microscope under magnification of 400 times were measured acini dimensions except buccal gland because of it irregular structure in microns and acinar epithelial cell diameter in microns.

For rest 10 rabbits under the same general anesthesia revascularization of *a. carotis communis dextra* by end-to-end anastomosis using Monosof 8-0 sutures and 6X magnification loups was performed. All rabbits survived and after 28 days were sacrificed with intrapulmonal injection of T61. Histological samples were taken and prepared for histological examination. After in the same manner in light microscope acini and secretory cell dimensions were measured.

RESULTS

Submandibular gland in the side of ligature presented decrease of secret granules in glandulocytes. It shows slight decreased diameter of acini mean 0.9 microns ($p<0.05$) and secretory epithelial cells mean 0.7 microns ($p<0.05$) in side of ischemia comparing to control side (*Fig. 1*). Control side represented more mucous secretion type (*Fig.7*).

In parotid gland after ligature also was decrease of cell and acini diameter in test side, correspondingly acini mean 0.9microns ($p<0.05$) and secretory epithelial cells mean 0.2microns ($p<0.05$)(*Fig.2*). Parotid gland also showed intersticial tissues enhance and manifest of glandular lipomatosis more than in control glands (*Fig.8*).

Buccal gland also showed decrease of cells in side of ligature mean in 1micron ($p<0.05$) (*Fig.3*). The size of acini can not be measured because of irregular structure (*Fig.9*).

Submandibular gland after revascularization procedure showed morphometric parameters of glandular acini and acinar epithelial cells size more high as before artery ligation. Acini becomes in mean 0.1 micron bigger then in control side ($p<0.05$) but acinar epithelial cells in mean 0.6 microns bigger than control side (*Fig.4*).

Response of parotid gland after revascularization was the same; only in test side maintenance of lipomatosis was more than in control side. Acini reverted in mean 0.4microns ($p<0,05$) and acinar epithelial cells 0.2microns ($p<0.05$) bigger then in control side(*Fig.5*).

Buccal gland histological changes also were in the same manner and acinar epithelial cells becomes bigger in mean 0.2microns ($p<0.05$) (*Fig.6)*.

DISCUSSION

In human medicine histopathological investigation of salivary glands besides another pathology is essential for differential diagnosis in cases of dry mouth syndrome more often due Sjögren's syndrome with replacement of parenchymatous secretory units by lymphocytic infiltration (8,11). Age related changes in human salivary glands are studied on material of autopsies or biopsies. The main histological findings include fat infiltration between serous, mucous and myoepithelial cells in the intercalated and striated ducts (18,24), replacement of functional parenchymatous cells by by fat and connective tissue also oncocytes (25,19). Reduction of functionaly active parenchyma is explained due chronic obstruction of salivary ducts (21), acinar and ductal atrophy, ductal hyperplasia and dilatation (7). There are only some publications with data on intraglandular vascular changes as tortuosity of arteries and dilatation of veins(18), vascular congestion as result of atheromatous plaques and vascular obstructions (2) in human salivary glands with atrophic and degenerative changes. The vascularity network is regarded as critical to the functioning of the secretory acinar cells and the production of saliva , but it is difficult to detect in routine histological sections (26).

In experiment age-related changes of salivary glands were studied on rats and mouses (13,14). Distribution pattern of blood vessels in mouse submandibular gland is in similar manner to that in humans and in such way histomorphological data obtained in experiment may be used in interpretation of human pathology. We can't find data about comparision of salivary gland morphology in human and rabbits. Rat and mouse salivary glands undergo severe degeneration if their blood supply via the main feeding artery is interrupted (5,12,15). There is difference in ischemic damage severity in peripheral and central portions of salivary glands (23,9). Three-dimensional evaluation of blood vessels using stereoscopic and scanning electron microscopy in mouse submandibular gland showed no communicating vesels between the gland proper and the capsula. It is suggested that the parenchymal cells surviving in the ischemic peripheral portion of the gland are nourished by permeation of tissue fluid contained in the capsula (17).

Our histomorphological data confirms ischemic damages of major salivary glands due ligation of regional feeding artery. No necrotic tissue changes were observed as are obtained in previous reports where ligation of feeding arteries was done more close to salivary gland and possibility of collateral compensation is less.All rabbit's major salivary glands showed decrease of secret granules in glandulocytes, diminished diameter of acinar epithelial cells and size of acini. Revascularisation due reconstruction of occluded common carotid artery resulted in restored functional morphology of ischemic salivary glands by increase in size of acinar epithelium and parenchymatous acini which showed to be bigger as on contralateral control side.

Histomorphological changes of salivary glands after occlusion of common carotid artery in experiment may have some consequences to explain salivary gland function in quiet common human pathology as are occlusive diseases of carotid arteries.

CONCLUSIONS

1. After ligation of common carotid artery in rabbits ischemic changes as decrease in functional activity and size of major salivary gland acinar epithelial cells and size of acini occures.
2. Revascularisation by reconstruction of occluded common carotid artery provides increase in size of acinar epithelial cells and acini up to that on control collateral side.

Conflict of interest: None

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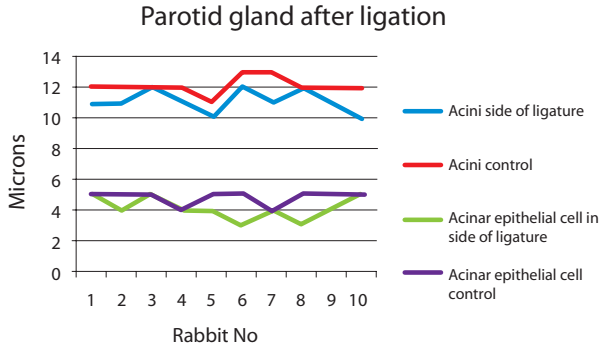

Fig. 1. Diameter of parotid gland acini and acinar epithelial cells 4 weeks after ipsilateral common carotid artery ligation

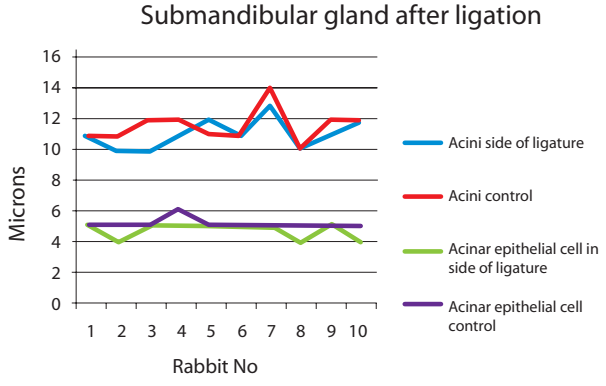

Fig. 2. Diameter of submandibular gland acini and acinar epithelial cells 4 weeks after ipsilateral common carotid artery ligation

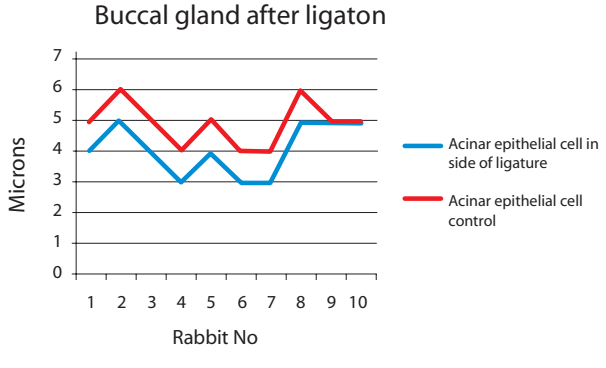

Fig. 3. Diameter of buccal gland acinar epithelial cells 4 weeks after ipsilateral common carotid artery ligation

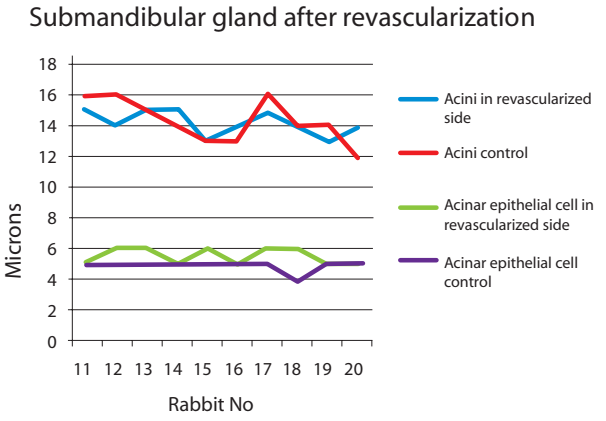

Fig. 4. Diameter of submandibular gland acini and acinar epithelial cells 4 weeks after ipsilateral common carotid artery revascularization

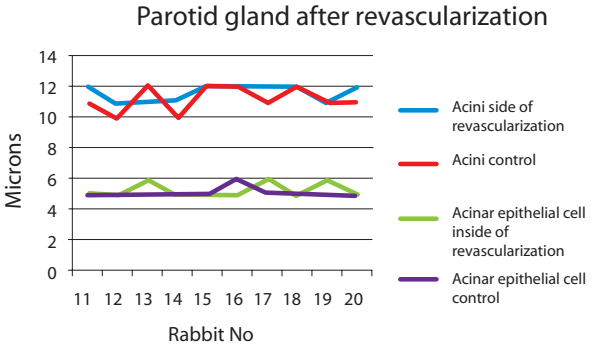

Fig. 5. Diameter of parotid gland acini and acinar epithelial cells 4 weeks after ipsilateral common carotid artery revascularization

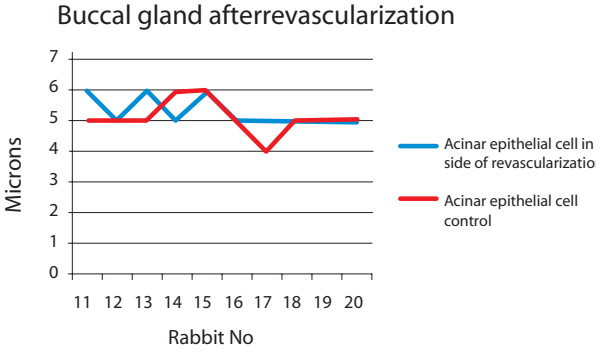

Fig. 6. Diameter of buccal gland acinar epithelial cells 4 weeks after ipsilateral common carotid artery revascularization

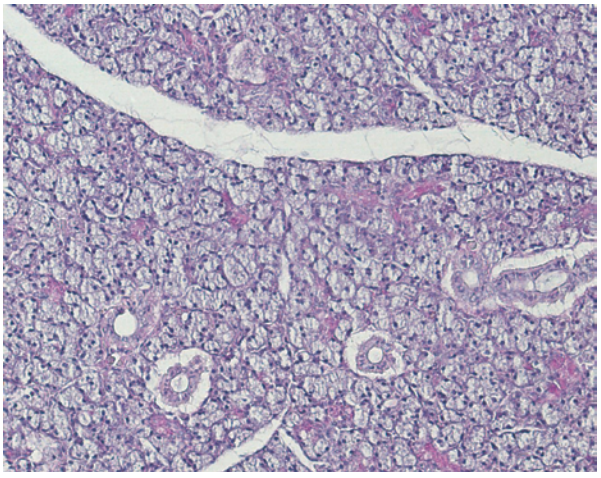

Fig. 7. Submandibular gland control side after ischemia, x 200

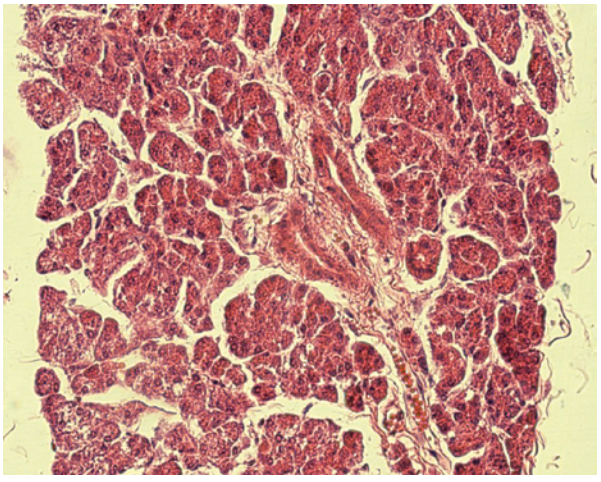

Fig. 8. Parotid gland test side after ischemia, x 400

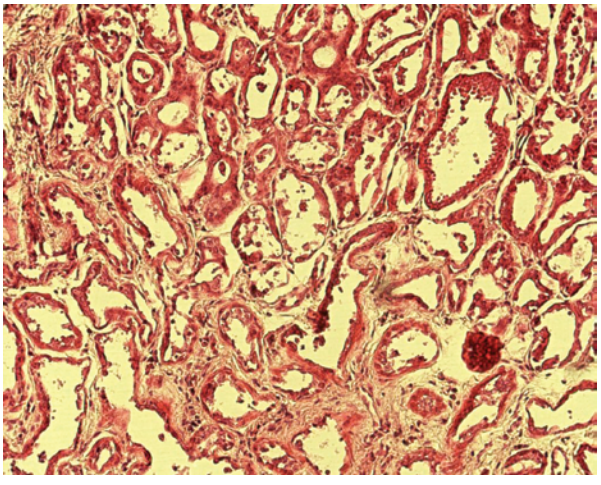

Fig. 9. Buccal gland test side after ischemia, x 200

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ORIGINAL ARTICLE

The Study of the Risk Factors of Health Care – Associated Infections in Patients' Care with Invasive Devices

