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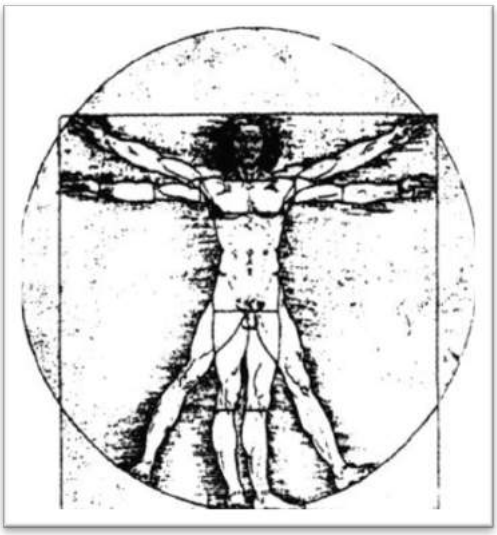

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ORIGINAL ARTICLE

LYSOENZIMURIA AS AN INDICATOR FOR LYSOSOMAL DYSFUNCTION OF THE PROXIMAL RENAL TUBULES IN SYSTEMIC LUPUS ERYTHEMATOSUS TREATED WITH CHLOROQUINE PHOSPHATE

Dejan Spasovski[1], Sandevska Emilija[1], Pivkova-Veljanovska Aleksandra[2], Subevska-Stratrova Slavica[3], Brezovska-Kavrakova Julijana[4]
[1]University Clinic for Rheumatology, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2]University Clinic for Hematology, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[3]University Clinic for Endocrinology, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[4]Institute of Medical and Experimental Biochemistry, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: The approach for estimation of drug nephrotoxicity is possible only with drugs that have dominant proximal tubular excretion, such as methotrexate, chloroquine phosphate, diclophenum, acetaminophen. Very often, therapeutic drugs can have some nephrotoxic effect. This can be seen in a long-term SLE therapy.

Aim: To estimate the effect of initial therapy with chloroquine phosphate and some most used nonsteroidal anti-inflammatory drugs (diclophenum) on tubular and glomerular function in patients with systemic lupus erythematosus (SLE), to determinate toxicity of these medicals through measurements of the urine enzyme excretion that correlates with the damage degree on the tubular epithelium. Microalbuminuria is used as a marker for glomerular damage, and the urinary excretion of N-Acetyl-β-D-glucosaminidase (NAG) as an indicator of proximal tubular damage.

Material and methods: Using the colorimetric method for determination of NAG, as well as immunoturbidimetric assay for detection of microalbuminuria, we examined samples of 70 patients (35 SLE pts treated only with chloroquine phosphate, 35 SLE pts treated with diclophenum), followed up at five-time intervals within the course of 12 weeks.

Results: There was a weak correlation between NAG and microalbuminuria (r=0.16) in the group of patients treated with chloroquine phosphate, while in the group treated with diclophenum, there was a moderate correlation (r=0.28). NAG enzymuria appeared earlier in the group treated with Diclophenum compared to the group treated with chloroquine phosphate.

Conclusions: Diclophenum triggers greater tubular enzymuria than chloroquine phosphate.

Keywords: N-acetyl-β-D-glucosaminidase, microalbumin, systemic lupus erythematosus, chloroquine phosphate, diclophenum

INTRODUCTION

The approach for estimation of drug nephrotoxicity is possible only with drugs that have dominant proximal tubular excretion, such as methotrexate, chloroquine phosphate, diclophenum, paracetamol.

Such approach for estimation of drug nephrotoxicity is not applicable for other drugs from the baseline used in the treatment of SLE, such as sulfasalazine, due to the predominant hepatobiliary secretion. There are no literature data about the toxic effect of these drugs on proximal tubular dysfunction.

Traditional treatment of SLE includes non-steroid anti-inflammatory drugs (NSAIDs), drugs that modify the disease (DMARDs), chloroquine phosphate, steroids, immunosuppressive and cytotoxic drugs. Methotrexate in a low dose regimen is the most frequent drug from the group of DMARDs**,** while from the group of NSADs the most used drugs are diclophenum (Diklofenak^{R}) and Paracetamol^{R} (Acetaminophenum).

Very often, therapeutic drugs (NSAIDs, drugs that modify disease activity - DMARDs, cytotoxic and immunosuppressive drugs, Acetaminophenum, chloroquine phosphate, can have some nephrotoxic effect. This can be seen in a long-term SLE therapy [1-8].

Such high amount of enzymes in the urine shows the dominant role of the kidneys in their excretion [9-16]. The examination of the cell membranes of the brush epithelium of the proximal tubules has proven the localisation of the alanine amnopeptidase (AAP) in 90%, alkaline phosphatase (AP) in 70% and γ-glutamyl transpeptidase (γ-GT) in 50% of the whole enzyme activity in the kidney.

OBJECTIVES

To estimate the effect of the initial therapy with Resochin (chloroquine phosphate) and diclophenum on tubular and glomerular function in patients with systemic lupus erythematosus (SLE), to determine toxicity of these medicals through measurements of the urine enzyme excretion that correlates with the damage degree on the tubular epithelium. Microalbuminuria was used as a marker of glomerular damage, and the urinary excretion of N-Acetyl-β-D-glucozaminidase (NAG) as an indicator of proximal tubular damage.

MATERIAL AND METHODS

The diagnosis of patients included in this study was based on the revised diagnostic criteria for classification of systemic lupus erythematosus proposed in 1987 by the American Association for Rheumatism (ARA) [17].

The study comprised 35 patients with SLE (25 women, 10 men), treated with chloroquine phosphate, and 35 patients with SLE (23 women, 12 men) treated with diclophenum. Their average age was 25.53 years (±8.42) range (40-55 years) in the group treated with chloroquine phosphate, while 23.24 years (±10,36) range (29-55 years) in the group treated with diclophenum. Mean disease duration from the beginning was 10.11 months (±5.32 months), range (1-6 months). None of the patients had previous or current history of renal disease. None of the patients had previously used NSAIDs. Specimens were collected in the period of 2 years.

Inclusion criteria:

The study included patients with SLE, aged 18-65 years, not previously treated with NSAIDs or DMARDs.

Exclusion criteria:

1. Patients younger than 18 years.
2. Patients with history of blood transfusion and patients with body overweight.

3. Patients with history of using drugs from the baseline.

4. Patients that at 0 point had an increased level of glucose, serum and urine urea and creatinine, blood hypertension, smokers and blood and history of some enzyme disorders

5. Patients previously treated with salicylates, antibiotics or diuretics, conditions like lupus nephritis

6. Patients with history of hypersensitivity (allergy) to chloroquine phosphate or to one of the other constituents of the drug.

7. Diseases of the retina and/or limitations to the eye field diagnosed, retinopathy (retina damage)

8. Patients with history of disorders related to formation of blood cells, history of deficiency of glucose-6-phosphate-dehydrogenase

9. Patients with myasthenia gravis.

All patients took part in this study voluntarily, so the ethics criteria for this study were fulfilled.

Clinical estimation of disease activity

Clinical estimation was made by a subspecialist in rheumatology. Disease activity was estimated using systemic lupus erythematosus disease activity index, SLEDAI score [17]. The SLEDAI was originally developed to estimate disease activity in lupus patients.

Laboratory estimation

For clinical estimation of the disease, it was necessary to examine the following laboratory variables: complete blood, reactants of the acute phase, antinuclearantibodies-ANA, Anti ds-DNA, Anti DNP, anti-histone antibodies, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), creatin kinase (CK), serum urea and creatinine. Urine samples were taken not only for routine analyses, but also for determination of NAG and microalbuminuria.

Statistical analysis

Statistical package Statistica 7.0 was used for data analysis. The p value <0.05 and <0.1 was considered to be statistically significant. Wilcoxon-matched test for independent species was used to compare mean values of certain numerical parameters between two groups and to test the significance of the differences between two arithmetical means i.e. proportions. Sensitivity and predictivity for positive and negative test of the examined markers was determined with the sensitivity and specificity test.

RESULTS

The analysis of the group of patients treated with chloroquine phosphate regarding the NAG values in the four samples showed that they were registered in 22 patients in the 12^{th} week, when the degree of the mean urine NAG value was the highest (1.32 ± 1.14).

The analysis of the group of patients treated with diclophenum regarding the NAG values in the four samples showed that they were registered in 25 patients in the 12^{th} week, when the degree of the mean urine NAG value was the highest (1.78 ± 0.32).

Testing the significance of the differences in both groups in the 0 (zero) sample in the group of patients treated with chloroquine phosphate, the mean value of the NAG enzymuria was in the range 0.92 ± 0.38, while in the group of patients treated with diclophenum 1.09 ± 0.47. This has confirmed that diclophenum is a more potent NAG indicator in comparison to chloroquine phosphate, both in range and in time of appearance.

In the group of patients treated with chloroquine phosphate, the values of microalbuminuria in the four groups were increased in 8 patients in the 4th week, when the degree of microalbuminuria was the highest (15.1±1.05).

The analysis of patients regarding the values of microalbuminuria in the group of patients treated with diclophenum showed that in they were registered 9 patients in the 4th week, when the degree of microalbuminuria was the highest (17.78±0.23) (Fig. 1).

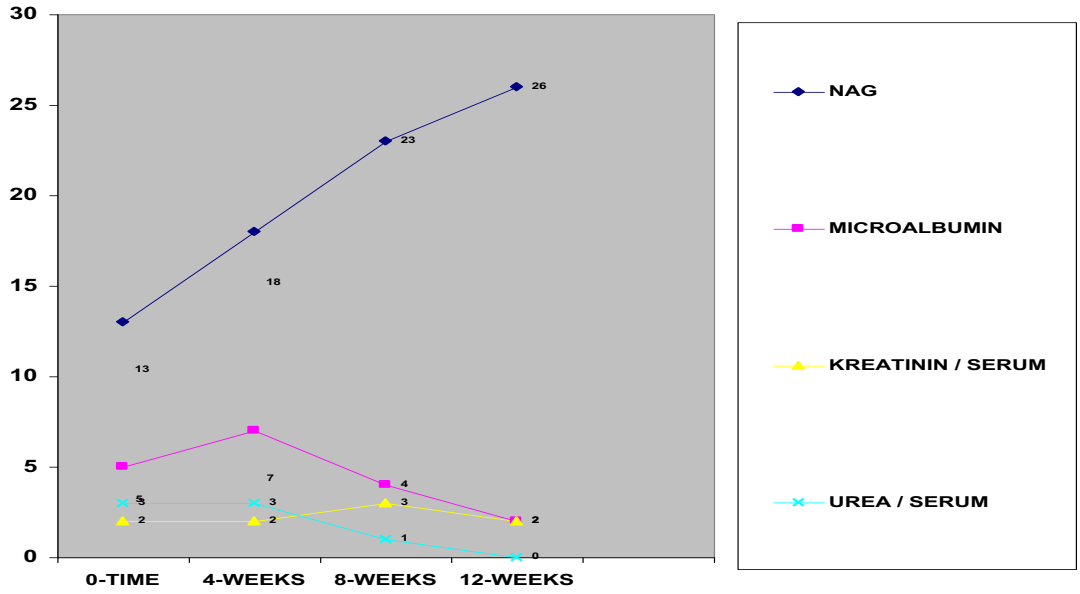

Fig. 1. Distribution of patients in the group with diclophenum according to initial values of NAG, microalbuminuria and other laboratory variables in the four samples.

Testing the significance of the differences in both examined groups in the zero sample, in the group of patients treated with chloroquine phosphate, the mean value of microalbuminuria was in the range 0.53±0.48, while in the group of patients treated with diclophenum 0.67±0.57. Chloroquine phosphate had identical values of microalbuminuria in comparison to diclophenum.

Analysis with the Pearsons $\chi 2$ test showed a moderate correlation between NAG and microalbuminuria ($r=0.28$) between the increase of the values of NAG and microalbuminuria in the follow-up period of 12 weeks in the group of patients treated only with chloroquine phosphate.

Analysis with the Pearsons $\chi 2$ test showed a statistically significant correlation ($r=0.16$) between the increase of the values of NAG and microalbuminuria in the follow-up period of 12 weeks in the group of patients treated only with diclophenum (Fig. 2).

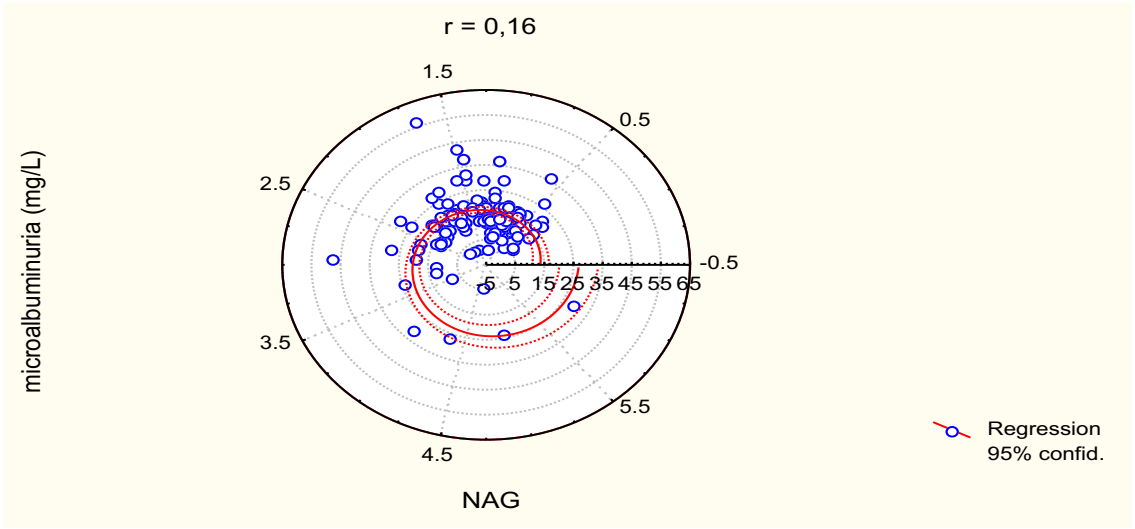

Fig. 2. Pearson's coeficient of correlation (r) between the values of NAG and microalbuminuria in the group treated with dicophenium. There is weak correlation between NAG and microalbuminuria (r=0,16).

DISCUSSION

Chloroquine phosphate tablets are used for treating chronic polyarthritis (rheumatoid arthritis) including juvenile chronic arthritis, systemic lupus erythematosus. Systemic lupus erythematosus with rash as butterfly is an inflammatory connective tissue disease, which affects internal organs and skin.

Chloroquine phosphate is primarily eliminated via the kidneys, with proximal tubular excretion. Taking chloroquine phosphate tablets at the same time with the following medicines can influence the effect of chloroquine phosphate: corticosteroid derivatives (active substances that have an anti-inflammatory effect), can enhance myopathies (muscle diseases) and cardiomyopathies (heart muscle diseases), can enhance the effect of folic acid antagonists (methotrexate, the most used drugs in inflammatory rheumatic disease).

This study investigated the potency of low molecular weight protein and enzymes which were located in the epithelium of the proximal tubules for detecting early renal damage in patients with untreated systemic lupus erythematosus (SLE). It has been proved that NAG can work as a more sensitive marker than microalbuminuria in asymptomatic renal damage in SLE. The study is an interesting and pioneering research in SLE associated renal disease because there are only a small number of studies that have put their focus on tubular dysfunction.

Tubular dysfunction will occur earlier in the process of renal damage in SLE, like lupus nephritis [17-20].

In untreated SLE both tubular and glomerular functions are primarily affected. Glomerular integrity is basically intact in the examined groups of SLE patients with the use of chloroquine phosphate, diclophenum. The initial increase in the activity is the result of the changes in cell synthesis and not always enzymuria could be the result of the lytic or necrotic processes.

Chloroquine phosphate usually does not trigger a significant damage of the renal proximal tubules in most of the examined patients. Nephrotoxicity of diclophenum is higher in comparison with chloroquine phosphate.

Diclophenum is more potent NAG inductor in comparison to chloroquine phosphate. NAG induction is higher and appears earlier in the use of diclophenum compared to chloroquine phosphate

CONCLUSION

The results obtained in our study proved the safety of chloroquine phosphate and diclophenum in the treatment of SLE patients.

Measures taken for prevention of nephrotoxicity are the following: monitoring the renal function by regular check-up of the enzyme activity in the urine. As complementary diagnostic tools determination of the urine NAG together with urinary creatinine excretion could be more sensitive tests for renal damage in patients with SLE.

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ORIGINAL ARTICLE

EXPRESSION OF MAJOR VAULT PROTEIN IN OVARIAN CARCINOMA

Tamara Angelovska[1], Jovanovic Rubens[2], Kostadinova – Kunovska Slavica[2], Zdravkovski Panche[2], Klisarovska Violeta[3], Dimitrov Goran[4], Aluloski Igor[4], Petrushevska Gordana[2]

[1]Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

[2]Institute of Pathology, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

[3]University Clinic for Radiotherapy and Oncology, Skopje, Republic of North Macedonia

[4]University Clinic for Gynecology and Obstetrics, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: The major vault protein (MVP) is a component of large ribonucleoprotein complexes called Vaults. Vaults are organelles in eukaryotic cells, three times bigger than the ribosomes, associated with the nuclear pores. Their function has not been fully elucidated. Vaults are probably involved in RNA trafficking and cell signaling by regulating the selective permeability of the nuclear pores. Since Scheffer et al. showed that MVP is identical to the LRP (Lung Resistance Protein), a new role for the vaults emerged - a multi-drug resistance in malignant tumors.

Objective: To evaluate the expression of MVP in advanced high-grade ovarian carcinoma and analyze possible correlations to the disease course and chemotherapy response.

Material and methods: We tested the expression of MVP in 31 cases with ovarian cancer treated with the same postoperative chemotherapeutic protocol, 17 of which had a satisfactory response to the therapy (group I) and 14 had a poor response (group II). MVP expression was tested with immunostaining using the LRP-56 primary antibody and En-Vision Flex (DAKO) visualization kit and in two of the positive samples, electron microscopy was performed to confirm the localization of vaults at the nuclear pores and correlate the immunostaining pattern.

Results: We found MVP over-expression in 50-100% of the tumor cell population in the second group, compared to cases with negative staining and cases with MVP staining in less than 30% of tumor cells in samples from the first group ($p<0,01$). Anti-MVP staining prior to chemotherapy could be a beneficial prognostic marker in cases with ovarian carcinoma, especially having in mind that its visualization (detection) exploits routine methodology.

Conclusion: We found MVP expression in more than 50% of tumor cells in advanced high-grade ovarian carcinoma in 38.7% of cases. This overexpression is correlated to worse chemotherapy (platinum-based and taxanes) response and shorter event-free time.

Keywords: major vault protein, lung resistance protein, ovarian carcinoma, vaults

INTRODUCTION

Large, oval-shaped ribonucleoprotein particles, were discovered in 1986 in rat liver tissues, and when purified, revealed symmetric, hollow, barrel-shaped structures, with 39-fold dihedral symmetry, resembling the ceilings of gothic cathedrals, thus being named vaults [1].Vault particles are organelles in eukaryotic cells which are three times larger than the ribosomes and are often associated with nuclear pores. A vault particle, when intact has a 2 fold symmetry, each half, opening like a flower resembling structure, with eight petals around a central ring. Using SDS PAGE it has been discovered that they are comprised of major vault protein (MVP),

telomerase-associated protein 1 (TEP1), vault poly-(ADP–ribose)polymerase(VPARP), and three non-coding vRNAs (hvg-1, hvg-2, and hvg-3) [1-3].They have been discovered in multiple species such as mammals, birds, fish, slime molds, and echinoids, yet although their exact functions have not been fully explained, it is suggested that they have multiple important biological roles [3, 4].Vaults are probably involved in RNA trafficking, cell signaling by regulating the selective permeability of the nuclear pores, and the defense of eucaryotic cells against xenobiotics. The vault particle mass and symmetry are very similar to the proposed mass of the putative central plug as a part of the nuclear pore complexes, suggesting that vault particles participate in the nucleocytoplasmic transport[5].The major vault protein is present in multiple normal tissues such as the bronchi, the digestive tract, macrophages, as well as in malignant cells, including different cell lines, lung, ovarian, colon carcinoma, acute myeloid leukemia, etc. [6-11]. This proposition further supports the suggested role of the vault particles in multi-drug resistance. Scheffer et al. showed that MVP is identical to the LRP (Lung Resistance-related Protein), proposing a new role for the vaults - a multi-drug resistance (MDR) in malignant tumors [7]**.** Kickhofer et al. isolated the genes encoding the human vault RNAs and measured the expression of MVP in different tumor cell lines, such as non-small cell lung cancer, small cell lung cancer, breast cancer, and myeloma, and found an overexpressed MVP in all of them [8]. A chemo-resistant phenotype correlated with MVP/LRP expression in other studies on various cancer cell lines and primary tumor samples [10-12]. This suggests a crucial importance of the involvement of vault particles in the multi-drug resistance, yet the mechanism is not clear. Izquierdo et al. in 1995 proposed that the prediction of response to chemotherapy and prognoses in advanced ovarian carcinoma could be evaluated using LRP as a drug resistance-associated marker [13]. It is important to note that the patients included in the study were not treated with preoperative chemotherapy or radiotherapy. Although the role of MVP in MDR has been already established in multiple studies, the latter study suggests a complex regulation of MVP and vaults in tumor cells. This poses an important question about the significance of MVP expression and its role in multi-drug resistance, as well as its value as a predictor of the conventional chemotherapy outcome in different malignant tumors.

OBJECTIVE

To evaluate the expression of MVP in advanced high-grade ovarian carcinoma and analyze possible correlations to the disease course and chemotherapy response.

MATERIAL AND METHODS

Material

We retrospectively tested the expression of MVP in 31 cases of advanced ovarian cancer (Stage 3) treated with the same postoperative chemotherapeutic protocol (taxane and platinum agents).The mean age of the patients was 41.6 years (min. 29; max. 56; SD 6.8), with a normal distribution (Figure 1).

According to the histologic type, there were 28 high-grade serous carcinomas, 2 high-grade serous carcinomas with clear cell components, and 1 high-grade clear cell carcinoma. Cases were divided into two groups by the oncologists according to the chemotherapy response. Therapy response was evaluated by the oncologists based upon the appearance of recurrent tumor and time to recurrence (less than 6 months for poor response) and the serum level of CA125 (no decrease or increase during therapy in the second group).

The first group (n=17) had a satisfactory response to the therapy and the second group (n=14) had a poor response. In the first group, there were 15 high-grade serous carcinomas, 2 high-grade serous carcinomas with minor clear cell components, while the second group consisted of 13 high-grade serous carcinomas and one clear cell carcinoma.

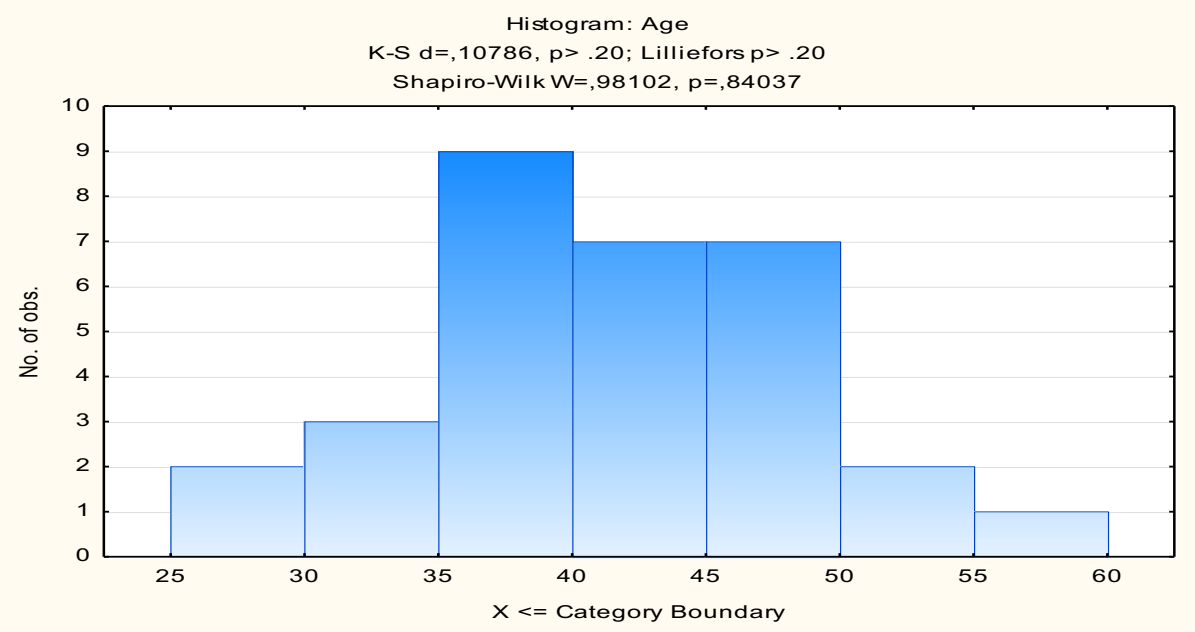
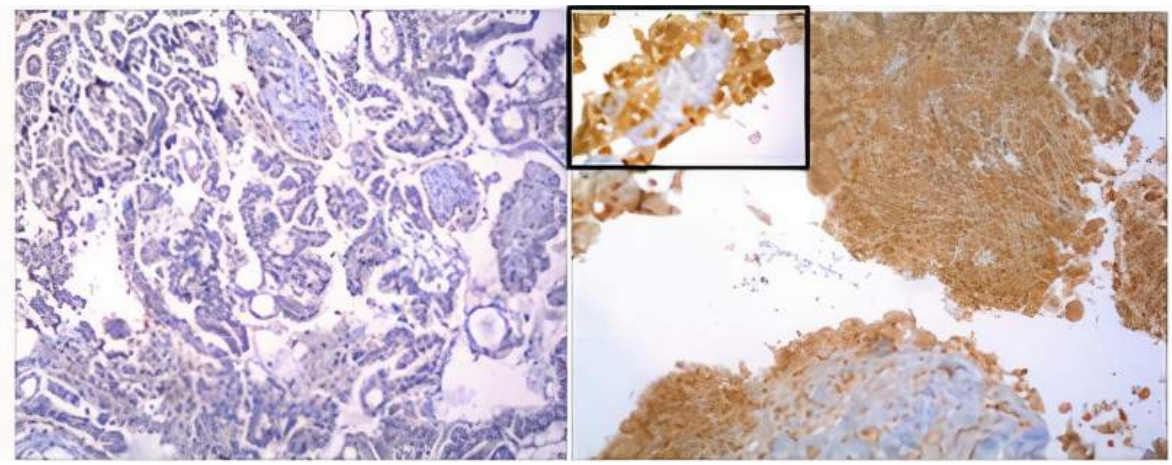