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Summary

Introduction. Health care-associated infections (HAIs) are a major threat to patient safety. The spread of HAIs cases in intensive care units (ICUs) and in surgical units is influenced by the severity of the patients' condition, as well as the number of invasive procedures and the environment of the units. The immune systems of critically ill patients are in a severe state which increases the probability of beneficial conditions for a colonisation by pathogenic microorganisms due to invasive procedures. Furthermore, the action of inserting an invasive device (urinary catheter, peripheral vein catheter) disrupts the natural defence mechanisms by itself.

Aim of the study. To determine risk factors of HAIs during invasive procedures: peripheral vein catheter and urinary catheter insertion and care at the time.

Materials and methods. The research was performed in the ICUs and in the surgical units of Latvia's regional multi-profile hospitals (n=3), using the quantitative research method: questionnaire (n=188), and qualitative research methods: clinically structured empiric research (n=45) and microbiological tests (n=86): a) with a Count-Tact applicator and a special culture medium, b) with a swab, and c) with catheter sedimentation method.

Results. The study results showed that surgical nurses take care of 18 to 40 patients per day, while intensive care nurses take care of 2 to 3 patients per day. A common problem identified in ICUs and in surgical units related to the lack of unified nursing protocols on performing invasive procedures in all cases (100%; 45/45). Microbiological investigations showed a high level of bio-contamination during invasive procedures: the amount of microorganisms on the nurses' hands considerably exceeded the acceptable levels in all cases (100%; 4/4), and medium to high levels of bio-contamination were discovered on patient's changed bed sheets (100%; 2/2) as well as in nurses' hair (85.7%; 48/56) and their work wear (78.5%; 44/56). In 25% of the observed cases Staphylococcus aureus was discovered in the sterile zone of the peripheral vein catheter 72 hours and 96 hours after the catheter's insertion. 72 hours after the catheter's insertion, as well as 7 days after the insertion of a urinary catheter, 100% of the cases revealed the presence of at least one and in some cases several of the following pathogens: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, β haemolytic *Streptococcus* and fungi, such as *Candida albicans* and *Candida tropicalis.*

Conclusions. The main risk factors of HAIs in patients' care using invasive devices are: the lack of unified nursing protocols, aseptic and antiseptic mistakes and excessive workload, as well as a high level of bio-contamination in the ICUs and in surgical units.

Key words: invasive devices, health care-associated infections, urinary catheter, peripheral vein catheter.

INTRODUCTION

In contemporary medicine the invasive devices - especially intravascular devices of all types and urinary catheters - continue to be essential for the management of critically ill patients (15). Invasive devices are far more important in determining susceptibility to health care-associated infections (HAIs) than the underlying diseases (7). The most common device-related infection pathogens are *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Enterobacter, Serratia, Candida albicans, Pseudomonas aeruginosa, Citrobacter freudii Corynebacterium, Escherichia coli* (8, 19, 22). Staphylococci, enterococci, and many other species of bacteria are known to attach to indwelling medical devices and form biofilms consisting of complex communities of single cells and microcolonies within a matrix of hydrated polysaccharides, proteins, and other macromolecules, including DNA. Within this matrix, bacterial cells evade the host immune response and survive antimicrobial chemotherapy, resulting in persistent infections that are difficult to treat (2, 20). The risk of device-related infection, more than anything else, has forced medicine to accept the necessity for infection control that can be very cost-effective. Intensive education and "bundling" of evidence-based interventions can reduce the infection rate through

improved asepsis in handling and an earlier removal of invasive devices, but the maintenance of such gains requires ongoing efforts (13, 14).

AIM OF THE STUDY

The aim of the study was to determine risk factors of HAIs during invasive procedures: peripheral vein catheter and urinary catheter insertion and care at the time.

MATERIALS AND METHODS

The research was carried out in the intensive care units (ICUs) and in surgical units of Latvia's regional multi-profile hospitals (n=3), using the quantitative research method: questionnaire (n=188), and qualitative research methods: clinically structured empiric research (n=45) and microbiological tests (n=86). To determine the risk factors of HAIs from the care-givers' side (knowledge concerning patient care using invasive devices and the carrying-out of high-risk procedures, as well as practical skills concerning the daily care of patients by using invasive devices), a structured questionnaire was developed which contained predetermined (closed as well as open) questions with prepared answers and a clinically structured empiric research plan, which included: a) the observation of nurses' work activities when performing invasive procedures, and b) the inspection of the nurses' work environment (sufficient material and technical means, the presence or lack of nursing protocols and the number of nursing staff in the units). By means of microbiological investigation the contamination level of the ICUs and surgical units was determined: a) using a Count-Tact applicator and a culture medium specially selected for this method, the bio-contamination level of the work environment, work surfaces, nurses' work wear and patients' changed bed sheets were determined, b) the microbiological contamination of the equipment used in the invasive procedures and the care-giver's hands were determined by using the swab method, c) the microbial contamination of the invasive devices was analyzed by using the catheter sedimentation method. The samples taken with the aim of identifying bacterial species present were put on selective culture media. Interpretation of the Count-Tact method results was performed according to the risk level present and the colony forming unit (CFU) count on a 25 cm^2 surface.
The obtained data were analyzed with the help of the following software: SPSS 16.0 for MS Windows, and MS Office Excel.

RESULTS

In total were analyzed 188 questionnaires which included patients' care with peripheral vein catheters (n=100) and patients' care with urinary catheters (n=88). The obtained results by questionnaire showed that most of the nurses were informed about the hospital-developed guidelines for insertion and patients' care with peripheral vein catheters in 77 cases (77%; n=100) and for insertion and patients' care with urinary catheters in 77 cases (87.5%; n=88) and adhered to these guidelines in their daily work. Data collected by clinically structured empiric research during cannulation of the peripheral vein and patients' care with peripheral vein catheters (n=30), and urinary catheterization and patients' care with urinary catheters (n=15) showed that cannulation of the peripheral vein in 16 cases (53.3%; n=30) and urinary catheterization in 5 cases (33.3%; n=15) were recorded in the ordination list, the patient's care protocol or the manipulation journal. Less than half of the observed nurses adhered to the principles of hand hygiene during invasive procedures: during the cannulation of the peripheral vein only 3 nurses (10%; n=30) treated their hands before putting on gloves and 10 nurses (33.3%; n=30) treated their hands after removing the gloves, but none of them used an adequate technique for treating their hands. By comparison, when dealing with insertion of urinary catheters, 3 nurses (13.3%; n=15) treated their hands before putting on gloves and 6 nurses (40%; n=15) treated their hands after removing the gloves, and 3 of them (13.3%) used adequate hand-treating techniques (Fig. 1 and 2). In 23 cases (77%; n=30) of the observed peripheral vein cannulation and in 4 cases (27%; n=15) of urinary catheterization, there was jewellery on the nurses' hands. Change of the peripheral vein catheters, not rarer than once in 72 hours, was observed in 25 cases (83.3%; n=30), the timely changing of urinary catheters - in 4 cases (27%; n=15) (Fig. 1 and 2). In 21 out of 30 cases (70%) the patient's puncture dressing was dry and clean, but nine patients (30%) had blood-soaked or wet puncture dressings. An observation study showed that a nurse from the surgical units takes care of 18 to 40 patients per day and 2 to 3 patients per day in the case of ICUs. Microbiological investigation with the Count-Tact method of samples taken from the hands of ICU nurses before the cannulation of the peripheral vein determined that the number of microorganism colonies exceeds the acceptable levels as much as sixteen-fold (44 CFUs and 83 CFUs; n=2) (the permissible level on 25cm^2 ≤ 5 CFUs). The bio-contamination of the hands of nurses working in surgical units also exceeded acceptable levels as the 25cm^2 surface produced more than 200 colony forming units (128 CFUs and 241 CFUs; n=2) (permissible level on 25 cm^2 ≤ 50 CFUs) (Table 1). Microbiological investigation of the patients' changed bed sheets and nurses' work wear performed with the Count-Tact method during urinary catheterization revealed a medium to high level of bio-contamination: the number of bacteria colonies on work wear exceeded the norm in 44 of the investigated cases (78.5%; n=56), *Mucor* fungi were found in 44 cases (78.5%; n=56) and the bacteria colonies count in the nurses' hair exceeded permissible levels in 48 cases (85.7%; n=56) (Table 2). The microbiological investigation of the urinary catheters' connection with the collector 72 h and 7 days after the insertion of the catheters showed that in 6 cases (50%) pathogenic microorganisms like the *E. coli*, β haemolytic *Streptococcus spp.* and fungi, like the *C.albicans* were present (Table 4). The research performed with the swab method and by catheter

sediment analysis 72 h and 96 h after the insertion of a peripheral vein catheters revealed the presence of such pathogens as *Pseudomonas aeruginosa, Staphylococcus aureus* in the sterile zone of the peripheral vein catheter in one case (25%). Likewise the analysis of the urinary catheter sediments showed the presence of pathogens like *S.aureus, P.aeruginosa, E.coli,* β haemolytic *Streptococc us spp.* and fungi like *C.albicans* and *C.tropicalis* in 6 cases (100%) both after 72 hours and 7 days of the insertion of the catheter (Tables 3 and 4).

DISCUSSION

Prevention of health care-associated infections is the key procedure in quality of patients care. The study of risk factors in patients' care with invasive devices is essential for evaluation of current infection prevention activities and for planning further interventions in units and in hospital levels as well as at national level. Obtained results help to identify risk factors for HAIs and to optimize the hospital work, the use of more efficient and financially advantageous methods for their prevention. This is the first report on risk factors for HAIs in patients' care with indwelling medical devices in Latvia.

Every nurse can play a significant role in minimizing the risk of devices- related infections. An important factor in the nurses' daily work is an adequate distribution of work shifts and duties, because excessive workloads are a risk factor for HAI (9). Our studies confirmed the excessive workload in nurses' daily work, which significantly exceeded the limits of patients' care (1-2 patients per nurse in ICU and 15 patients per nurse in surgical units). As shown by several studies, a significant role in limiting the risk of HAIs are care guidelines and nursing protocols for the development and implementation in practice (6, 11, 13, 16, 18). Our results showed that a common problem in ICUs and in surgical units is the lack of unified nursing protocols on performing invasive procedures. More and more, the authors support the principle of adequate hand hygiene, because many hospital personnel fail to follow basic infection control, such as hand washing between patients' contacts (4, 10, 21). In our studies, less than half of the observed nurses adhered to the principles of hand hygiene during invasive procedures and microbiological investigations showed a high level of bio-contamination during invasive procedures. The amount of microorganisms on the nurses' hands exceeded the acceptable levels sixteen-fold, and medium to high levels of bio-contamination were discovered on patient's changed bed sheets as well as in nurses' hair and their work wear. Our data demonstrate some evidence that the pathogenic flora (β haemolytic *Streptococcus, E. coli*) remains unchanged both 72 h and 7 days after the insertion of the urinary catheter. It leads to the conclusion that the urinary catheter is contaminated during the first 72 hours after insertion and should be changed at least once every 72 hours instead of once a week as indicated by the manufacturer of the catheters. However, most of devices-related infections are not severe and self-limiting, and microbiological investigations in such cases are not performed routinely in regional multi-profile hospitals of Latvia. Conclusions of epidemiologic research are inconsistent when evaluating the effect of changing intravascular or urinary artificial implants less frequently than once every 72 hours. Some authors point out that 24 hours is a sufficient period of time for a biofilm to form on the catheter surface, consisting of microorganisms such as *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, etc.* making the catheter a source of bacteremia (1, 5). Nevertheless the frequency of changing the catheters should not be considered as the main cause of HAI development, since more attention should be paid to antiseptics and aseptics during the patients' care with invasive devices (17).