Fig. 1. Age distribution of the patients - Shapiro-Wilk's W test p>0,05

Methods

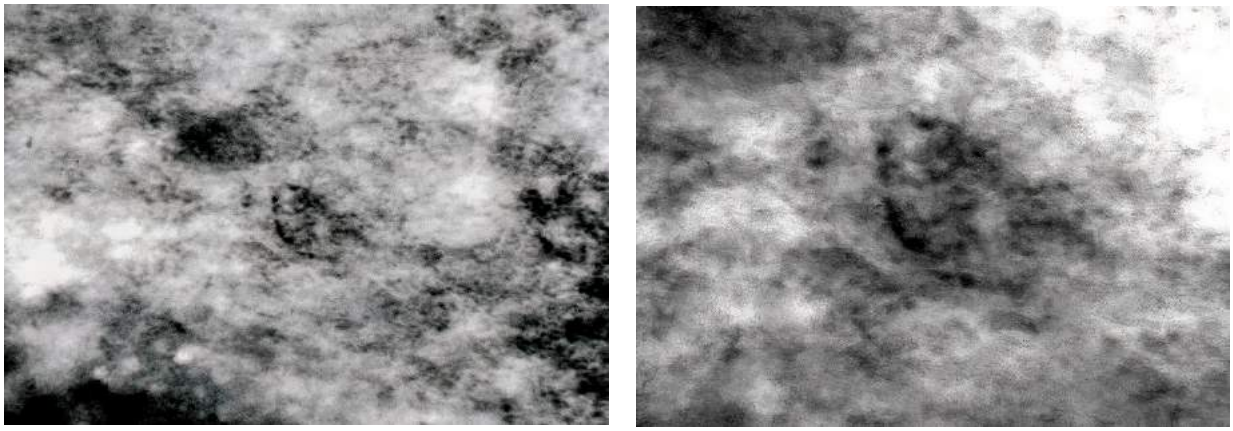
Tumor samples for light microscopy analyses were fixed in 10% neutral formalin, paraffin-embedded, sectioned and stained with HeEo. MVP expression was tested by immunohistochemical staining (IHC) using an anti-MVP antibody(clone LRP-56,dilution 1:50, Invitrogen)and En-Vision Flex (DAKO) visualization kit. IHC slides were analyzed for the percentage of tumor cells expressing MVP. Results were expressed as low MVP expression (less than 50% positive tumor cells) and high MVP expression (more than 50% positive tumor cells) (Figure 2).



A. MVP negative. 100x magnification. B. MVP positive. 100x magnification (inset 400x)

Fig. 2. Immunohistochemical staining for MVP

On one of the positive samples, transmission electron microscopy (TEM) was performed to confirm the presence of the vault particles and their localization (Figure 3).



A. TEM - 100.000 magnification B. TEM -250.000 magnification

Fig. 3. Transmission electron microscopy - Visualization of vault particles in high-grade serous ovarian carcinoma at ascending magnifications. A – Visualization of vault particles at lower magnification; B – higher magnification.

TEM sample preparation started from already paraffin-embedded tissues, which were firstly deparaffinized with xylol, fixation with glutaraldehyde followed. Osmium tetroxide was used as a second fixative (post-fixation). The dehydration step was done by adding acetone. Then, an ultra microtome was used for making semi-thin sections of the resin block with the embedded tissue, dyed with toluidine blue and ultrathin sections contrasted with uranyl acetate and lead citrate. For visualization, we used Transmission Electron Microscope JEM-1400, JEOL Ltd. Tokyo, Japan.

RESULTS

The IHC analysis showed low MVP staining in 19 of the cases, while 12 cases expressed high MVP staining (Figure 4).

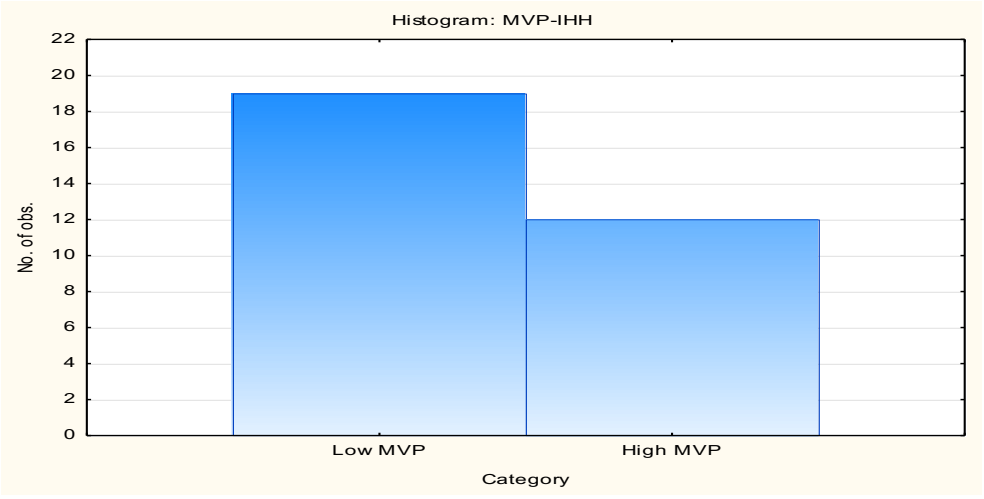


Fig. 4. Frequency of low- and high immunohistochemical expression of MVP

The MVP staining in high MVP cases had diffuse cytoplasmic or perinuclear distribution. There was high MVP expression in 11 out of 14 samples from the second group (poor chemotherapy response), compared to only 1 out of 17 from the first group (satisfactory chemotherapy response) (Figure 5).

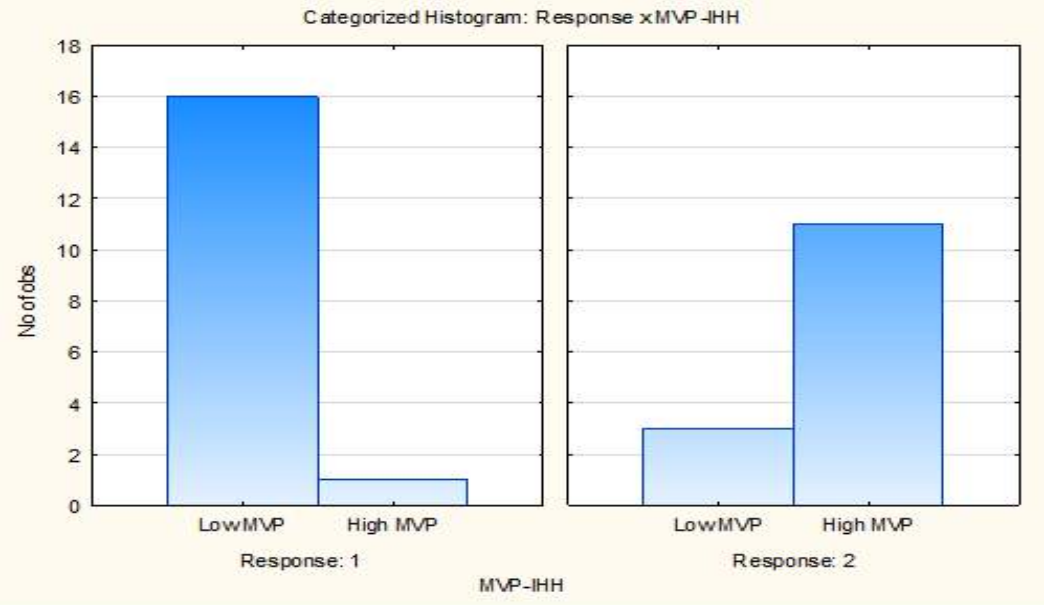


Fig. 5. Chi-Square: p<0,01; Yates corrected chi-square: p<0,0

The difference between the chemotherapy response groups was significant (Chi-Square: p<0,01; Yates corrected chi-square: p<0,01).In the cases from the first group, the MVP staining was positive in ≤30% of cells, dominantly focal with weak to moderate intensity. The IHC staining for MVP was not correlated to the age of the patients (Spearman's R=0,28; p>0,05).

The time to an adverse event (confirmed recurrence, metastasis, increased serum CA125)for both chemotherapy response groups ranged between less than a monthand23 months (mean 10,3; SD 7,25). The difference between the two groups was significant (p<0,01). Patients in the first group (satisfactory response to therapy), had a mean time to event of 16.12 months (min.11, max. 23; SD 3,76), and patients in the second group (poor response to chemotherapy) had a mean time to event of 3.21 months(min. 1; max. 8; SD 2,42) (Figure 6).

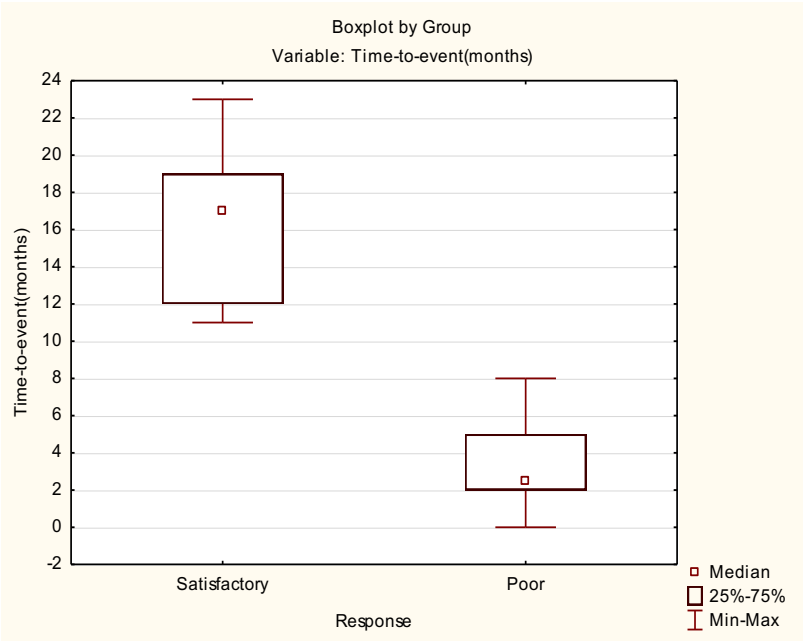


Fig. 6. Time-to-event in Poor vs. Satisfactory chemotherapy response
Mann-Whitney U test - M-W; U= 0,0; Z= -4,704; p<0,01

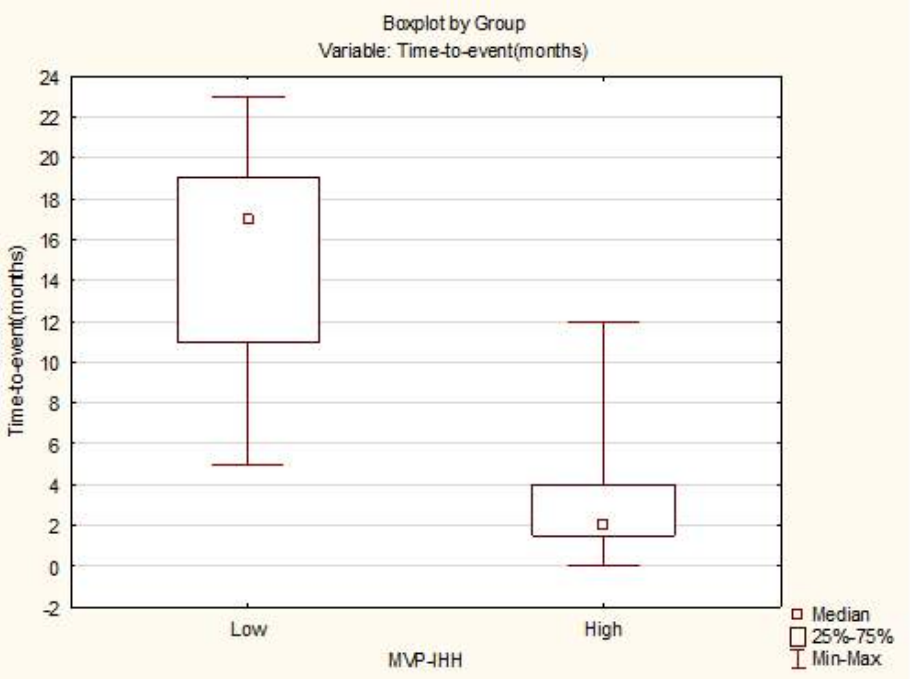


Fig. 7. Time-to-event in Low vs. High MVP expression
Mann-Whitney U test: Z=4,319; p<0,01

The expression of MVP was correlated to the time to an adverse event (Spearmans' R= -0,79; p<0,05), and there was statistically significant intergroup difference between the cases with low vs. high MVP expression regarding the time to event. The mean time to event in cases with low MVP expression was 14,8 months (min. 5; max 23; SD 5), but in the group with high MVP expression, the mean time to event was 3,08 months (min 1; max 12; SD 3,2), (Mann-Whitney U test; p<0,01) (Figure 7).