Regardless of all previously mentioned, the rate of HAIs in multi-profile hospitals of Latvia is comparable to that of in the developed countries (3).

CONCLUSIONS

The following HAIs risk factors in patients' care with invasive devices were determined: 1) lack of unified nursing protocols on performing invasive procedures in the intensive care units and in surgical units, 2) disregard of basic principles of hand hygiene while carrying out invasive procedures, 3) antiseptic and aseptic mistakes during patients' care with invasive devices, 4) excessive workload of nurses working in ICUs and in surgical units, 5) high levels of microbiological contamination during invasive procedures, including medium to high levels of bio-contamination of changed patients' bed sheets, nurses' work wear and hair.

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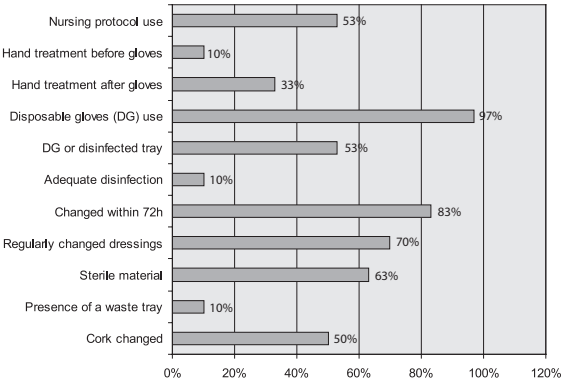

Fig. 1. Cannulation of the peripheral veins and patients' care with peripheral venous catheter (clinically structured empiric research, n=30)

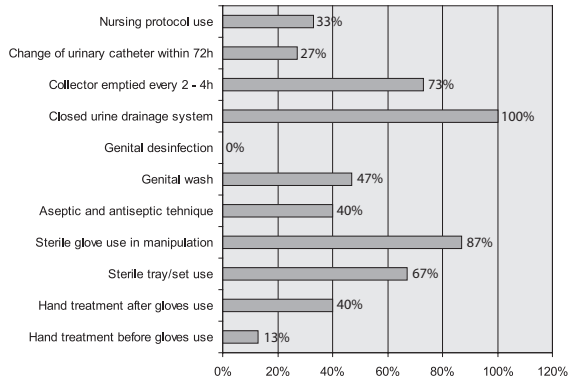

Fig. 2. Urinary catheterisation and patients' care with urinary catheter (clinically structured empiric research, n=15)

Table 1. Microbiological investigation with a Count-Tact method (samples taken prior to the introduction of a PVC, n=4)

Object	Obtained results
Hands of a surgical unit nurse in clinic A	241 colonies on 25cm²
Hands of a surgical unit nurse in clinic B	128 colonies on 25cm²
Hands of a ICU nurse in clinic A	44 colonies on 25cm²
Hands of a ICU nurse in clinic B	83 colonies on 25cm²

Table 2. Microbiological investigation with a Count-Tact method (samples taken prior to the insertion of a urinary catheter, n=6)

Object	Obtained results
Procedural trolley, sterile tray, set in ICUs of clinic A and B	0 colonies on 25cm²
Patient's changed bed sheets in ICU of clinic A	1000 colonies on 25cm²
Patient's changed bed sheets in ICU of clinic B	15 colonies on 25cm²
Nurse's work wear in ICU of clinic A	100 colonies on 25cm²
Nurse's work wear in ICU of clinic B	69 colonies on 25cm²

Table 3. Microbiological investigation performed using the catheter sedimentation method (n=4)

No.	Object	Blood agar medium	Chrom Agar Candida medium
Sample 1	Patient – male, 35 yrs. o., Surgical unit in clinic A Dg. Amputation of left leg PVC introduced 96 h ago	*Staphylococcus aureus*	Negative
Sample 2	Patient – male, 53 yrs. o. ICU in clinic A Dg. Hemorrhagic stroke. PVC introduced 72 h ago	Negative	Negative
Sample 3	Patient – male, 22 yrs. o. ICU in clinic B Dg.Polytrauma after a traffic accident. PVC introduced 72 h ago	Negative	Negative
Sample 4	Patient – male, 40 yrs. o. Surgical unit in clinic B Dg. Osteomyelitis of left shin. PVC introduced 96 h ago	Negative	Negative

Table 4. Microbiological investigation performed using the catheter sedimentation method and swab (n=12)

No.	Object	OESA medium	*P.aeroginosa* medium	Chrom Agar medium	Bloodagar medium
Sample 1	Connection between collector and catheter after 72 h (ICU, clinic A)	Neg.	Neg.	Neg.	β haemolytic *Streptococcus, E. coli*
Sample 2	Biomaterial sample taken from the catheter after 72 h (ICU, clinic A)	Positive	Neg.	*C. albicans*	β haemolytic *Streptococcus, Staphylococcus aureus*
Sample 3	Connection between collector and catheter after 7 days (ICU, clinic A)	Neg.	Neg.	Neg.	Neg.
Sample 4	Biomaterial sample taken from the catheter after 7 days (ICU, clinic A)	Neg.	Neg.	*C. albicans*	β haemolytic *Streptococcus, Staphylococcus aureus*
Sample 5	Connection between collector and catheter after 72 h (ICU, clinic B)	Neg.	Neg.	Neg.	Neg.
Sample 6	Biomaterial sample taken from the catheter after 72 h (ICU, clinic B)	Neg.	Neg.	Neg.	*E. coli*
Sample 7	Connection between collector and catheter after 7 days (ICU, clinic B)	Neg.	Neg.	Neg.	β haemolytic *Streptococcus*
Sample 8	Biomaterial sample taken from the catheter after 7 days (ICU, clinic B)	Neg.	Neg.	*C. tropicalis*	*E. coli*
Sample 9	Connection between collector and catheter after 72 h (ICU, clinic C)	Neg.	Neg.	Neg.	β haemolytic *Streptococcus, E. coli*
Sample 10	Biomaterial sample taken from the catheter after 72 h (ICU, clinic C)	Neg.	Positive	Neg.	β haemolytic *Streptococcus*
Sample 11	Connection between collector and catheter after 7 days (ICU, clinic C)	Neg.	Neg.	*C. albicans*	Neg.
Sample 12	Biomaterial sample taken from the catheter after 7 days (ICU, clinic C)	Neg.	Neg.	*C. albicans*	Neg.

Conflict of interest: None

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ORIGINAL ARTICLE

Clostridium difficile Associated Disease Clinical and Molecular Data

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Summary

Introduction. A balanced microflora of the gastrointestinal tract plays a significant role in the protection of an organism. Dramatic changes may take place in the composition of normal microflora during antibacterial therapy, because part of sensitive microorganisms die and microflora detrimental to an organism proliferates there causing gastrointestinal tract disorders (7).

C. difficile is an important agent causing gastrointestinal tract disorders. It may induce manifestations ranging from asymptomatic colonization of the gastrointestinal tract to severe diarrhea, pseudomembranous colitis, toxic megacolon, intestinal perforation, and death. All these severity levels are included in the term *Clostridium difficile associated disease (CDAD).* A patient at risk for *C. difficile* is an elderly person with a severe principal disease, who receives long-term inpatient treatment, has been receiving antibiotics, antacid and antiulcer agents for a long time, has had a gastrointestinal surgical intervention or any organ transplantation, which is always related to immune suppression (11).

Aim of the study. Molecular typing of Latvian strains of *C. difficile* and clinical data analysis.

Materials and methods. The research includes data collected during the time period from August 2006 to the end of 2008 from three Latvian hospitals. A pure culture of *C. difficile* bacteria was isolated and cultured from fecal material. Ribotyping was done by amplification of specific regions of the 16S and 23S RNA genes and amplification products were separated in 1.5% agarose gel.

Results. Fecal samples of 500 patients having manifestations of clinical symptoms of CDAD were microbiologically and molecularly studied from August 2006 to the end of 2008. 130 samples were *tcdA*$^+$*tcdB*$^+$ toxin-positive.

Conclusions. All samples were cultured on agar in anaerobic conditions. As a result, 55 pure cultures were obtained. The average age of patients is 65 years. Women get infected more frequently than men. In 95% patients CDAD was induced by antibacterial therapy. In 86% cases, having received a positive response for *C.difficile* antibacterial therapy is changed. Disease development risk factors are previous antibacterial therapy, previous hospitalization, GIT manipulations and *C.difficile* infection in anamnesis. The biggest number of CDAD patients was found in Nephrology Department of P.Stradins Clinical University Hospital. A hypervirulent *Cl.difficile* ribotype 027 was not found in Latvia yet, bat 16 different ribotypes were determined using Bionumerics software. 4 of them are domineering ribotypes with 17, 14, 5 and 4 isolates in every group.

Key words: *C.difficile; CDAD; tcdA and tcdB.*

INTRODUCTION

A balanced microflora of the gastrointestinal tract plays a significant role in the protection of an organism. Dramatic changes may take place in the composition of normal microflora during antibacterial therapy, because part of sensitive microorganisms die and microflora detrimental to an organism proliferates there causing gastrointestinal tract disorders. The most widespread endogenous infection related to use of antibiotics is candida, induced by fungi of the genus *Candida* and *Clostridium difficile* (7).

C. difficile is an important agent causing gastrointestinal tract disorders. It may induce manifestations ranging from asymptomatic colonization of the gastrointestinal tract to severe diarrhea, pseudomembranous colitis, toxic megacolon, intestinal perforation, and death. All these severity levels are included in the term *Clostridium difficile associated disease (CDAD).* A patient at risk for *C. difficile* is an elderly person with a severe principal disease, who receives long-term inpatient treatment, has been receiving antibiotics, antacid and antiulcer agents for a long time, has had a gastrointestinal surgical intervention or any organ transplantation, which is always related to immune suppression (11).

The increase in *C.difficile* infection rates for the last three decades may be explained by:

1. Improvement of diagnostic techniques;
2. Widespread use of antibiotics and chemotherapy;
3. Contamination of hospitals with C.*difficile* spores (9).

C. difficile is a gram-positive, spore-forming, anaerobic, rod-shaped bacterium of the genus *Bacillus*. It may be found in many objects, therefore it is called a ubiquitous (being everywhere) microorganism. It is present in soil (up to 21%), water, intestinal tract of many animals. It is also present, though not very often, in the intestinal tract of healthy people – *C. difficile* may be isolated from feces of not more than 5% of healthy people; *C.difficile* is

present in feces of up to 50% of healthy newborns, but it does not cause disease, because babies are protected by antibodies received from their mothers. Sometimes the microorganism may be also found in the vagina and in the urethra(14). The colonization percentage among elderly people, especially those who received inpatient treatment, may reach 10-20%, because the hospital environment is often contaminated with *C. difficile* spores. Vegetative bacteria die, but spores may live for months and even years (20).

Colonizing the large intestine *C.difficile* generates toxins. *C. difficile* generates two toxins – A(*tcdA)* and B(*tcdB)*. Toxin production is determined by a chromosomal gene. Toxin production and microorganism's virulence depends on the degree of gene expression (6).

Less than 1% are symptom-free carriers of *C.difficile*. Factors of host and microorganism determining symptom-free carriage are not clear. Symptoms appear either during use of antibiotics or shortly after it. In rare cases the interval may be several weeks. Patients most frequently have mild or moderately severe diarrhea, they may also have loose stool with occult blood. Rarely, it may be accompanied by pains in the lower belly. Severe colitis, without formation of pseudomembranes, characterized by profuse, severe diarrhea, stomach aches, and abdominal swelling, may develop. The most frequent systematic symptoms are sickness, fever, loss of appetite, tiredness. Rarely, hemorrhagic colitis develops. Diffuse or fragmentary nonspecific colitis is visible on the sigmoidoscopy. Severe colitis with formation of pseudomembranes is characterized by pronounced severe diarrhea, stomach aches. Characteristic yellow pseudomembranes, 2 to 10 mm in diameter, are visible on the sigmoidoscopy, the mucous membrane between them looks healthy or slightly eritematous. An acute stomach pattern with pronounced stomach aches, fever, and tachycardia may develop in patients. As a result of loss of muscular tone toxic megacolon may develop, which is a state usually characteristic of ulcerative colitis (16).

When paralytic ileus and large intestine dilatation develop, a pathologic decrease in the volume of diarrhea is observed. If stomach aches increase, intestinal perforation and peritonitis should be excluded. Anyway, disease symptoms may be different and may change every several hours, therefore it is important to follow up the patient and to evaluate the situation in an adequate way (5).

Since 2002 special attention has been attracted to a hypervirulent strain of *C. difficile* which belongs to toxinotype III (ribotype 027) and initially spread in the USA and Canada (18,19), but now has already caused hospital outbreaks in Ireland (17), Belgium (8), France (23), Denmark (22), Austria (10) and other countries (4,13).

Taking into consideration that a very pathogenic *C. difficile* ribotype 027 causing very serious disease is currently spreading in the USA and Europe, the research of this microbe becomes very topical in Latvia as well in order to perform not only diagnostics, but also ribotyping of this microorganism to start adequate therapy in case of necessity and perform infection control measures.

All CDAD patients need patient-centered approach, developing the most optimal and effective treatment regimes. Since CDAD is a severe infectious disease requesting the use of many different drugs (PDS, antibacterial, cardiological agents, anaesthetics, and resolvents, etc.), the presence of a hospital pharmacist could significantly optimize patient's treatment as well as reduce treatment costs . It is important to carry out a separate research on the situation in Latvia, because in case of minor microbiological changes in the circulating inducer, the process and the course of treatment may differ from the experience of other countries.

AIM OF THE STUDY

Molecular typing of Latvian strains of *Cl. difficile* and clinical data analysis.

MATERIALS AND METHODS

The research includes data collected during the time period from August 2006 to the end of 2008 from three Latvian hospitals – P. Stradins Clinical University Hospital,

Gailezers hospital and Traumatology and Orthopedic Hospital. A questionnaire applied only to patients of P. Stradins Clinical University Hospital and a retrospective study was performed within the framework of this research.

Bacterial isolates. DNA was isolated from feces which were sent to the laboratory being suspect to CDAD, using a kit QIAamp DNA Stool mini Kit (Qiagen, Germany) and it was determined whether they contained toxin genes *tcdA* and *tcdB* by PCR (16). A pure culture of *Cl. difficile* bacteria was isolated and cultured from fecal material. Each material was cultured on *Cl. difficile* selective agar (*Clostridium difficile* Selective Agar, Becton Dickinson, USA), blood agar and CNA blood agar, according to anaerobic culture growth methods (20). On blood agar and CNA blood agar *Cl. difficile* is of medium-size, forming colonies with a concentrated whitish center and a transparent, wavy area around it. Colonies are characterized by 'smell of a horse'. On *Cl. difficile* selective agar colonies grow in a similar way, but the colour of colonies change as a result of fission of fructose (mannose).

Ribotyping. Ribotyping was done by amplification of specific regions of the 16S and 23S RNA genes and amplification products were separated in 1.5% agarose gel (12, 15). The filogenetic analysis of ribotypes was performed using BioNumerics (Applied Maths BVBA) software (1).

RESULTS

Fecal samples of 500 patients having manifestations of clinical symptoms of CDAD were microbiologically and molecularly studied from August 2006 to the end of 2008. 130 samples were $tcdA^{+}tcdB^{+}$ toxin-positive. All samples were cultured on agar in anaerobic conditions.

As a result, 55 pure cultures were obtained. All cultures comprised toxinogenous *Cl.difficile* bacteria, which were ribotyped, i.e. the arrangement of ribosomal genes in a specific strain was determined (Figure 1).
As a result of filogenetic analysis 16 different ribotypes were determined using Bionumerics software. 4 of them are domineering ribotypes with 17, 14, 5 and 4 isolates in every group. (Figure 2).
Having analyzed data from appointment card forms, it can be concluded that the average age of patients is 65 (+/-17.12) years. Having distributed patients by age groups with a 5 year interval it is seen that mainly people older than 60 get infected. (Figure 3).
Distribution by gender: 35 women (64%) and 20 men (36%). Almost all patients, i.e.
52 patients (95%), received antibacterial therapy, 3 patients (5%) did not receive it. All patients had serious clinical diagnoses and were treated for a long time both at home and in hospital.
21 patient of all 55 patients were from P.Stradins Clinical University Hospital. The analysis by departments clearly demonstrates that the biggest number of confirmed *Clostridium difficile* patients is in the Nephrology Department (Figure 4). The development of clinical presentation of CDAD was similar in all patient, diarrhea and fever were prevailing (Figure 5). Retrospectively analyzing possible risk factors for CDAD development, it was traced that the majority of patients were prescribed antibacterial therapy in hospital (18 patients), 10 patients had already been hospitalized during previous two months, 8 patients were performed different GIT manipulations and 3 patients had *C.difficile* infection in their anamnesis (Figure 6). Having received a positive response for *C.difficile* antibacterial therapy is changed in 86% cases, it is changed to metronidazole or vancomycin (Figure 7).

DISCUSSION

Analysis of the clinical data was performed during the research for patients having laboratory-proved *C. difficile* infection. The average age of patients of the study is 65 years. CDAD patients are elderly people also according to literary data from all over the world (2). In 95% of patients the infection was induced by antibacterial therapy used for treatment of the principal disease. Having received a positive response for *C.difficile* positive toxin, the therapy was changed to metronidazole or vancomycin – agents selected for treatment of *C.difficile* infection – in 86% cases. The infection may develop in 5 to 30% of patients receiving antibacterial therapy (3). Due to the fact that the range of antibiotics becomes wider, the incidence of the disease has increased over the past years. Currently, *C.difficile* is resistant to all antibiotics, except vancomycin and metronidazole, scientists, however, consider that new bacterial strains may become resistant to these antibiotics as well. Thus antibacterial agents are the main cause of outbreaks of iatrogenic diarrhea, and it significantly increases mortality and inpatient treatment costs (2).
20% of hospitalized patients get infected by *C.difficile* infection in hospital and diarrhea develops in 30% of them (5). The development of *C. difficile* bacterium is also stimulated by patient's complicated severe principal diagnosis making the patient to receive long-term inpatient treatment and have diverse examinations related to it in hospital, in particular gastrointestinal tract examinations. It is proved by my research data.
A toxicogenic ribotype 027 is not spread in Latvia yet, but 16 other different ribotype strains inducing severe health impairments are spread. British scientists have already proved that according to their studies *C. difficile* is able to collect mutations and exchange genes incredibly quickly, in this way ensuring its viability. 10% of the genome consist of mobile phases, i.e. the nucleotide sequence may migrate from one organism to the other, as a result a bacterium with genes making it so pathogenic appears (21). Therefore, the relation of other ribotypes to clinical presentations should be evaluated and infection control measures should be implemented. The control possibilities of this infection are not complex, but are labour-consuming. The use of high-risk antibacterial agents should be reduced and the spread of *C. difficile* spores in hospitals should be restricted. Experimental data demonstrate that in the ward, in which an infected patient was accommodated, one third of environment samples contain spores of the bacterium, but in control wards only 1.3% of samples are positive (13). Therefore, hygiene plays a very important role in the restriction of spread of the infection.

CONCLUSIONS

1. The average age of patients is 65 years.
2. Women get infected more frequently than men, distribution by gender: 35 women (64%) and 20 men (36%).
3. In 95% patients CDAD was induced by antibacterial therapy.
4. In 86% cases, having received a positive response for *C.difficile* antibacterial therapy is changed.
5. Disease development risk factors are previous antibacterial therapy, previous hospitalization, GIT manipulations and *C.difficile* infection in anamnesis.
6. The biggest number of CDAD patients was found in Department of Nephrology in Pauls Stradins Clinical University Hospital.
7. A hypervirulent *Cl.difficile* ribotype 027 was not found in Latvia yet, bat 16 different ribotypes were determined using Bionumerics software. 4 of them are domineering ribotypes with 17, 14, 5 and 4 isolates in every group.

Conflict of interest: None

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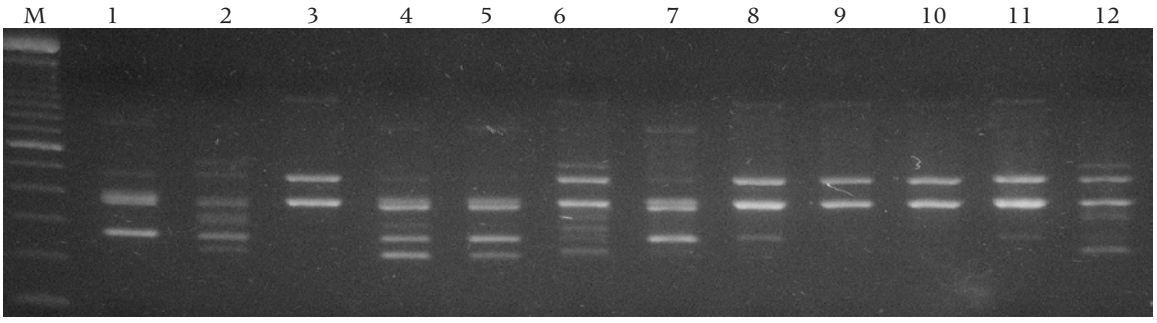

Fig. 1. Separation of ribotyping amplification products in 3% agaroze gel. 1 – 12 different isolates. M – DNA marker (100bp DNA Ladder, Invitrogen)