DISCUSSION

Our results are in concordance with most of the previously published studies. The MVP and vaults in general seem to have a more complex role than initially suggested. The complex interaction with the nucleus and its expression in different cellular settings show that MVP is involved in multiple cell signaling pathways and cell processes[7]. In 1995, Scheffer et al. isolated and sequenced cDNA that encoded the protein associated with MDR, named Lung Resistance-related Protein, currently known as human Major Vault Protein – MVP. In the same year, Izquierdo et al. suggested that LRP could be used as a drug resistance-associated marker for the prediction of the response to chemotherapy and prognosis in advanced ovarian carcinoma[13]. In later years, Kitazono et al. confirmed the connection between MDR and vault in human colon carcinoma cells [14]. However, as Scheffer et al. explain later, overexpression of the MVP is not the only factor influencing the drug-resistant phenotype [9]. This is not unexpected considering that the MVP covers 70% of the vault particle mass. It was suggested that additional components of the particle such as the minor vault proteins and vRNA might be necessary for drug resistance. They also show that vault levels vary greatly between different cell lines, although the exact mechanism of the vault up-regulation is unknown [8, 9]. In 2005, Gopinath et al. analyzed the ability of mitoxantrone and doxorubicin to interact with non-coding RNAs associated with the vault complex [15]. They suggested that human hvg1 and hvg2 vRNA can bind to anticancer drug – mitoxantrone and may play a role in the efflux of toxic compounds, yet doxorubicin did not interact with any of the hvg-RNAs.

These results are in concordance with the study of Mandoky in germ cell testicular tumors [16]**.** Mandoky et al. investigated whether there was any correlation between LRP expression and the clinical outcome and patients' clinical parameters in germ cell testicular neoplasms. Their findings showed that a high level of LRP expression had an adverse effect on the overall survival of the patients except for seminoma cases. However, authors alert of possible bias caused by the small number of seminoma cases in their study (n=15) and early stage of the seminoma cases which were all cured. Furthermore, they found a higher LRP expression in mature, well-differentiated cells of mature teratomas, already known for their intrinsic resistance to chemotherapy. This was in concordance with the study of Izquierdo et al. who found a higher LRP expression in well-differentiated and less chemo-sensitive tumor areas in various tumor types [10]. Mandoky et al. concluded that LRP has a prognostic significance in testicular germ cell tumors and can predict the patients' adverse clinical outcomes. The study of Bai et al. about the MVP expression in non-small cell lung carcinoma versus normal tissues showed that MVP had been up-regulated significantly in tumor tissues in comparison to tumor-adjacent normal tissues [17]. Another contribution to the hypothesis of MVP being associated with chemo-resistance in carcinomas was made by Xiao et al. in a recent study in triple-negative breast cancer cells (TNBCs) [18]. The lack of therapeutic target in patients with TNBCs conjoined with the resistance to chemotherapeutic agents, significantly compromises the treatment in these patients, leading to recurrent or metastatic disease and ultimately death. As mentioned earlier, Kickhoefer et al. in 1998 reported that vault particles are involved in chemotherapy resistance in different tumor types, so with that in mind, Xiao et al. explored the role of Notch 1 and MVP as regulators contributing to chemo-resistance in TNBC cells, suggesting that Notch could transcriptionally promote the expression of MVP, AKT pathway and the process of epithelial to mesenchymal transition (EMT), suggesting a probable strategy in tackling chemo-resistance in TNBC [8]. They found that the expression of Notch1 and MVP were both elevated in cisplatin-resistant breast cancer cells compared to the parental cells in a time-dependent manner with an increasing concentration gradient of doxorubicin. Moreover, they described that silencing Notch1 could down-regulate MVP expression and thus could restore their sensitivity to cisplatin. In summary, this study demonstrated that Notch1 signaling significantly contributed to chemoresistance in TNBCand that MVP predicted poor survival outcome in patients with TNBC, suggesting it could serve as a unique predictive marker for chemotherapy response in those patients.

A recent study by Li et al. explored the role of E2F2 directed activation of non-coding RNAs, specifically nc886, in the regulation of MVP in cervical cancer [19].They examined the potential role of nc886 in chemotherapeutics resistance in cervical cancer tissues obtained from 10 early-stage tumors and 10 advanced-stage tumors versus 10 normal tissues. It has been previously proposed that nc886 has a role as a vault RNA (vtRNA), a part of the complex involved in chemo-resistance [20]**.**Nc886 is also associated with carcinogenesis and progression of human cancers including cervical cancer, esophageal cancer, cholangiocarcinoma, thyroid cancer, and small cell lung cancer. Their study identified nc886 as a novel regulator of cervical cancer chemo-sensitivity as well as its role in the regulation of MVP expression upon treatment with chemotherapeutic agents. They also showed that E2F1 regulates nc886 expression through direct promoter interaction. Regarding the role of MVP in chemo-resistance and patients outcome, in this study, by inhibition of nc886, the expression of MVP had been significantly suppressed.

An uncommon detection of MVP in normal cervical tissues, yet abundant MVP in advanced-stage cervical tumors, supports the idea that MVP modulates nc886 mediated chemotherapy resistance in cervical cancer cells.

Although the number of tested samples in our study is rather low, leaving no space for deeper and advanced statistical analyses, we find these results very indicative and worth further

study on a larger scale. Our findings suggest that over expression of LRP/MVP protein in ovarian cancers is associated with poor chemotherapy response at least when taxane and platinum agents are concerned. It should be considered that in the future vault complexes and their constituents, especially MVP could be a target in cancer treatment. Considering the studies mentioned earlier MVP could be used as a marker of chemotherapeutic resistance as well as a patients' outcome and survival in the forthcoming years. Although there are no clinically available means for decreasing the MVP expression at present, we believe that anti-MVP staining prior to chemotherapy could be a beneficial prognostic marker in cases with ovarian carcinoma, especially having in mind that its detection exploits routine methodology.

CONCLUSION

We found MVP expression in more than 50% of tumor cells in advanced high-grade ovarian carcinoma in 38.7% of cases. This overexpression is correlated to worse chemotherapy (platinum-based and taxane) response and shorter event-free time.

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ORIGINAL ARTICLE

SURFACE ADHERENCE PROPERTIES AND ANTIMICROBIAL SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI WITH INTRAHOSPITAL AND COMMUNITY ORIGIN

Liljana Labachevska-Gjatovska[1], Cekovska Zaklina[1], Pavlovska Kristina[2]
[1]Institute of Microbiology and Parasitology, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2]Institute of Preclinical and Clinical Pharmacology and Toxicology, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: Coagulase-negative staphylococci (CoNS) were long regarded harmless commensals which commonly colonize human skin and mucous membranes. Although CoNS are usually in a benign relationship with the host, changes in the medical practice and also in the underlying host populations resulted in their emerge as opportunistic pathogens with key role primarily in nosocomial but in community-acquired infections as well.

Objective: To determine the association between surface adherence properties/ antimicrobial susceptibility and the isolates origin in clinically significant CoNS and CoNS from normal skin microbiota of healthcare workers and healthy volunteers.

Material and methods: CoNS were identified by sample cultivation on standard microbiological nutrition media, gram staining and biochemical tests. Modification of the microtiter plate assay described by Christensen et al. was used to investigate the formation of biofilm. The antimicrobial susceptibility testing (AST) was performed according to EUCAST standard disc diffusion method guidelines for 13 drugs frequently prescribed as commonly active against CoNS.

Results: In present study biofilm production was observed in 9 (90%), 7 (70%) and 2 (20%) CoNS isolates from blood cultures from septic patients, skin swabs from hands of medical professionals and skin samples from hands of healthy volunteers, accordingly. Importantly, our study indicated that 63.3% CoNS isolates were resistant to methicillin. Along with the resistance to methicillin, a multidrug resistance pattern towards benzylpenicillin (63.3%), flucloxacillin (63.3%), ceftriaxone (63.3%) and cefotaxime (63.3%) was noted. The results obtained in the study also showed that, the biofilm forming coagulase negative staphylococci have higher resistance rates to various antibiotics compared to biofilm non- producing bacteria.

Conclusion: Highest production of biofilm and multidrug resistance were observed in bacterial isolates obtained from hospital environment. We also observed that the majority of MDR pathogens were biofilm producers and were almost equally present in blood samples from critical patients and skin samples of the hands of healthcare personnel.

Keywords: biofilm production, coagulase negative Staphylococcus, antimicrobial resistance

INTRODUCTION

Coagulase-negative staphylococci (CoNS) were long regarded harmless commensals which commonly colonize human skin and mucous membranes. Besides their natural habitat, the skin, this gram positive bacteria have the ability to persist and multiply on a variety of environmental surfaces [1]. However, although CoNS are usually in a benign relationship with the host, changes in the medical practice and also in the underlying host populations resulted

in their emerge as opportunistic pathogens with key role primarily in nosocomial but in community-acquired infections as well [2].

Major recognized targets of CoNS are the immune-compromised and hospitalized, chronic debilitated patients particularly in the intensive care unit in which they are most common causative agent of surgical site infections and central line-associated bloodstream infections [3].

While CoNS are known to own relatively low factors of virulence compared to *S. aureus*, the most virulent representative specie of the genus, the pathogenesis of CoNS disease spectrum trends to differ from *S. aureus* related infections with host susceptibility having crucial impact on the outcome [3, 4].

The increased use of indwelling medical devices, such as central venous catheters and other prosthetic implants as well as the expanded life stand and rising number of elderly and immuno-compromised patients are considered as crucial factors for upraise of CoNS, once considered contaminant from the normal microflora to the position of most common pathogen in healthcare-associated blood stream infection [5].

CoNS role as major nosocomial pathogen and its potential as serious healthcare problem is domainely associated with the tendency for fast development of multiple antimicrobial resistance along with extraordinary ability for biofilm production [6].

Biofilms are defined as functional consortiums of microorganisms that are attached to a surface enclosed in extensive hydrated polymeric matrix [7, 8]. In other words, a biofilm is a thin coating comprised of living material. Microorganisms growing in a biofilm are sessile and self-produce the egsopolimeric matrix (glycocalix) they are embedded in.

This dynamic organised community can be formed by a single bacterial species, but in natural environment biofilms often are comprised from various bacterial species as well as fungi, algae, yeasts, protozoa etc.

The glycocalix and its three dimensional structure is one of the key elements in creating chemical and nutrition gradient resulting in establishment of multiple microenvironments and consecutive high level of diversity within the biofilm structure.

These unique growth conditions are a beginning step which further delegates the essential differences between the sessile and planktonic microorganisms, primary through specific gene expression [9, 10, 11] than by ability of intercellular communication via chemical signaling molecules.

Numerous studies have shown that indwelling biomedical implants and transcutaneous medical devises in vivo coated with human extracellular matrix and serum proteins represent a risk factor for biofilm formation on different anatomical sites in the human body [7, 12].

Today's research is focused on the medical importance of biofilms and their role in the conventional antibiotic treatment failure of numerous infections, particularly in hospital environment [13, 14].

It has been established that biofilm mode of growth results with significant increase of the resistance toward antimicrobial agents (up to 1000 times) as opposed to the cultures grown in suspension [7]. The resistance of biofilm bacteria is largely phenotypic. Research findings demonstrate that when biofilm bacteria are grown in conventional laboratory suspension they become susceptible to antimicrobial which explains the clinical failure of antibiotic treatment of biofilm-related infections [15].

The clinical spectrum of biofilm associated infections varies from chronically infective processes in different tissues and organs (sinuses, biliary calculi, wounds, genitourinary and respiratory system) to permanent or temporary infection threw indwelling medical devices (e.g., contact lenses, central venous catheters and needleless connectors, endotracheal tubes,

intrauterine devices, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, tympanostomy tubes, urinary catheters, and voice prostheses) [7].

Among the most important steps involved in the pathogenesis of catheter- or implant-associated sepsis caused by CoNS is their adhesion and attachment to bioimplants via (non) covalently anchored cell wall proteins as well as non-protein adhesions and formation of previously mentioned recalcitrant community structures.

Layers of agglomerated bacterial cells in the biofilm protects CoNS from phagocytosis, chemotaxis and antimicrobial agents and along with the intercellular exchange of signals via quorum sensing is crucial for acquisition of multiple antibiotic resistance of the intrahospital CoNS species.

Biofilm mode of growth of the staphylococci has been related to foreign bodies [16], infections of skeletal system [17], infections of the skin and subcutaneous tissues [18], cystic fibrosis [19], urinary tract infection [20], abscess collections [21], endocarditis [22] and a variety of surfaces [23-25].

According to the published data coagulase-negative staphylococci are registered as third most common causative pathogen for hospital acquired infections and the most frequent isolate in patients with nosocomial sepsis [26, 27]. Studies also point out that particular advantage for CoNS as an etiological cause of these infections is associated with its ability for adheration to plastic surface of different medical devices more potently than any other micoorganism [6].

Colonization of the skin and mucous membranes of the host by nosocomial strains is the first step and main source of endogenous infections by CoNS. Usually, these potential pathogens are cross-transmitted from other patients or from the medical staff by or from medical equipment [28]. Taking this into consideration and the fact the immune response in inpatients is often impaired and any infection may result with fatal outcome it is important to have an insight into antimicrobial sensitivity of the circulating strains in relation to the place of origin.

OBJECTIVE

To determine the association between surface adherence properties/ antimicrobial susceptibility and the isolates origin in clinically significant CoNS and CoNS from normal skin microbiota of healthcare workers and healthy volunteers

MATERIAL AND METHODS

From 30 MR-CoNS species used in this study, 10 were isolates from haemocultures from septic patients treated on 3 different hospital wards (departments), 10 from skin samples of healthcare personnel hands and 10 from hands micorbiota of randomly selected volunteers'.

All of the hospital-acquired samples included in the study (blood samples and skin swabs from medical personal) originated from the University clinics of the clinical centre in Skopje and were submitted for routine laboratory testing at the Institute of microbiology and parasitology.

CoNS were identified by sample cultivation on standard microbiological nutrition media (Columbia agar, Mannitol Salt Agar with 4 mg/ml of oxacillin) and subsequent evaluation of the cultures with yellow and white colonies by gram staining and biochemical tests (presence of catalase and negative DNA-aze and slide coagulase test)29.

Following identification the isolated strains were numerated and subcultivated on solid nutrition media to get pure bacterial culture. To avoid multiple subcultivation and genetic modification of the original isolate, after the identification, one CoNS isolate of each colony-morphology type, was stored in tripticase soya broth supplemented with 20% glycerol at -80°C until further analysis.

Each experiment involved the use of bacterial cultures that were refreshed from stocks on nutrient agar (after defreezing, 5µl of each bacterial suspension were streaked on Columbia agar and incubated aerobically for 18 hours at 37°C). Modification of the microtiter plate assay described by Christensen et al. [30] was used to investigate the formation of biofilm. One colony from Columbia agar referenced above was inoculated in 5 ml of trypticase soya broth and incubated for 18 h at 37 C. Subsequently, 10 µl of Stationary (18-h) TSB cultures were diluted 1:100 into 1000 µl of media (TSB was utilized as a replacement of artificial urine /M63 media) to acquire McFarland 0.5 suspension containing roughly 108 CFUs/ml. This blend was homogenized by vortexing, and 100 µl from each bacterial suspension was vaccinated into the wells of sterile, polystyrene, 96-well, flat-bottomed tissue culture plates. As a compensation for the considerable fluctuation in the test, for each strain 3 duplicates were utilized and average value for biofilm formation and the standard deviation were determined. The last two wells in the line contained control strain+TSB (positive control) and just media (negative control). For biofilm formation the 96-well polystyrene microtitre plates were incubated for 24 h in a normal atmosphere at 37 °C without shaking. After the incubation time the supernatant containing the unattached cells was removed using a pipette and discarded and the plates were gently washed three times with 200 µl l 85% NaCl. After elimination of the medium containing the planktonic cell fraction we used crystal violet, a cationic dye that quantitatively stains non specifically negatively charged biofilm constituents based on ionic interactions for spectrophotometric evaluation of biofilm mass [30]. Measurement of the absorbance of each well was done by microplate spectrophotometer (ELISA microplate reader) at 570 nm.

The antimicrobial susceptibility testing (AST) of the planktonic cells of coagulase negative staphylococci was performed for 13 drugs from eight antimicrobial categories including beta-lactams, macrolides, qinolones, aminoglycosides, sulfonamides, incosamides, glycopeptides and ansamycins according to EUCAST (European Society of Clinical Microbiology and Infectious Diseases) standard disc diffusion method guidelines [31]. Commercial antibiotic discs (Oxoid, England) of the following frequently prescribed antibiotics which are commonly active against CoNS: benzylpenicillin (P, 10 µg), flucloxacillin (µg), ceftriaxone (CRO, µg), cefotaxime (CTX), erythromycin (E, 15 µg), ciprofloxacin (CIP, 5 µg), gentamycin (G, 10 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), clindamycin (DA, 2 µg), rifampicin (RD, 5 µg), linezolid (LZD, 30 µg) and vancomycin (VAN; 30 µg) were used in disc diffusion test. For detection of methicillin resistance, disc diffusion test with cefoxitin (FOX, 30 µg) was preformed. Briefly, the antibiotic disks were placed on the plates with Muller Hinton agar (Oxoid) and incubated for 18-24 hours and examined for inhibition zones.

RESULTS

Biofilm forming ability of the isolates

A total number of 30 CoNS isolated from blood samples from inpatients with sepsis (10 isolates), hospital personnel's (10) and healthy volunteers' (10) skin were examined for biofilm production by the tissue culture plate (TCP) method (Figure 1).

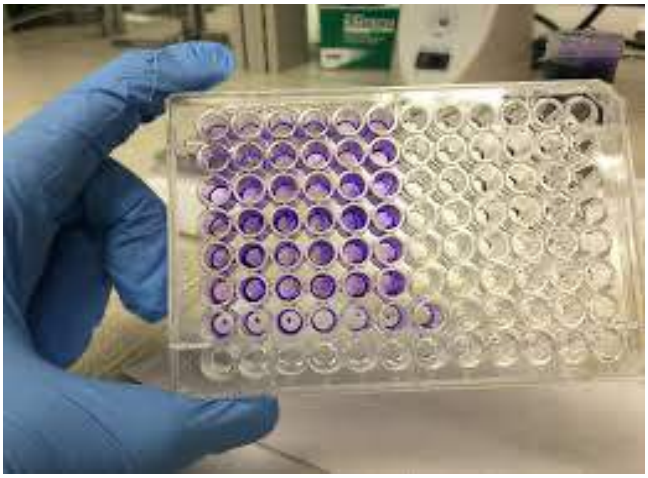

Fig. 1. Biofilm formation of CoNS isolates by the TCP method.

Biofilm production was observed in 18/30 coagulase negative staphylococci including 9 (90%), 7 (70%) and 2 (20%) isolates from blood cultures from septic patients, skin swabs from hands of medical professionals and skin samples from hands of healthy volunteers, accordingly (Table 1, Figure 2).

Table 1. Biofilm forming ability of CoNS isolates from patients with blood infection, healthcare personnel's and healthy volunteers' skin

Origin of the sample	Biofilm producers n (%)	Biofilm non-producers n (%)
Patients with blood infection	9 (90%)	1 (10%)
Healthcare personnel	7 (70%)	3 (30%)
Healthy volunteers	2 (20%)	8 (80%)

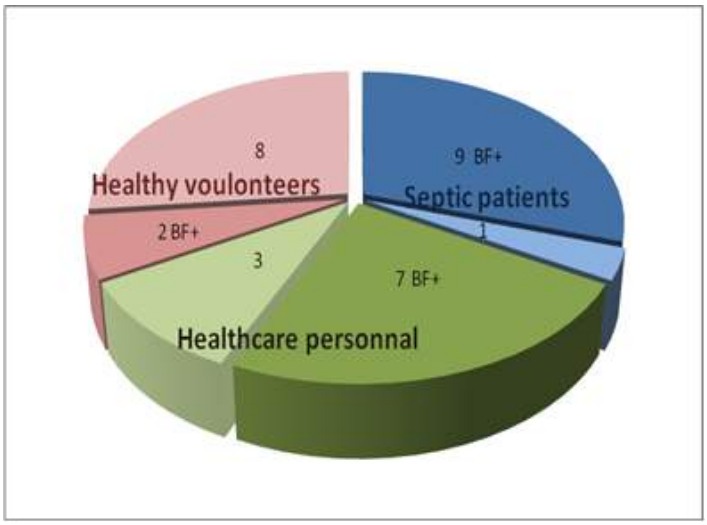

Fig. 2. Biofilm forming ability of CoNS isolates from patients with blood infection, healthcare personnel's and healthy volunteers' skin

Antimicrobial susceptibility testing

Sixty-three (63.3%) CNS isolates were resistant to cefoxitine (MRCoNS) according to disk agar diffusion. Associated with the resistance to methicilin present in most of the coagulase negative staphylococci investigated in this study, a multidrug resistance pattern was noted.

Antibiotics with the least activity against the CoNS were benzylpenicillin (63.3%), flucloxacillin (63.3%), ceftriaxone (63.3%) and cefotaxime (63.3%), followed by erythromycin (56.6%) and clindamycin 10 (56.6%). All 30 isolates were found to be uniformly susceptible to vancomycin and linezolid.

There was uneven distribution of the resistance among the various study groups, with CoNS isolated from skin microbiota of random volunteers being sensitive to most of the applied antibiotics. This has been depicted in Table 2 and Figure 3.

Table 2. Antibiotic resistance pattern of different CoNS isolates against several antimicrobial agents (%)

	CoNS isolates			
Antimicrobial	**Patients with sepsis (n=10)**	**Healthcare personnel n (%)**	**Healthy volunteers n (n=10)**	**Overall study population (n=30)**
Cefoxitine*	10 (100%)	7 (70%)	2 (20%)	19 (63.3)
Benzylpenicillin	10 (100%)	7 (70%)	2 (20%)	19 (63.3)
Flucloxacillin	10 (100%)	7 (70%)	2 (20%)	19 (63.3)
Ceftriaxone	10 (100%)	7 (70%)	2 (20%)	19 (63.3)
Cefotaxime	10 (100%)	7 (70%)	2 (20%)	19 (63.3)
Erythromycin	8 (80%)	7 (70%)	2 (20%)	17 (56.6%)
Ciprofloxacin	7 (70%)	7 (70%)	2 (20%)	16 (53.3%)
Gentamycin	6 (60%)	6 (60%)	0	12 (40%)
Co-trimoxazole	5 (50%)	4 (40%)	2 (20%)	11 (36.6%)
Clindamycin	10 (100%)	7 (70%)	0	17 (56.6%)
Rifampicin	2 (20%)	1 (10%)	1 (10%)	4 (13.3%)
Linezolid	0	0	0	0
Vancomycin	0	0	0	0

*Cefoxitime was used as screening for methicilin resistance

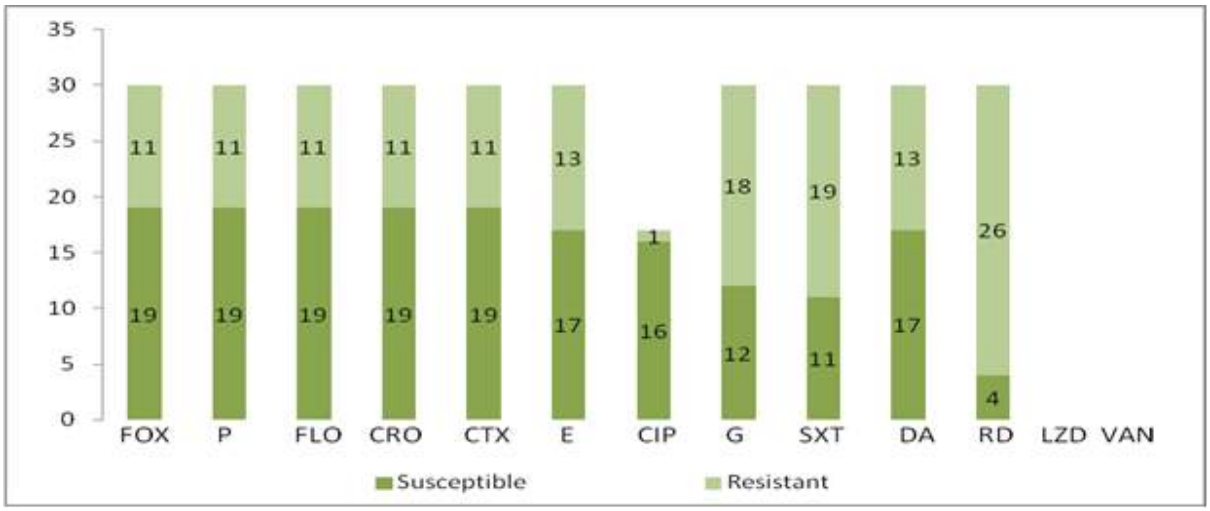

Fig. 3. Overall number of resistant vs. susceptible CoNS species towards most commonly applied antibiotics

The results obtained in the study also showed that, the biofilm forming coagulase negative staphylococci have higher resistance rates to various antibiotics compared to biofilm non-producing bacteria.