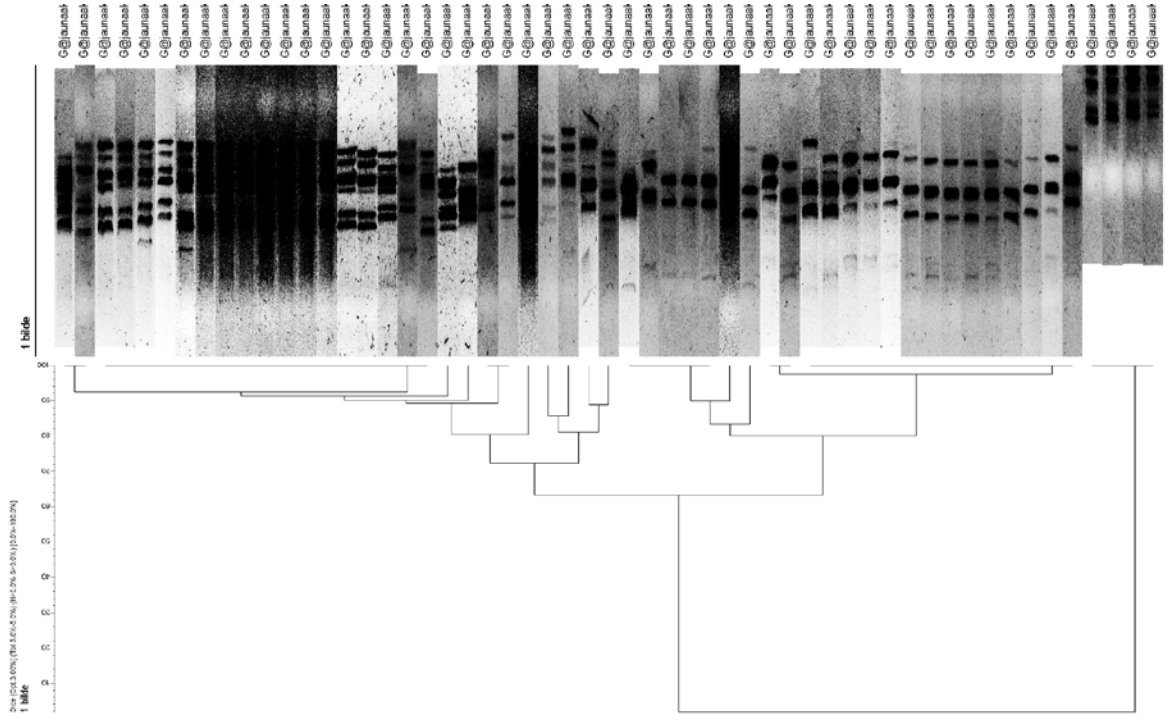

Fig. 2. Filogenetic tree of *Cl.difficile* isolates

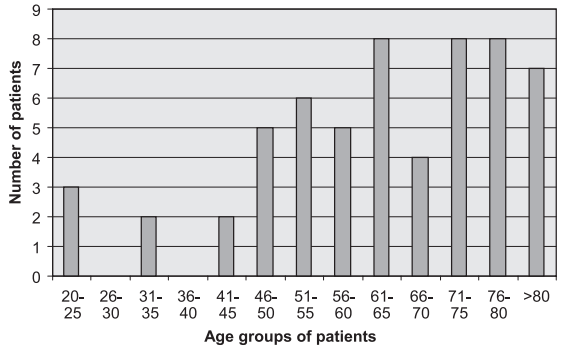

Fig. 3. Age distribution of patients with *Clostridium difficile*

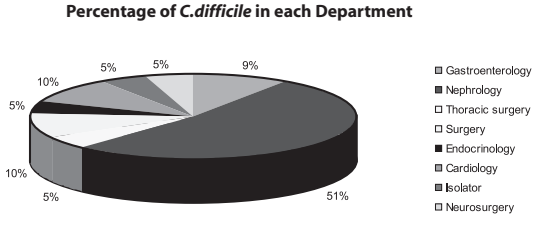

Fig. 4. The percentage of *Clostridium difficile* patients in each department in Pauls Stradins Clinical Unversity Hospital

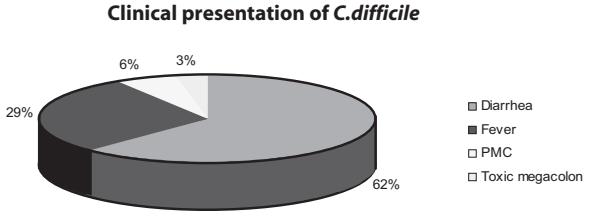

Fig. 5. Clinical presentation of *C.difficile* infection

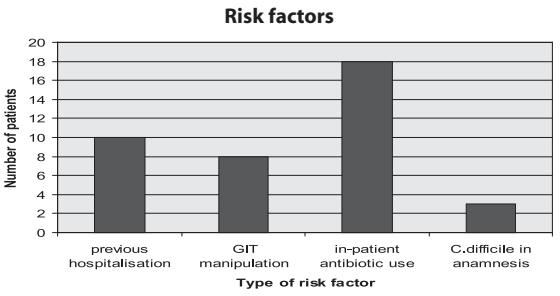

Fig. 6. Risk factors for the development of *Clostridium difficile* infection

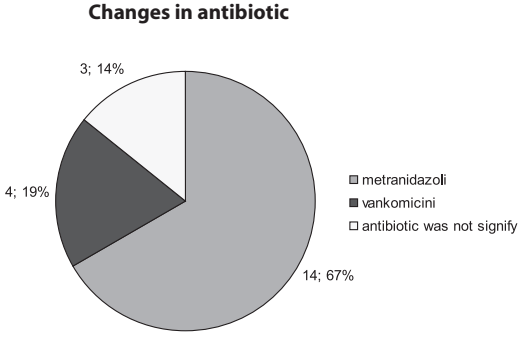

Fig. 7. Changes made to the administration of high risk antibiotics that have been implicated as risk factors for development of *Clostridium difficile*

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ORIGINAL ARTICLE

Central Auditory Processing Disorders: Effects of Age and Hearing Loss to Electrophysiological and Behavioral Responses

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Summary
Introduction. Central auditory processing involve normal sound perception, speech recognition, ability of cognition and memory. Accordingly speech recognition difficulties may manifest due to changes at any segment of auditory processing. Cortical auditory evoked potentials (CAEPs) and behavioral measures provide insight into the neural mechanisms underlying speech recognition. These disorders are observed both in young and elderly population.
Aim of the study. To evaluate central auditory processing for subjects of different age and hearing level through the presentation of noise using CAEPs and behavioral measures of speech discrimination. However due to complicated connectivity in auditory pathway it is difficult to identify the central auditory processing damage. Behavioral tests in conjunction with electrophysiological examination will reveal more complicated information for differenciation between peripheral and central auditory processing disorders.
Materials and methods. Three groups of subjects participated: young normal hearing, young hearing-impaired and elderly hearing–impaired subjects. To minimaze subject variables, the CAEPs (wave peaks P1,N1,P2,N2,P3,N3 in miliseconds) was investigated using passive listening paradigme. The CAEPs were elicited by 1,1s change in frequency 1000 Hz and 2000 Hz in pure tones presented at 65, 70 and 75 dB SPL. Sentence recognition tests in quite and noise, Digit Pairs (DP) and Word Pairs (WP) were developed in Latvian language prior to investigation. They were presented to all subjects.
Results. The most prominent finding was the increased latency of P3, N3 in elderly and also in younger hearing impaired adults groups and highly differed within groups. More prolonged latencies were find of N1, P2 in elderly hearing impaired group than in younger hearing impaired group adults.
Conclusions. During this investigation the CAEPs are performed for the first time in our clinic therefore the main standarts are determined for our laboratory. The speech recognition tests (Sentence recognition test, DD, DW) are developed in Latvian. The present findings suggest patterns of CAEP are variable within individuals however shows that auditory perception and cognitive function is not only a result of aging and must be associated with a general slowing of neuronal processing or decreased neuronal synchrony within the central auditory nervous system. Determination of central auditory processing capacity level is of crucial significance to prognose and evaluate the hearing result after hearing prosthetics and to appraise indications for hearing prosthetics, cochlear and middle ear implantation including.
Key words: central auditory evoked potentials (CAEP); event related potentials (ERP); auditory processing; speech recognition threshold (SRT); peak latencies; P3.

INTRODUCTION

Difficulty in understanding speech especially in adverse listening conditions is a common complaint of elderly persons. However more and more young people complains of it. The auditory system must be able to select out signals of interest from surrounding information. In this selective attention process are involved peripheral, central auditory and cognitive factors, hence significant interactions among these factors have not been fully established. Aging is one of the reasons of impaired selective attention processes (Humes, 2005). Declined cognitive functioning in elderly population also negativly affects auditory processing, including selective attention (Mueller et al., 2008). Relationship between cognitive function and performance on central auditory tests have been reported (Golding et al., 2005). Dichotic listening correlated with measures of general cognitive function like auditory processing speed and working memory (Martin et al., 2008).

One of the remarkable characteristics of human hearing comprehension is its speed (Norris et al., 1995). Scalp-recorded evoked brain potentials including CAEPs derived from electroencephalogram (EEG) takes relevant place in investigations of central auditory processes. The CAEPs reflect changes of ongoing EEG activity evoked by the stimulus. The most important components of ERP studies are peaks such as N1, P1, N2, P2, N3 and P3 (Naatanen et al., 2007). It is observed that peaks N1 and P2 are earliar sensory-perceptual potencials and reflect response of early attention to stimulus (Anderer et al., 1996). The peak N2 is very sensitive to different deviant stimuli (Naatanen, Gaillard, 1983). The P1-N1-P2 complex reflects neural activity of structures in the thalamo-cortical part of the central auditory system

in response to acoustic changes (Martin et al., 2008). Later components N2 and P3 show endogenous or cognitive stages of the auditory processing (Naatanen, 2007). Auditory event related potential and behavioral investigations often have focused to examination of either age or age-related hearing loss.

The aim of the current study was to investigate the effects on CAEPs in young and elderly listeners suffering of speech recognition difficulties and to study the differences between this lesion in elderly and younger adults groups. Furthermore, it is important to determine how ERP waveforms are related to behavioral performance. Hence this study may reveal correlation between speech recognition scores and CAEPs speed.

MATERIALS AND METHODS

Subjects

Three groups of right-handed human subjects were included: 12 younger patients (YHI; aged 24-32 years, 6 males and 6 females) and 14 older (EHI; aged 52-65 years, 9 males and 10 females), and 12 control subjects (NH; aged 22-28 years, seven males and five females). All participants of the study were native-Latvian-speaking, with no history of otological or neurological disorder and nobody report having tinnitus at the time of testing.

All control subjects had air and bone conduction hearing thresholds of no more than 15 dB hearing loss in the range between 125 and 8000 Hz. Pure tone thresholds for both patient groups varied from mild to moderate bilateral sensoneural high-frequency hearing loss.

Pure tone thresholds and speech recognition tests were measured with a Madsen OB 922 clinical audiometer calibrated to appropriate ANSI standarts (ANSI S3.6.2004) and equipped with TDH-39 headphones. Thresholds were measured in 5-dB steps.

Prior to test sessions tympanometry was performed to exclude middle ear functionating disorders. The auditory brainstem evoked potential responses (ABR) were determined at three intensities (60, 65 and 75 dB) and the latencies of the fifth wave of the ABR (ABR V) were within norms (SD=0.1-0,2; ABR V latencies 5,4-5,57ms) for all participants in three groups (Fig.1).

The patients and the control subjects were fully informed about the experimental procedures in accordance with the decision of the Ethical Committee of Riga Stradins University.

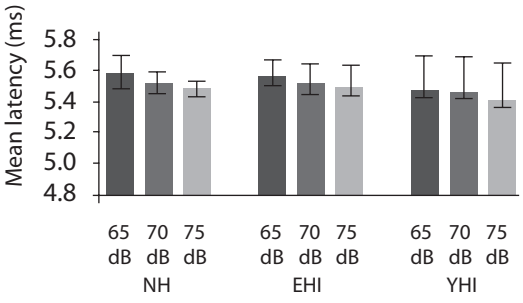


Fig. 1. Mean latencies (ms) of the fifth wave of auditory brainstem response for normal-hearing subjects (NH), elderly hearing-impaired subjects (EHI) and young hearing-impaired subjects (YHI) in stimulus intensities of 65,70 and 75 dB in noise

Speech recognition tests

Speech recognition tests (Sentence recognition test, word pairs (DW), digit pairs (DD)) were developed in Latvian prior to this study. Testing was conducted in a sound treated room. Testing lasted two hours including two breaks of 15 minutes.

Sentence recognition test (SRT) was developed in Latvian (Plomp and Mimpen,1979; Gelfand, 1998). The test material consists of ten short everyday sentences consisting of five or six words presented in quite and in background noise which increases the speech level for 20 dB. The listener is asked to repeat the last word of the sentence. The procedure is continuing increasing or decreasing the speech intensity level by 5 dB in accordance to whether the final word is recognized or not. The S/N ratio for 50 % of the identified final words was detected. Two lists of the SRT were presented monaurally to both ears. The results of the right ear were analized.

Word pair test (DW) in Latvian (Baran and Musiek, 1999; Jerger et al., 1993) consists of two-syllable words, the first of syllables of each word of the pair are similar. Five word pairs consisted of 10 words were presented. In addition simultaneously to rihgt and left ear one word of the pair was presented. The listener is asked to repeat each word. The number of identified words in percentage is fixed as a recognition score.

Digit pair test (DD) in Latvian (Musiek, 1983; Kimura, 1961a, 1961b) consists of two one-digit number. One pair of digits consists of similar syllable words (one, two or three) arranged in five pairs, ten words at all. Like in previous test simultaneously to every of ears one word of the word pair was presented. Partcipant must repeat each comprehended word and recognition score was determined in percentage.

Stimuli

For electrophysiological tests were used pure tones. The standart click stimuli were 1000Hz and 2000Hz pure tones with rise/fall times of 7 ms and duration of 700 ms in quite and in contralateral noise. The tone was presented at three intensity levels: 60, 65 and 75 dB SPL to the right ear and the noise 40 dB SPL to the left ear through TDH-39 earfones with S/N ratio 0 dB.