All 18 (100%) of the biofilm producers exhibited resistance to cefoxitine, benzylpenicillin, flucloxacillin, ceftriaxone and cefotaxime compared to only 8.3% (1/12) of biofilm non-producers. Similar results were obtained for erythromycin and clindamycin with 88.8% (16) of biofilm positive CoNS being resistant to both of the antibiotics while only 8.3% (1) of biofilm negative species.

All the isolates were sensitive towards linezolid and vankomycin regardless of the origin of the sample or the ability for biofilm production.

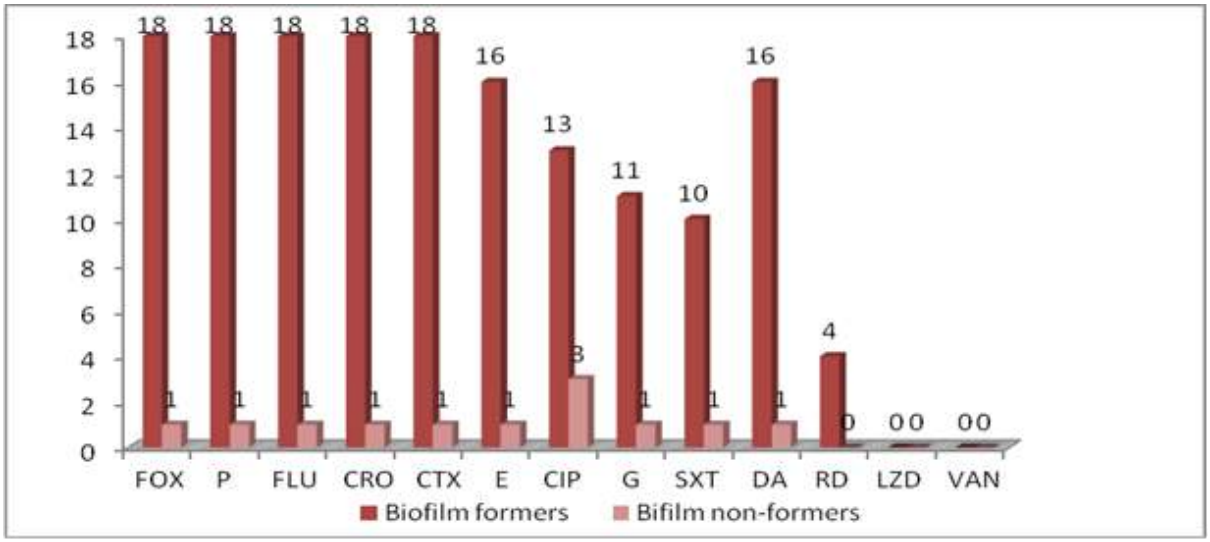

Fig. 4. Distribution of antibiotic resistance between biofilm- and non-biofilm producing CoNS isolates; *Cefoxitime was used as skrining for methilicin resistance

DISCUSSION

CoNS are generally known as non-invasive, low virulent microorganisms which take dominant part of the composition of normal human microflora. Despite the long known role of CoNS as commensals of skin and mucous membranes there is an increasing trend of their isolation as causative agents in patients with severe life threatening infections particularly intrahospital blood infections in immunocompromised patients with indwelling medical devices (such as vascular access devises). This is mainly related with their ability to form layers of cell clusters embedded in extracellular polymeric polisaharide matrix on medical equipment that penetrate skin surfaces [32].

Biofilms are reservoir for pathogenic organisms and significant potential risk factor for worse prognosis and treatment failure of wide spectrum of infections. Antimicrobial resistance is an innate feature of bacterial biofilms that, in addition to the increasing rates of reported antimicrobial resistance amongst clinical strains, may further complicate the infection [33].

In present study biofilm production was observed in 9 (90%), 7 (70%) and 2 (20%) CoNS isolates from blood cultures from septic patients, skin swabs from hands of medical professionals and skin samples from hands of healthy volunteers, accordingly.

This result shows that community associated CoNS isolates, have significantly lower ability for biofilm production compared to hospital CoNS which was supported by the results obtained by Wojtyczka et al. **(**2014) who noted biofilm production in 37.5% of *S. epidermidis* isolates from hospital environments [34], while only in 6.3% of *S. epidermidis* isolates from healthy people in Shanghai area of China as registered by Du X et al (2013) [35]. Rathanin Seng et al. (2019) in their study had lower biofilm producers ratio between the two groups of isolates with 68.3 % of the staphylococci isolated from community environments showing in vitro biofilm production vs. 92.4% of intrahospital CoNS [36].

Methicillin-resistant coagulase-negative staphylococci (MRCNS) are known to have cross-resistance to all other β-lactam antibiotics. According to the published data the increased incidence of methicillin-resistant coagulase-negative staphylococci (MRCNS) is associated with increased severity and worse outcome of the disease.

In the present study 63.3% CoNS isolates were resistant to methicillin. Along with the resistance to methicillin, a multidrug resistance pattern toward benzylpenicillin (63.3%), flucloxacillin (63.3%), ceftriaxone (63.3%) and cefotaxime (63.3%) was noted. This prevalence is similar to that reported by Singhal et al. (2006) [37] but higher than the 47.7% and 52% clinically significant MR coagulase-negative staphylococci reported in studies conducted in a tertiary care hospital by Udo et al. (2009) [38] and Sharma et al. (2019) [39], accordingly.

A comparison between antimicrobial susceptibility of biofilm-forming and biofilm-nonforming coagulase negative staphylococci revealed a significantly higher resistance rates to various antibiotics among biofilm producing bacteria which corroborates the findings of many authors [40-43].

CONCLUSION

Although it is well known that antimicrobial resistance is an innate feature of bacterial biofilms there are still many unexplored aspects regarding the mechanisms involved in its recalcitrance to treatment.

We documented a high rates of multidrug resistance and biofilm production in bacterial isolates obtained from hospital environment. We also observed that the majority of MDR pathogens were biofilm producers as well and were almost equally present in blood samples from critical patients and skin samples of the hands of healthcare personnel. This, implies that, among others, MDR CoNS associated with sepsis origin from the hands of the hospital stuff.

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ORIGINAL ARTICLE

STUDENTS PERSPECTIVES TOWARDS TEACHING MEDICAL HISTOLOGY: CONVENTIONAL LIGHT VERSUS VIRTUAL MICROSCOPY AMONG UNDERGRADUATE MEDICAL STUDENTS

Angelovska Tamara[1], Simeonovski Viktor[2], Kostovski Marko[3]
[1] Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2]University Clinic for Dermatology, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[3]Institute of Microbiology and Parasitology, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: Over the last year living in a world pandemic, along with the technology revolution, the worldwide trend is transitioning from conventional light microscopy (CLM) to virtual microscopy (VM) in undergraduate histology education rapidly increased. The opportunity to simultaneously deliver study materials to the students and the economic benefits of VM over CLM, as well as the easy accessibility for distance teaching and learning, both have been shown as major benefits of VM implementation.

Objective: Our study objective was to evaluate students' perceptions towards the concept of VM implementation as a new additional pedagogical tool during the practical portion of undergraduate histology education compared to the students' perceptions towards CLM.

Material and methods: Two hundred and fifty-seven, first-year medical students were enrolled in the study. During the summer semester, because of distance learning implemented during the pandemic, the students were taught using free online web-based virtual laboratories. Students' perceptions about VM implementation and decreased use of LM were then evaluated through a self-developed pre-validated questionnaire using a 5-level Likert scale. The data was further analyzed statistically.

Results: 80.5% of students think that both modalities should be used during practical sessions. Only 22.2% of the students think LM should be eliminated and replaced with VM. With the statement that they gain more knowledge if they use only VM, Pearson's chi-square with χ^2=17.54; df=4; p=0.001, showed a strong statistical significance with p<0.01 and Spearman's R'= -0.24 (p<0.05) with a negative correlation of the compared parameters, meaning more students that passed disagreed with the statement, opposed to the students that failed or didn't take the exam. However, 50.6% of the students that failed or didn't take the exam, find VM more inspiring, compared to the 29.3% that passed found VM more inspiring, Pearson's chi-square test showed a strong statistical significance with p value less than 0.01 (χ^2=15.64; df=2; p=0.000). Notably, 52.5% of students pointed out that their preference towards VM vs. CLM doesn't depend on examination modality (VM or CLM).

Conclusion: Considering medical students' opinions the use of CLM should not be abandoned from histology and embryology courses and should be used in the future of medical students' education as an integral part of histology courses.

Keywords: virtual microscopy, conventional microscopy, histology, education, students

INTRODUCTION

Human histology as a basic morphological discipline is of great importance for medical students in undergraduate studies as an introduction to normal tissue form and function but also in understanding the disease process and the diagnosis of diseases. Traditionally, students have

achieved their competencies and knowledge in histology using a light microscope and glass slides of tissue. In previous years, there have been attempts of implementing a more digital approach, using virtual microscopy as a teaching tool in our faculty. With the development of technology, a question arose for whether light microscopy (LM) should be partially or fully substituted with virtual microscopy, but also to explore the benefits and disadvantages of both. Conventional light microscopy and glass slides were used during practical sessions with the students and were available to them for further learning before the practical exams. Virtual microscopy was an option as a teaching tool but was not implemented during classes. Whether the student chose to learn by conventional light microscopy or virtual microscopy was by choice, but the exams were performed on a light microscope. Although this was an important question and surveys were made in previous years of the opinion of the students, it has become a necessity in the previous year. With a global pandemic of Covid-19 emerging, and Universities around the world taking a virtual approach to the typical lectures, virtual microscopy practically became a requirement. Virtual microscopy (VM) is an emerging technology and tool allowing teachers and students a much easily accessible approach to learning and exploring tissue slides at their convenience. A study by Mione et al. at the Faculty of Medicine and Health Sciences in Ghent University in Belgium explored the impact of VM versus optical microscopy (OM) in the gaining of knowledge in histology and their results showed that there was not a significant difference in the gained knowledge and passing the exams between VM and OM, suggesting that VM is equivalent to OM in the knowledge acquisition [1]. A more recent study done by Amer and Nemengani from the College of Medicine Taif University, Saudi Arabia, evaluated the use of VM in the assessment of practical histology during the Covid-19 pandemic [2]. Their results show successful implementation of the VM during the Covid-19 pandemic with a similar performance by the students, noting that students preferred VM as a learning tool during online distance learning. Krippendorf et al. assessed the rapid switch from LM to VM for teaching histology and concluded that VM can effectively replace LM. Interestingly, students who exclusively use VM to learn histology were immensely more inclined to VM, compared to the students that had learned histology by LM previously, but still had a strong preference towards VM [3]. Fonseca et al. showed similar results when evaluating the student's experience with transitioning from LM to VM in teaching oral pathology [4]. Over 90% of the students preferred the digital slides and considered the modality much more user-friendly. In the Histology and embryology practical sessions and teaching in 2020, virtual microscopy was mainly used, as opposed to light microscopy as the main tool in the previous semester of the same students. So the question arose, of the success in the implementation of VM and to ascertain the results of the students at the practical exams, comparing to the method used in the previous semester, hoping to assess the quality of knowledge gained with VM versus LM in the same students and their preferred method of learning. We conducted a survey that aims to evaluate their opinions towards the two teaching modalities for a better quality of the future Histology and Embryology (HE).

OBJECTIVE

Our study objective was to evaluate students' perceptions towards the concept of VM implementation as a new additional pedagogical tool during the practical portion of undergraduate histology education compared to the students' perceptions towards CLM.

MATERIAL AND METHODS

Study design and participants

VM was used as a teaching and assessment tool of practical knowledge of Histology and embryology for 1st-year medical students at the Medical faculty at Ss. Cyril and Methodius University. A survey was conducted in May of 2020 among the 1st year medical students and

data were collected from 257 respondents (59 males (22.95%), 198 females (77.04%) with a mean age of 19.13 ± 0.71) through a self-developed pre-validated questionnaire. Before filling out the survey, the students were given a brief introduction on its purpose and were given instructions on how to proceed. The questionnaire for the study consisted of 46 questions, designed to assess the student's perceptions of different aspects of the use of VM versus LM. The survey took approximately 13 minutes to complete. The questions about personal information included questions about Gender, Age, Ethnicity, and Year of studies. The questions assessed whether the students use VM at home and whether they used it during the previous semester with a yes/no type of question. Twenty-nine of the questions were designed to explore the perception of the student with a five-level Likert scale for both microscopies as follows: Strongly agree, agree, neutral, disagree, strongly disagree. Also, the students were asked about their preferred modality to be used in the following semesters, whether they would prefer to use VM versus LM, as well whether their choice depends on the modality used during the practical exam. Also, students were asked to answer about their grade in the practical exam: passed or failed/didn't take the exam. The answers of the students were analyzed and compared.