The tone was presented at three intensity levels: 60, 65 and 75 dB SPL to the right ear and the noise to the left ear that was 40 dB SPL lower then the current stimulus through TDH-39 earfones with S/N ratio 0 dB

Auditory evoked potential recordings

The auditory evoked potentials were obtained using two chanell recording configuration GNOtometrics EP Charter system connected with sound source with TDH-39 earphones. Surface EP electrodes were placed at the T3, T4, left mastoid (LM), right mastoid (RM) accordding to the International 10/20 system (Jasper, 1958). One electrode placed at the low forehead served as a ground electrode. Impedance was kept below 5 kΩ and was controlled during the recording session. Auditory evoked potential waves were digitally band-pass filtered at 0,1-30 Hz.

Procedure

Testing was conducted in a sound-treated room. During CAEP recordings subjects sat comfortably and read quietly. Testing lasted around two hours with 5 min brakes without auditory stimulation. Responses were averaged across a 500ms time window (100-ms prestimulus period and 400-ms post stimulus period). Epochs with artifact measuring in excess of 50 μV were excluded from average waveform. Acquisition was 1024 sweeps, every sweep time was 10 ms and rate 1,1/s.

Data analyses

For each subject and condition averages were created. P1,N1,P2, N2,P3,N3 peak latencies and amplitudes were determined. The group average waveforms (Fig.1) were used to appoint latency segments to detect the CAEP peaks (Picton et al., 1984). The P1-N1-P2 are lokalizated in time window within 200 ms, where P1 and P2 are positive peaks and N1 is the greatest negativity between them. Positive peak P3 originates around 300 ms after onset of stimuli. Negative peak N2 was identified after prominent peak P2 but N3 follows after P3. Peak amplitudes were calculated relative to baseline, and peak latencies to stimulus onset. ERP amplitudes, latencies and behavioral measures were analyzed by ANOVAs, the associations between ERP and behavioral measures were investigated using Pearson's product-moment correlations.

RESULTS

Speech recognition tests

The average S/N ratio of the SRT test in noise was 0,8 dB (SD ±2.6 dB) for NHI, 7 dB (SD ± 9,8 dB) for YHI and 9 dB(SD ±5,4) for EHI. The NH group had significantly smaller S/N ratio compare with YHI and EHI group ($p<0,05$). Variability of discrimination thresholds were larger in elder hearing impaired group however no reasonable differencies between YHI and EHI groups (Fig.2). Word and digit discrimination scores were significantly lower in young (YHI) and elderly hearing-impaired (EHI) groups in comparison with normal hearing (NH) subjects. The mean scores of digit discrimination (DD) was 95% (SD± 5,3) and word discrimination (DW) was 90% (SD± 6,7) for the NH; DD was 64,5% (SD± 10,1) and DW was 59,5% (SD± 14,6) for the EHI; DD was 70.0% (SD±13,5) and WD 65,5% (SD±14,2) for the YHI (Fig.3). Results of the behavioral measures reveal speech discrimination decreased level in both hearing impaired groups ($p<0.05$).

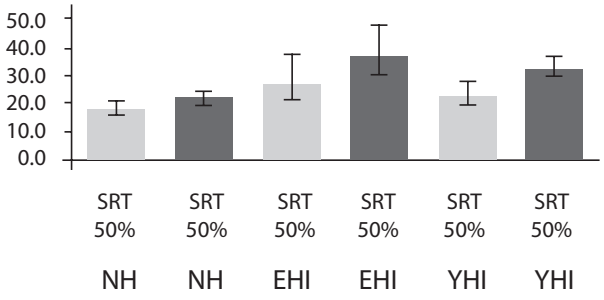


Fig. 2. Speech recognition threshold (dB) in quite (SRT50%) and in noise (SRT50%N) for normal-hearing subjects (NH), elderly hearing-impaired subjects (EHI) and young hearing-impaired subjects (YHI)

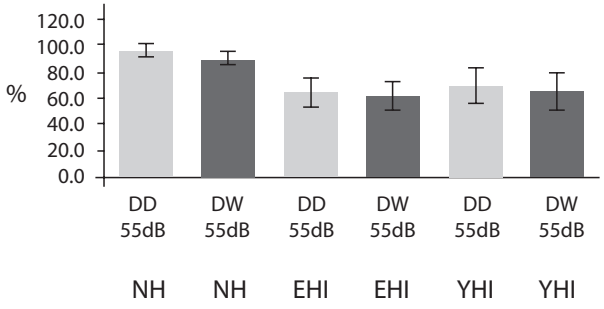
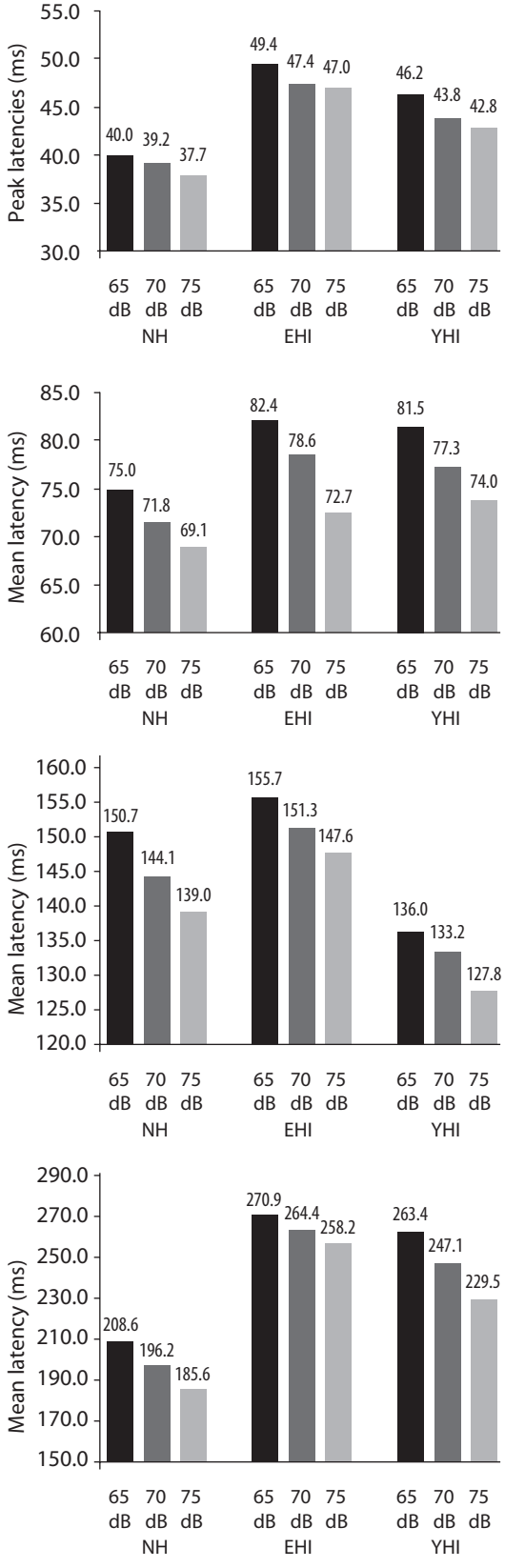


Fig. 3. Digit pair test (DD 55dB) and word pair (DW 55dB) recognition scores (%) for normal-hearing subjects (NH), elderly hearing-impaired subjects (EHI) and young hearing-impaired subjects (YHI)

Electrophysiological data: latency and amplitude characteristics

Similar response patterns were observed of other subjects for click stimuli at three intensities of 65, 70 and 75 dB SPL in presence of background noise 40 dB. The components P1, N1 and P2 represent earlier stages of auditory processing more sensitive to physical factors and can be evoked without attention. The average peak latencies in each subject group are shown in Fig.4. Waveforms clearly reflects a prominent negative peak N1 and positive peaks P1 and P2. According to analysis of ANOVA the latencies and amplitudes of peaks P1 and P2, were not significantly dependent to change of stimulus intensity or attendance. Results from these peaks were not of great diference between groups ($p<0,005$).



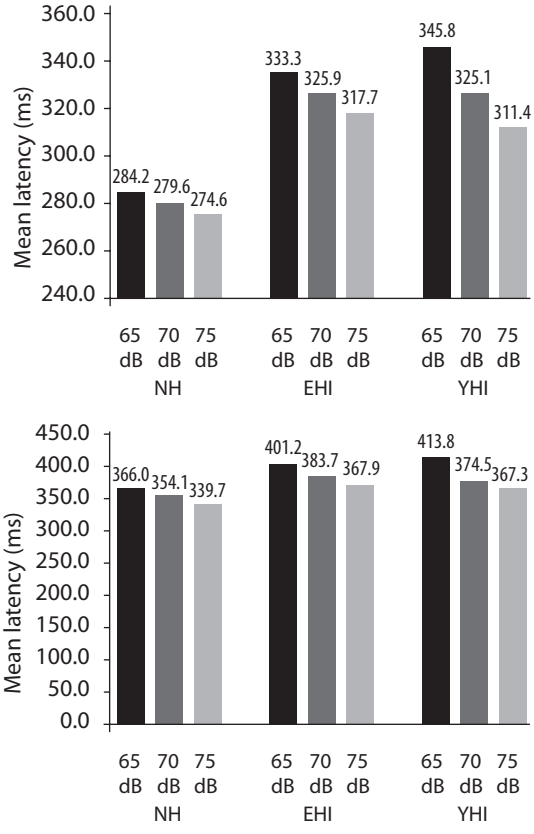


Fig. 4. Mean latencies (ms) of peaks P1, N1,P2, N2, P3 and N3 for normal-hearing subjects (NH), elderly hearing-impaired subjects (EHI) and young hearing-impaired subjects (YHI) in stimulus intensities of 65,70, and 75 dB in noise

More sensitive to attention level and signal intensity and noise was component N1. Latencies increased and amplitudes decresed in noise and without attention in YHI and EHI groups ($p<0,005$). P3 and N2 peaks are significantly smaller between NH and both hearing-impaired groups, in same cases very difficult to identify. Attention increases the amplitude of the N2 ($p<0,05$). The main effect in subject groups was observed on latencies of P3. Responses in YHI and EHI group were significantly longer in comparison with NH group ($p<0.005$) and these latences did not differ in YHI and EHI groups. Waveform for one of subjects of YNH group at signal intensity 70 dB SPL is shown in Fig.5.

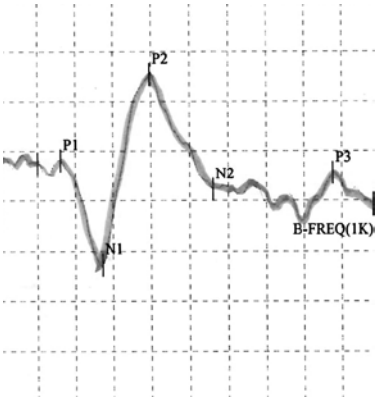


Fig. 5. Waveform for YHI subject at signal intensity level 70 dB SPL

Correlation between ERP and behavioral measures

The relationship between CAEP measures (P3 latences) and behavioral measures (SRT, S/N ratio, DW, DD) of speech discrimination performance was studied using Pearson's product-moment correlations. The correlation coefficients are shown in Fig.6. The most considerable correlations were between P3 latencies and speech recognition threshold and word pair (DW) scores in young and elderly hearing impaired groups (R2=0,244; p<0,05).