For data analysis, StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. was used. Descriptive statistics were used for processing the demographic data as well as answers to the questions about the student's preference and opinion of VM and LM answered by the five-level Likert scale. The results are presented as absolute numbers and percentages in Table 1 and Table 2. The Pearson's chi-square test was used for the analysis of categorical data. The results of the chi-square test are shown as histograms. The p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 gives an overview of the questions with five-level Likert scale type of answers, concerning the opinion of the students of LM versus VM. The answers are presented as numbers and percentages of students in agreement or disagreement with a specific statement.

Table 1. Assorted questions and statements from the questionnaire

Questions included in the student's survey
1. It is necessary to use both modalities in the practical sessions (LM and VM)
2. In my opinion, in the practical sessions, only one modality should be used (LM or VM)
3. My preferred choice of using LM or VM depends mostly on which modality is used during the practical exam
4. In my opinion, the practical sessions and practical exams should use the same modality (whether it is only LM or only VM)
5. In my opinion, my practical exam results would be better if LM is used during the exam
6. In my opinion, my practical exam results would be better if VM is used during the exam
7. It is my preference to use LM during the practical sessions and exam
8. In my opinion, LM should be eliminated in the practical sessions and replaced with VM
9. In my opinion, with the increased use of VM during the summer semester, my knowledge in the practical sessions is affirmed
10. In my opinion, with the decreased use of LM during the summer semester, my knowledge in the practical sessions is stagnant or decreased
11. In my opinion, my knowledge in the practical sessions is greater when using only VM
12. In my opinion, my practical knowledge does not depend on whether LM or VM is used
13. In my opinion, my knowledge in the practical sessions is greater when using only LM
14. VM should be included as an additional tool to the LM
15. LM should be included as an additional tool to the VM
16. Using VM at home facilitates my learning process
17. In my opinion, with the increased use of VM during the summer semester, my knowledge in the practical sessions is affirmed
VM – virtual microscopy; LM – light microscopy;

Table 2. Answers to the assorted questions from table 1 by five-level Likert scale

Answers to the questions by five-level Likert scale					
Question number	***Strongly agree N/(%)***	***Agree N/(%)***	***Neutral N/(%)***	***Disagree N/(%)***	***Strongly disagree N/(%)***
1.	**79 (30.7)**	**128 (49.8)**	**27 (10.5)**	**22 (8.6)**	**0**
2.	**12 (4.7)**	**44 (17.1)**	**42 (16.3)**	**130 (50.6)**	**26 (10.1)**
3.	**42 (16.3)**	**93 (36.2)**	**67 (26.1)**	**50 (19.5)**	**4 (1.6)**
4.	**63 (24.5)**	**136 (52.9)**	**32 (12.5)**	**23 (8.9)**	**1 (0.4)**
5.	**14 (5.4)**	**55 (21.4)**	**121 (47.1)**	**60 (23.3)**	**6 (2.3)**
6.	**30 (11.7)**	**95 (37)**	**104 (40.5)**	**24 (9.3)**	**3 (1.2)**
7.	**34 (13.2)**	**132 (51.4)**	**65 (25.3)**	**16 (6.2)**	**1 (0.4)**
8.	**20 (7.8)**	**37 (14.4)**	**62 (24.1)**	**90 (35)**	**46 (17.9)**
9.	**62 (24.1)**	**113 (44)**	**61 (23.7)**	**15 (5.8)**	**3 (1.2)**
10.	**8 (3.1)**	**29 (11.3)**	**58 (22.6)**	**136 (52.9)**	**25 (9.7)**
11.	**12 (4.7)**	**36 (14)**	**77 (30)**	**110 (42.8)**	**19 (7.4)**
12.	**6 (2.3)**	**40 (15.6)**	**73 (28.4)**	**106 (41.2)**	**29 (11.3)**
13.	**5 (1.9)**	**14 (5.4)**	**66 (25.7)**	**153 (59.5)**	**17 (6.6)**
14.	**80 (31.1)**	**107 (41.6)**	**40 (15.6)**	**25 (9.7)**	**0**
15.	**48 (18.7)**	**87 (33.9)**	**56 (21.8)**	**57 (22.2)**	**7 (2.7)**
16.	**104 (40.5)**	**130 (50.6)**	**18 (7.0)**	**0**	**2 (0.8)**
17.	**62 (24.1)**	**113 (44)**	**61 (23.7)**	**15 (5.8)**	**3 (1.2)**

Table 3. Opinion of the students about what would be the best methodological approach and their grade.

Student's responses to the survey regarding the use of virtual and the conventional (light) microscope						
Questions included in the student's survey	**VM *N/(%)***	**LM *N/(%)***	**Both *N/(%)***	**Total *N/(%)***		
Which method is better/more effective to gain knowledge (easier to use, recognize and find structures) in the practical sessions?	**48 (18.7)**	**22 (8.6)**	**84 (71.6)**	**254 (98.8)**		
Which modality is more stimulating/inspiring/gives you greater pleasure while learning?	**92 (35.8)**	**45 (17.5)**	**115 (44.7)**	**252 (98.1)**		
Which modality do you consider to be more useful for gaining skills for students in general?	**66 (25.7)**	**52 (20.2)**	**138 (53.7)**	**256 (99.6)**		
Which modality do you think should be used during the practical sessions?	**Only VM *N/(%)***	**VM + additional LM *N/(%)***	**Only LM *N/(%)***	**LM+ additional VM *N/(%)***	**Neutral *N/(%)***	**Total *N/(%)***
	14 (5.4)	**104 (40.5)**	**6 (2.3)**	**128 (49.8)**	**4 (1.6)**	**256 (99.6)**
Do you use VM at home?	**Yes *N/(%)***		**No *N/(%)***		**Total *N/(%)***	
	232 (90.3)		**24 (9.3)**		**256 (99.6)**	
My grade in histology and embryology 1:	**Passed *N/(%)***		**Failed/didn't take the exam *N/(%)***		**Total *N/(%)***	
	169 (65.8)		**88 (34.2)**		**257 (100)**	

Table 2 consists in student's answers to the assorted question in Table 1, regarding the use of virtual and the conventional (light) microscope by five-level Likert scale evaluated in number and percentage.

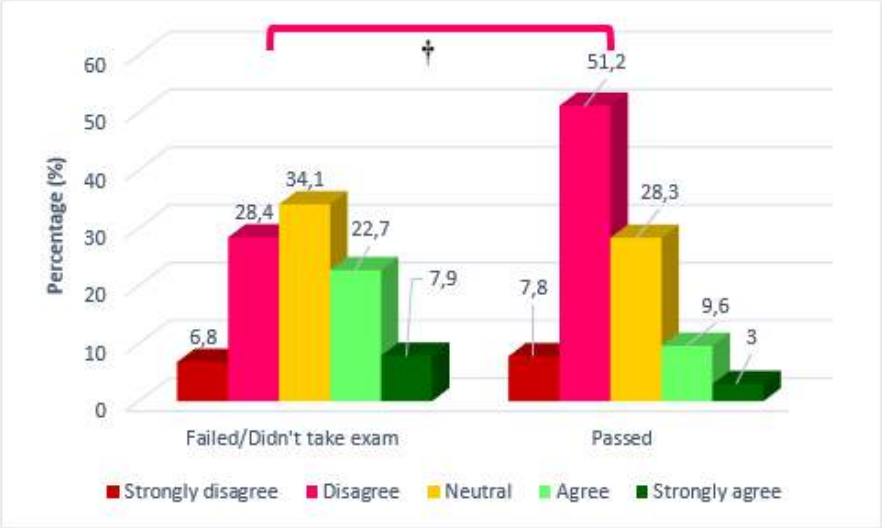
In Table 3 more questions are shown about the students' perception about which is the best modality or methodology to be used during the practical session and exams. On the question, which method is better/more effective to gain knowledge in the practical session, 71.6% answered both modalities. On the question with modality is more stimulating or inspiring, 35.8% chose VM, versus the 17.5% that chose LM. Yet again, most of them, 44.7% consider them both being inspiring/stimulating methods. Again, when asked which modality they consider to be more useful for gaining skills in general, 53.7% answered both.

Another question regarding their opinion on which modality or modalities should be used, only one of them, or one as a basis and an additional modality, 40.5% chose the basis with VM and adjunctive LM, and 49.8% preferred LM as a base with additional VM. Most of the students, 90.3% said that they use VM at home, versus the 9.3% that don't use it. When analyzing the students answer yes or no to the use of VM at home and their exam results, 81.8% of the student's that failed or didn't take the exam used VM at home, and 95.2% of the students that passed. Of the students that failed or didn't take the exam 18.2% didn't use VM at home, compared to the 4.8% of the students that passed the exam, didn't use VM at home. The Pearson's chi-square showed a strong statistical significance (χ^2=12.24; df=1; p=0.000) with p<0.01 (Figure 1).

†Pearson's chi-square showed a strong statistical significance (χ2=12.24; df=1; p=0.000) with p<0.01

Fig. 1. Comparison of the exam results and the usage of VM at home

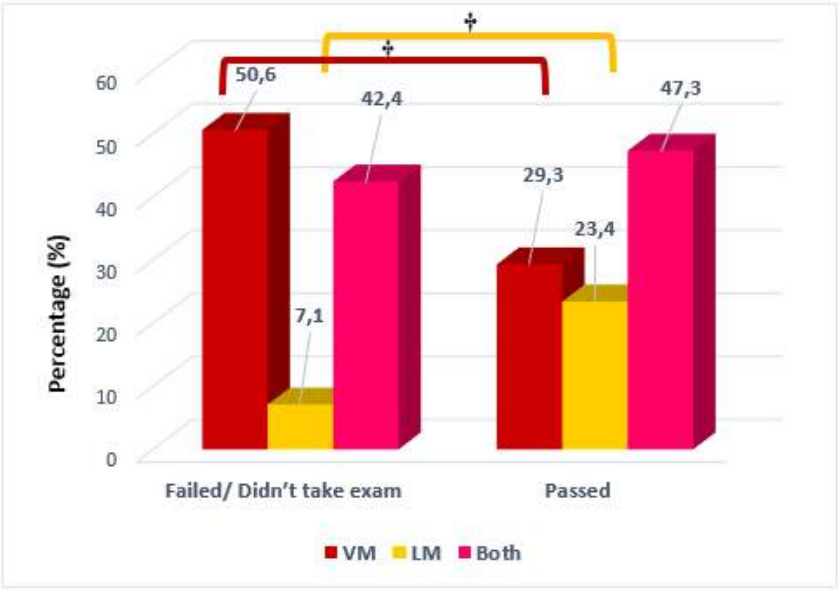
Next, we analyzed the answers to the question of whether the students think that they gain more knowledge when using only VM and then compared it to the exam results. As per results shown in Table 2, statement 11, 50.2% of the students disagree or strongly disagree with this statement, of whom 59% are students that passed the exam, compared to the 35.2% that disagree or strongly disagree and also either failed or didn't take the exam (Figure 2).



† Pearson's chi-square with $\chi 2=17.54$; df=4; p=0.002, shows a strong statistical significance with p<0.05

Fig. 2. Comparison of the exam results and opinion whether they get more knowledge when using only VM.

Pearson's chi-square with $\chi^2=17.54$; df=4; p=0.002, shows a statistical significance with p<0.05. Furthermore, Spearman's R'= -0.24 (p<0.05) with a negative correlation of the compared parameters, meaning more students that passed disagreed with the statement, opposed to the students that failed or didn't take the exam. We found another interesting comparison between the question, which method they find more inspiring or stimulating, mentioned earlier when compared with the exam results. Fifty point six percent (50.6%) of the students that failed or didn't take the exam, find VM more inspiring, compared to the 7.1% of students with the same exam result that find LM more inspiring. Among the students that passed, 29.3% found VM more inspiring, 23.4% found LM more inspiring, and 47.3 % answered both (Figure 3). Pearson's chi-square test showed a strong statistical significance with p less than 0.01 ($\chi^2=15.64$; df=2; p=0.000).



† Strong statistical significance with p less that 0.01; Pearson's $\chi 2=15.64$; df=2; p=0.000

Fig. 3. Comparison of the question of which method they consider more inspiring versus their exam results.

We analyzed the questions concerning the opinion about LM. When asked, is it their preference to use LM during the practical sessions and exams, 64.6% answered agree or strongly agree, saying they prefer the use of LM. The distribution of the answers by gender was significantly different. The female students prefer the LM with 70% of answers being, agree or strongly agree, in contrast to 56.9% of the males (Figure 4). Pearson's chi-square test showed a marginal statistical significance, χ^2=9.64; df=4; p=0.046.