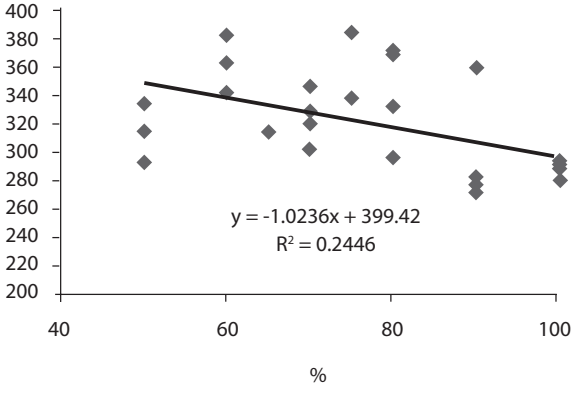


Fig. 6. Pearson's product-moment correlations coefficient between P3 latencies and speech recognition threshold (SRT), word pair (DW) and digit pair (DD) scores in young (YHI) and elderly hearing impaired (EHI) groups (R2=0,244; p<0,05)

DISCUSSION

Cortical auditory evoked potentials were recorded in response to periodic click stimulation at three intensities, in quite and presence of noise, and speech recognition scores were determined. The aim of this study was to investigate the age and hearing impairment influence to the different evoked potential components and speech recognition abilities. For this goal was determined intensity – latency characteristics, correlation between auditory evoked potential latencies and speech recognition scores in three participant groups. The main findings from this study is that the responses of definite CAEP components to noise and deviant stimulus intensity are different for normal hearing subjects in comparison with both hearing impaired subjects. Earlier peak P1, N1 and P2 latencies and amplitudes were not changed greatly between groups and within groups at different sitimulus intensity. The most considerable changes were observed for peaks N2 and P3. These peaks are very small or even absent unattended in, both hearing-impaired groups. They increase considerably if the listener is more attentive in all of tested groups (Naatanen,2004). Speech recognition abilities were significantly affected in hearing-impaired groups in comparison with normal hearing subjects. Recognition scores decreased significantly for DD and DW tests in less for SRT level in YHI and EHI groups in quite and more considerably in noise. These findings were rather similar in both hearing-impaired groups). P3 and N3 were significantly prolonged in elderly and younger hearing impaired adults. These later components P3 and N2 are connected with brain working memory (Stadler,2006) and ability of cognition. Accordingly speech recognition difficulties may manifest in these populations. These findings suggest that auditory signal recognition and discrimination is not only age-related hearing disorder. There is an evidence that CAEP may help to reveal processing disorders before they have manifest.

CONCLUSIONS

1. During this investigation the CAEPs are used for the first time in our clinic therefore the mean standarts were determined for our laboratory. The speech recognition tests (Sentence recognition test, DD, WP) are developed in Latvian.
2. Based on the results from behavioral and electrophysiological measures hearing difficulties were observed in both, elderly and young hearing-impaired subject groups. All of CAEP components are affected by tone level and the presence of noise by apearing prolonged later CAEP components that confirm about slower speed of auditory information. The present findings suggest that patterns of CAEP are variable within individuals however shows that auditory perception and cognitive function is not only a result of aging and must be associated with a general slowing of neuronal processing or decreased neuronal synchrony within the central auditory nervous system.

3. Determination of central auditory processing capacity level is of crucial significance to prognose and evaluate the hearing result after hearing prosthetics and to appraise indications for hearing prosthetics, cochlear and middle ear implantation including.

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Conflict of interest: None

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CASE REPORT

Teenager with Rosette-Forming Glioneural Tumour of the Fourth Ventricle: Case Study of a Rare Tumour

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Summary
We report the case of uncommon rare tumour of the fourth ventricle in young person. This diagnosis is included to WHO classification only in 2007. Tumour was identified by the MR imaging, later on surgically removed with post-operative MRI control scan. Diagnosis – rosette-forming tumour of the fourth ventricle- was confirmed both morphologically and immunohistochemically.
Key words: rosette – forming tumour of the fourth ventricle; diagnostics.

AIM OF THE DEMONSTRATION

The aim of the article is to demonstrate rare diagnosis case, which was confirmed for the first time only in 2007, its options of management and diagnostics in Latvia in order to add our experience to the published data.

CASE REPORT

12 y.o. female teenager admitted to the Department of Neurosurgery, Riga Children's Clinical University Hospital, complaining of recurrent headache during the past 8 months. Nausea and recurrent vomiting occurred in past month before admission. Patient showed no neurological focal signs when admitted. After the following MRI assessment the tumour of the 4th ventricle with secondary contrast enhancement was discovered (Fig.1). For the further surgery girl was transferred to the Department of Neurosurgery, Riga Eastern Hospital, Clinic Gailezers. The operation- subtotal tumour removal was successfully managed. Post-operative period showed no complications, operative wound healed per primam. MRI control assessment followed (Fig.2). Patient was discharged from the hospital without neurological focal signs. Surgical biopsy was morphologically and immunochemically investigated. Diagnosis – Rosette-forming glioneuronal tumour of the 4th ventricle confirmed.

DISCUSSION

Rosette-forming glioneuronal tumour of the 4th ventricle (RGNT) has been initially described in scientific literature in 1995 as cerebellar form of dysembryoplastic neuroepithelial tumour (4), but as a nosological entity was characterized by Komori et al (5) in 2002 after study of 11 cases. Till the present time number of cases of morphologically and immunohistochemically confirmed RGNT of the 4th ventricle is only a little more than 20 (6,7). This relatively small amount of cases can be added by our case, which fully fits with all characteristics of reported classic RGNT.

Analyzing the clinical status of our patient in comparison with reports published we found common complaints of headaches, nausea, vomitus (2,3), followed by visual disturbances, vertigo, ataxia. In our case only complaints of headache, nausea and recurrent vomitus were found out. Based on the experience reported to date, RGNT is more common in young female and teenage females (1), which fits with our case. Common localization of RGNT – the 4th ventricle- was also identified in our case (Fig. 1,2).

After the morphological analysis of our case classic structure of RGNT was found: Tumour formed by neurocytic (Fig. 3) and astroglial (Fig. 4) components (1,6). Neuronal structure formed by typical neurocytic Homer Wright-like rosettes (Fig. 5) and perivascular pseudorosettes (Fig.6).

Immunohistochemical study of RGNT in published reports (1,3,6) as well as in our case showed the immunoreactivity of neurocytic rosettes for synaptophysin (SYN) in granular fashion (Fig. 8) and reactivity of glial components for glial fibrillary acidic protein (GFAP) (Fig. 7).

In the series of Komori et al (5), tumoral MIB-1 indices ranged from 0,35%-3,07% (mean, 1,58%). In our case proliferative activity of 2% was found (Fig. 9).

In our case grade 1 of tumour anaplasia can be formulated, which in combination with almost total resection (Fig. 2) and low index of proliferation has favorable prognosis and long recurrence-free period.

Conflict of interest: None

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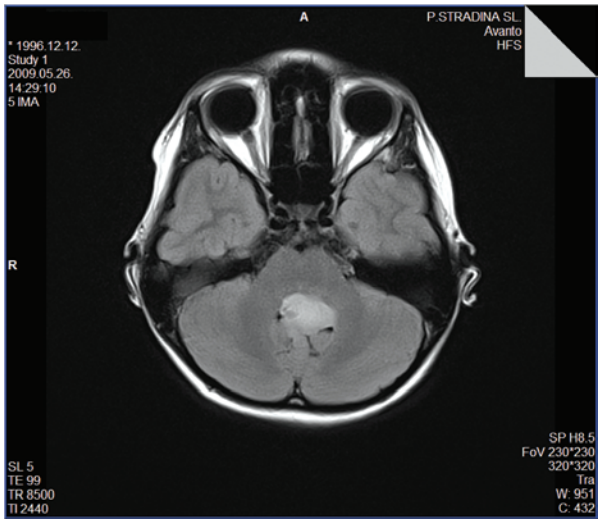

Fig. 1. MRI scan before the operation. Tumour of the 4th ventricle. Note the contrast enhancement of tumour

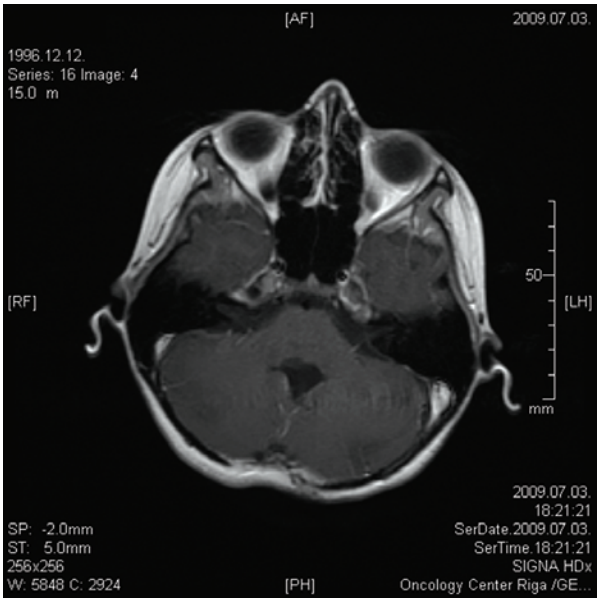

Fig. 2. Post-operative control MRI scan. Status after subtotal resection. Minimal residual tumour tissue without mass effect and disturbance to CSF circulation

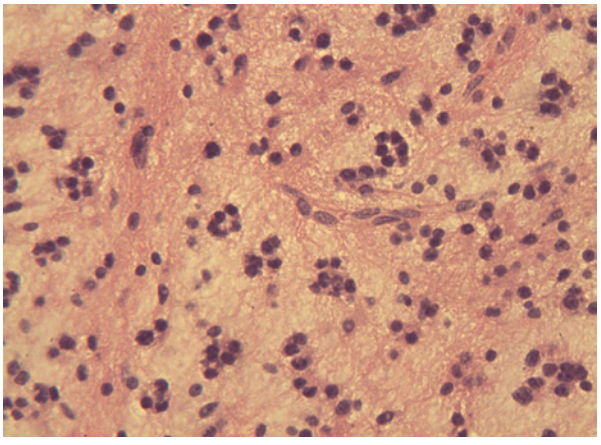

Fig 3. Neurocytic component of RGNT (Obj. 20x, H-E)

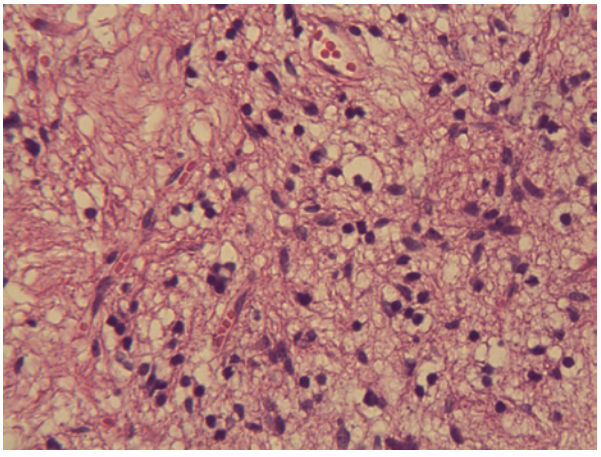

Fig. 4. Astrocytic component of RGNT (Obj. 40x, H-E)

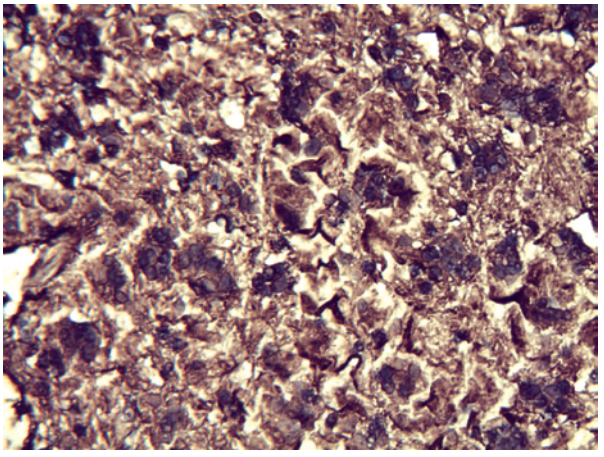

Fig. 7. Immunophenotypic features of RGNT. Glial components of RGNT (Obj. 40x, GFAP)

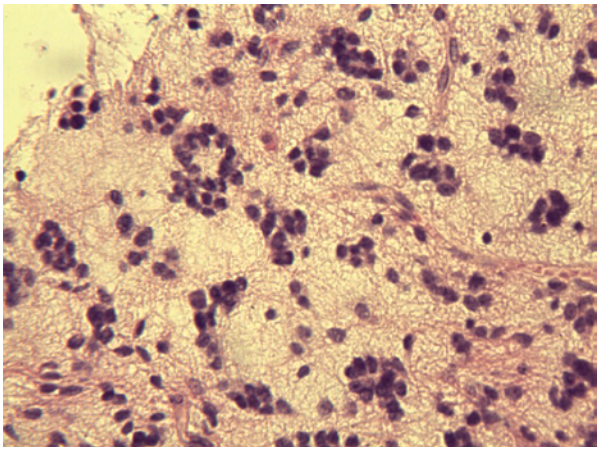

Fig. 5. Histological features of RGNT. True Homer Wright-like rosettes (Obj. 40x, H-E)

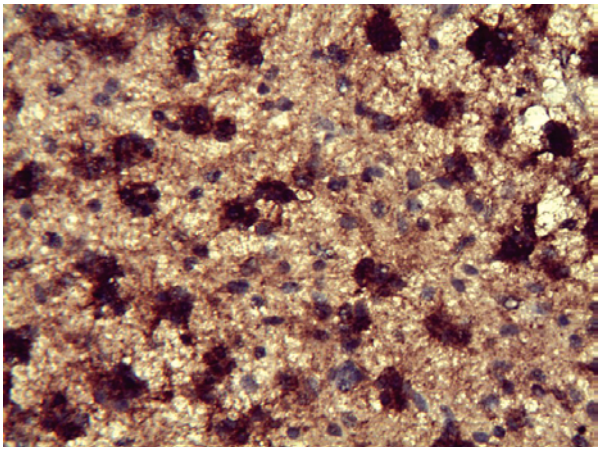

Fig. 8. Immunophenotypic features of RGNT. Rosettes manifest granular synaptophysin Imunnoreactivity (Obk.40x, SYN)

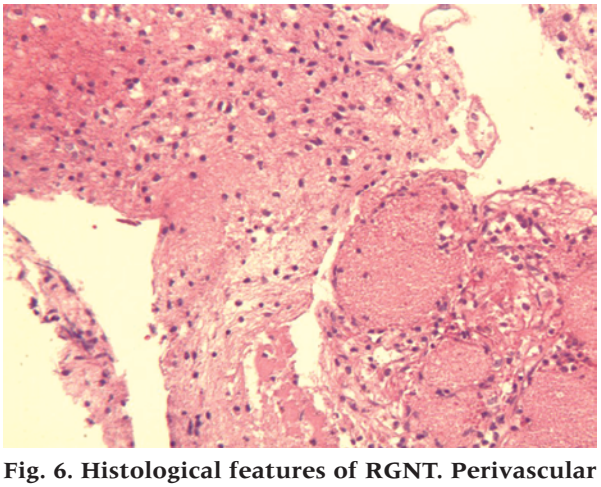

Fig. 6. Histological features of RGNT. Perivascular pseudorosettes (Obj. 40x, H-E)

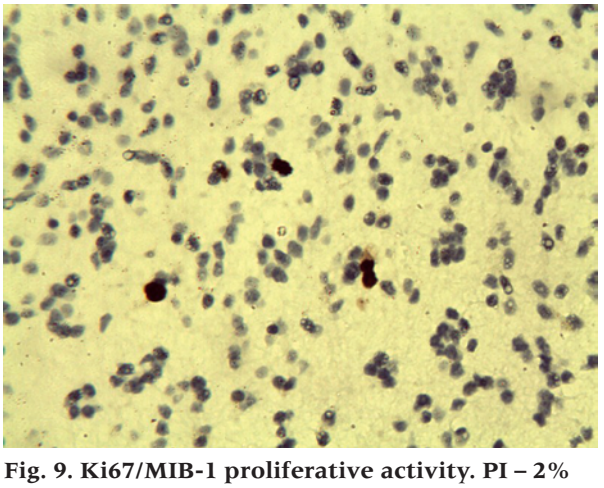

Fig. 9. Ki67/MIB-1 proliferative activity. PI – 2%

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CASE REPORT

Congenital Long QT Syndrome in an Infant

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Summary
Long QT syndrome (LQTS) is a disorder of myocardial repolarization characterized by prolonged QT interval on ECG with prevalence close to 1/3000–1/5000. LQTS is characterized by the occurrence of syncopal episodes due to torsades de pointes ventricular tachycardia (VT) and by a high risk for sudden cardiac death among untreated patients (1, 2, 3). In 12% of patients with LQTS, sudden death is the first manifestation of the disease and only in 4% this happens in the first year of life (2). There is consensus that all symptomatic children with LQTS should be treated with β-blockers which are effective in preventing cardiac events and reducing mortality in 70%, but do not protect patients from sudden death completely (1,2,3,4). The prognosis is poor in untreated patients with annual mortality 20% and 10 year mortality up to 50% (1, 2). Here we present a case of relatively rare congenital heart rhythm disorders in an infant which required immediate treatment.
Key words: long QT syndrome; congenital arrhythmia.

AIM OF THE DEMONSTRATION

We present a case of congenital heart rhythm disorders in an infant which carry serious risk of sudden death if left untreated.

CASE REPORT

The child was sent to the hospital at the age of 21 days by the general practitioner due to bradycardia detected within a routine visit. The girl was born from the first pregnancy in urgent cesarean section due to fetal distress (umbilical cord wrapped around the neck) at 40 weeks of gestation, birth weight 3090g, height 54cm, Apgar score 7/9 and was discharged from the maternity hospital at the age of 4 days. The child received breastfeeding and parents did not have any complains about the newborn. The mother of the child is 21 years old and the father 23 years old, both completely healthy. The course of the pregnancy was uneventful. There were no cases of heart rhythm disorders or sudden death in the family history, no chronic illnesses in the family history. At the time of hospitalization (at the age of 21 days) the weight of the child was 3265g, bradycardia-heart rate 72 times per minute was detected, no heart murmurs, no hepatomegaly. Repeated electrocardiograms (ECG) showed first to second degree second type (Mobiz II) atrio-ventricular block and prolonged QT interval 0,52, cQT 0,54 as well as complete right and left anterior bundle branch block. Echocardiography was completely normal with LVDd 22mm, fractional shortening 37% and ejection fraction 61%. 24 hour ECG monitoring reveled first to second degree second type (Mobiz II) atrio-ventricular block and prolonged QT interval and repeated paroxysms of ventricular tachycardia with the ventricular rate up to 251 beats per minute (the longest one 49 seconds). No changes were found in thoracic x-ray, neurosonography and abdominal ultrasound. There were neither signs of acute infections nor TORCH infections present. There were no positive auto antibodies detected. At the age of 25 days epicardial pacemaker (DDD regimen) was implanted in the abdominal front wall and therapy with β-adrenoblocker Propranolol started. The postoperative period was uneventful and the girl was discharged at the seventh day after the operation. No prolongation of cQT was observed in family members of the girl. The hearing was detected to be normal. The analyses to detect the genetic background showed mutation characteristic for LQTS type 2 (mutation in gene HERG on chromosome7). The age of the girl is seven months now, she is followed up by pediatric cardiologist, receives Propranolol, no serious hearth rhythm disorders observed. The pacemaker regimen is DDD (lowest rate 90 beats per minute, upper track 200, AS-VS 69%, AS-VP 29%, AP-VS 1,5%, AP-VP 0,2%, threshold impedance A 0,375V, V 1,375V, impedance A 550Ω, V 439Ω). During the last check up the pacemaker diagnostic data and 24 hour ECG monitoring showed no runs of tachycardia.

DISCUSSION

QT prolongation may be congenital or acquired. Long QT syndrome is a genetic disease due to mutations of several genes all encoding ionic (potassium or sodium) channels involved in the control of ventricular repolarization. The characteristic ECG shows a prolonged QT interval (usually cQT greater than 0,46 seconds), abnormal T – wave morphology (bifid, biphasic or notched), 20% bradicardia due to second degree AV block, monomorphic or polymorphic ventricular tachycardia(present in 10-20%) (1,2,3). Based on genetic background, six types of Romano-Ward syndrome (LQTS 1-6, autosomal dominant, with normal hearing) and two types of Jervell and Lange-Nielsen syndrome (JLN 1-2, autosomal recessive, with deafness) are identified. Two additional syndromes (Andersen-Tawil and Timothy syndrome are

considered different subgroups (1,3). In our case there is evidence of characteristic genetic changes of LQTS 2. The LQT2 type is the second most common gene location that is affected in long QT syndrome, making up about 35 to 45 percent of all cases. This form of long QT syndrome most likely involves mutations of the *human ether-a-go-go related gene* (HERG) on chromosome 7. The HERG gene (also known as KCNH2) is part of the rapid component of the potassium rectifying current (I_{Kr}). (The I_{Kr} current is mainly responsible for the termination of the cardiac action potential, and therefore the length of the QT interval.) The normally functioning HERG gene allows protection against early after depolarizations (1, 3, 4). In most cases, several members of the same family are gene-carriers (family history positive in about 60% of cases). Low penetrance exists in LQTS, which means that gene-carriers may not show the clinical phenotype and may have a normal QT interval (2). Therefore a normal QT in the parents does not rule out familial LQTS. In addition, approximately 30% of cases are due to 'de novo' mutations which imply unaffected parents and no family history. 'De novo' LQTS mutations have been demonstrated in infant victims of cardiac arrest and sudden death diagnosed as Sudden Infant Death Syndrome (1,2). However, about 40% of the families with LQTS have not yet been linked to any of the known genes. Our patient showed serious hearth rhythm disorders already during the neonatal period. Relatively few LQTS patients have cardiac events during the first year of life, the vast majority become symptomatic later on, either during childhood or adolescence according to genetic subgroups (1,2,3). The acquired causes of LQT (including drugs, electrolyte imbalance, marked bradycardia, cocaine, organophosphorus compounds, subarachnoid hemorrhage, myocardial ischemia, protein sparing fasting, autonomic neuropathy, and human immunodeficiency virus disease) were excluded. Beta-blockers are the first choice therapy in LQTS and are effective in preventing recurrences in 80% of already symptomatic patients; different degrees of protection exist according to genetic subgroups. If beta-blockers are unable to prevent new cardiac events, additional drug therapy, left cardiac sympathetic denervation, pacemakers or the implantable cardioverter defibrillator should be considered based on evidence, with consideration for body size (1,2,3,4). In our case there were indications for both implantable pacemaker and the therapy with beta-blockers. Despite this combined therapy there is still risk for serious heart rhythm disorders but the risk for sudden death would be much higher if left untreated. With a prevalence 1/3000-1/5000 and a manifestation within the first month of life this case is a rarity in our small population but in the case of pathology with potentially life threatening consequences it is very important to diagnose it as early as possible.

Conflict of interest: None

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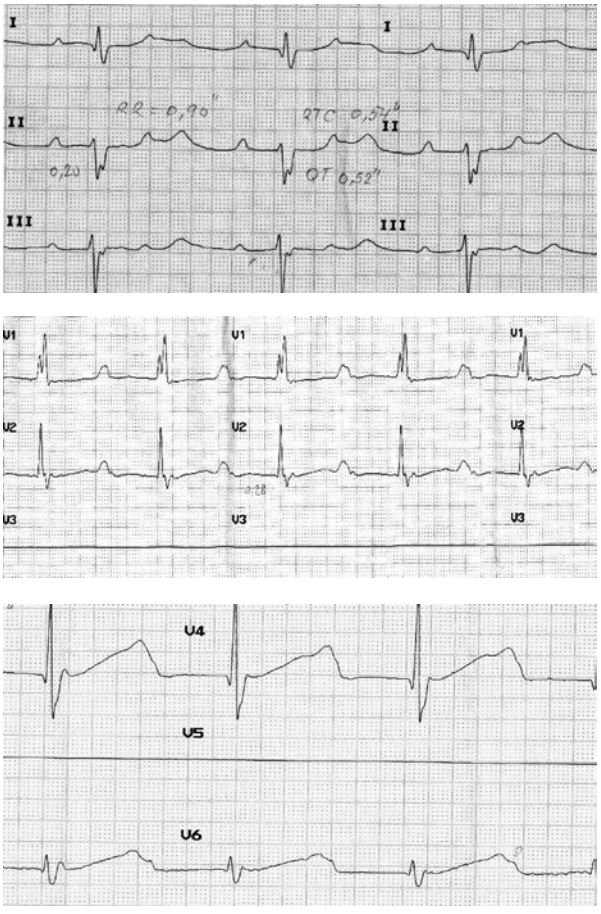

The ECG of the 24 days old infant shows prolongation of cQT>500ms and atrio-ventricular block first to second degree Mobitz II