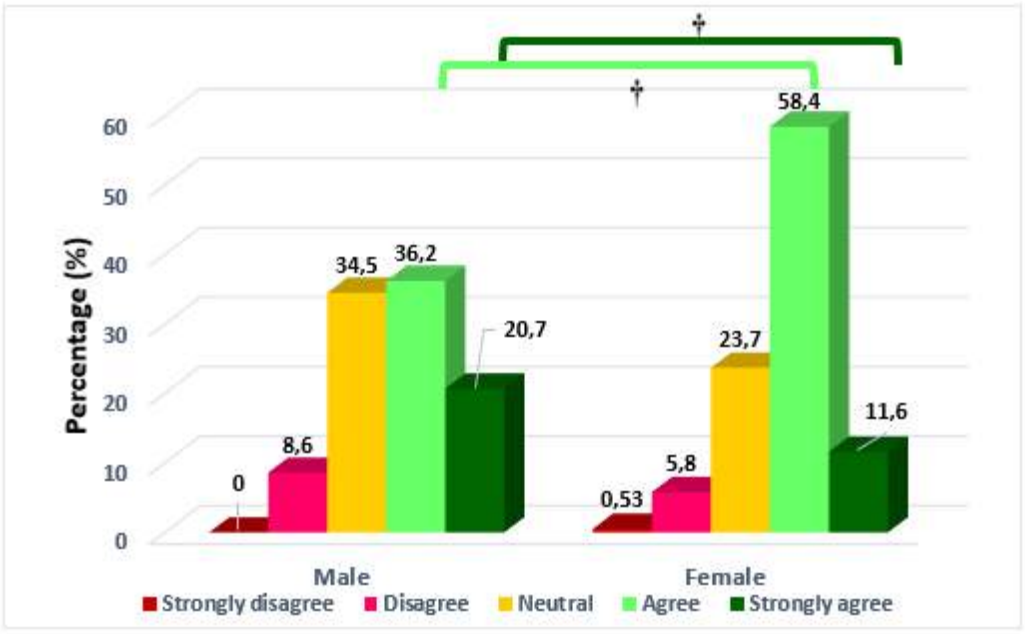


Fig. 4. Preference of usage of LM by gender

Further analysis was made on the students' perception of the complete elimination of LM from the practical session and exams and replaced with VM. As it is shown in Table 2, answers about statement 8, 52.8% disagree or strongly disagree with the elimination of LM, and only 22.2% agree or strongly agree that LM should be eliminated (Figure 5).

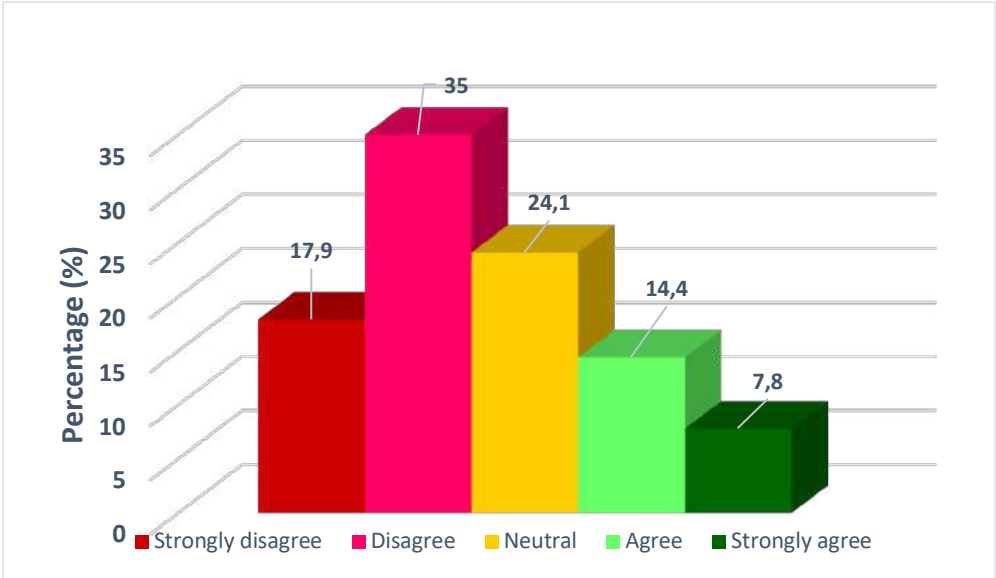


Fig. 5. Students' opinion on whether LM should be eliminated and replaced with VM

On the other hand, on statement 10, that their knowledge decreased during the summer semester when VM was predominantly used, 62.6% disagreed or strongly disagreed with this statement, and only 14.4% agreed or strongly agreed. There was not a remarkable difference between genders either, with 57.7% of the males, and 64.7% of females disagreeing or significantly disagreeing with the statement (Figure 6).

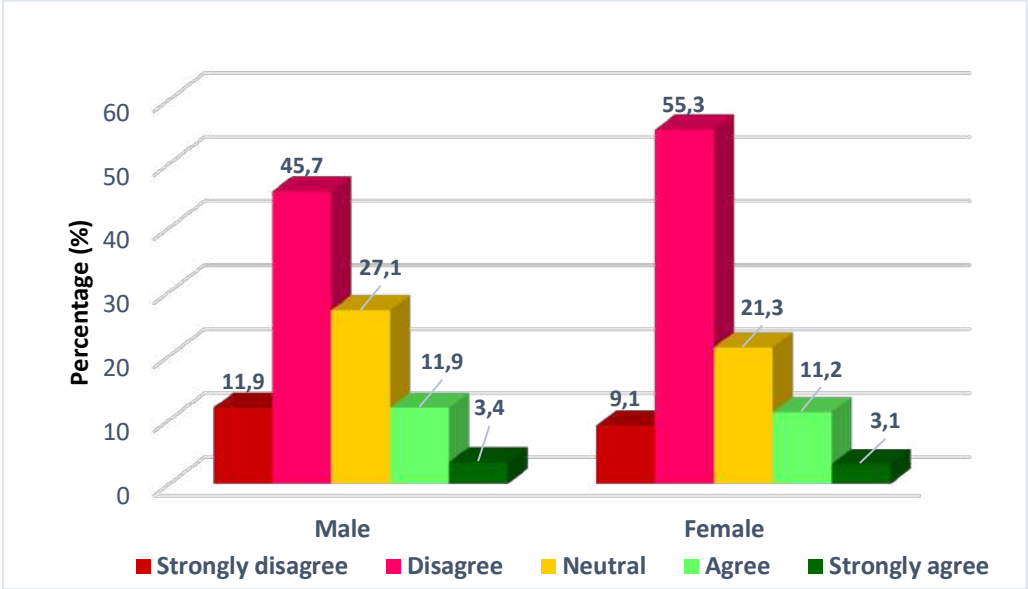


Fig. 6. Statements about less LM meaning less knowledge, distribution by gender

Students were also asked to give their perspectives on a combination of modalities. As shown in Table 1, statements 14 and 15, one of the options offered was the usage of VM as an additional tool to the LM as a principal method, 72.8% of students strongly agreed or agreed with the option, and only 11% disagreed. On the other hand, when reversed, the usage of LM to be additional to VM as a principal method, 52.5% strongly agreed or agreed, while 24.9% strongly disagreed or disagreed (Figure 7).

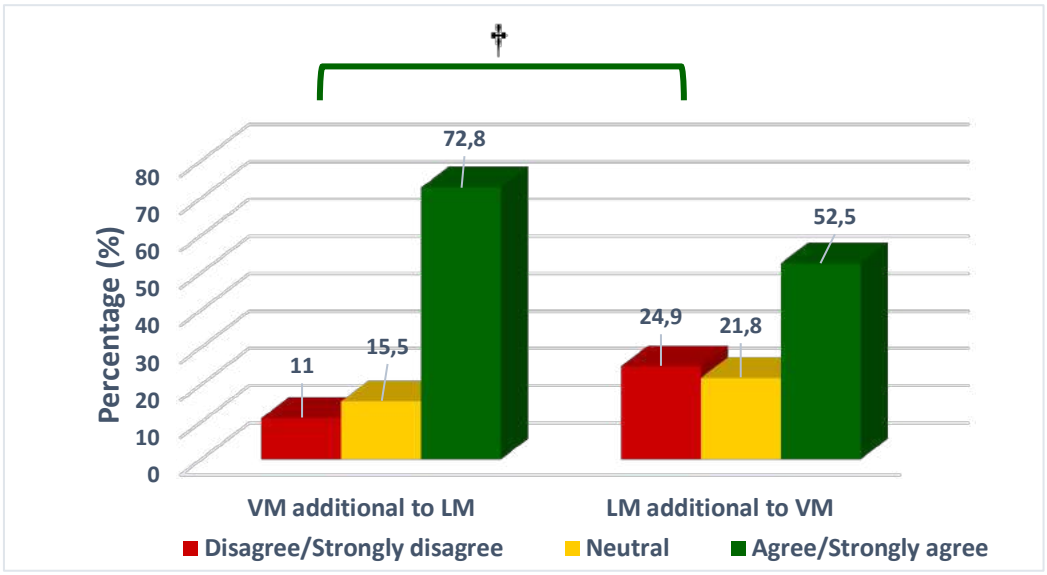
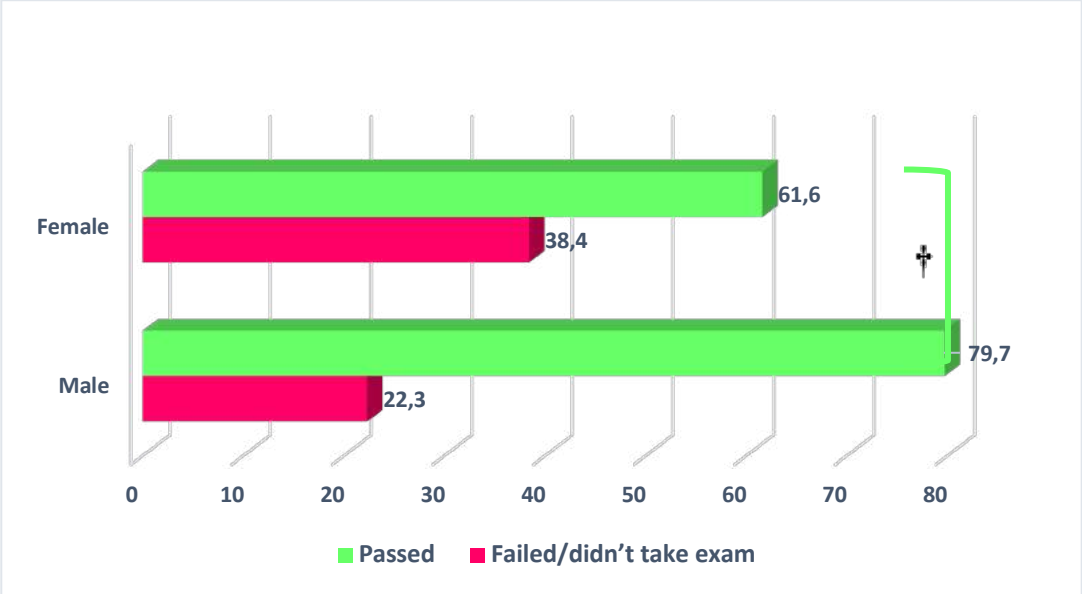


Fig. 7. Preference of different modalities

The Pearson's chi-square test on these two categorical values showed a strong statistical significance between the answers, with $\chi^2 = 18.44$; df=4; p=0.001, with p<0.01.

Considering that there were differences in opinions and preferences between genders, we analyzed the exam results by gender to establish whether there was a difference. Male students had an overall much better performance, with 79.6% of males passing the exam and only 22.3% either not taking the exam or failing.

Compared, 61.6% of female students passed the exam, and 38.4% didn't take the exam or failed it (Figure 8). Pearson's chi-square test also showed a statistical difference, χ^2=6.57; df=1; p=0.01, with p less than 0.05.



† Pearson's, χ2=6.57; df=1; p=0.01, p < 0.05, significant statistical difference.

Fig. 8. Exam results distribution by gender.

DISCUSSION

Traditional academic activities and vis-à-vis education in universities all over the world were suspended temporally during 2020 and moved to online teaching and learning. Previously the HE theoretical knowledge is taught in amphitheaters, followed by practical sessions in a histology laboratory. During the Covid-19 pandemic, HE lectures and practical sessions were adjusted and traditional face-to-face classes were substituted with online courses. Tissue observation under a microscope in a practical laboratory is a crucial part of HE teaching. The progress of techniques and necessities during the pandemic prompted the extensive application of virtual microscopy in histology education. At the end of the semester, we surveyed the students' perceptions concerning the implementation of VM. Mione et al. suggest that the acquisition of knowledge in histology is independent of the modality, furthermore supported by Amer et al, that VM is an effective learning method as well as an assessment method for measuring students' performance [1, 2]. Krippendorf et al. found VM to be highly effective for first-year students and they consider it to be suitable substitute for LM [3]. Similar results were published by Fonseca et al. regarding the use of VM in teaching oral pathology [4]. VM is considered as an improvement of the teaching process with the possibility of completely replacing glass slides with virtual images in teaching histopathology in the future. Virtual microscopy was positively received by our students, with 68.1% agreeing that their knowledge has been affirmed by using VM. Our results are in concordance with other studies. Pospíšilová et al. evaluated the use of VM in teaching practical histology and report that 93% of general medicine and dentistry students positively evaluated the use of virtual slides, explaining that they could study and discuss details of cells and tissues clearly at various magnifications [5]. Similar results were reported by Foad at Tabuk University [6]. They compared students that

used VM with those using LM and found that students quickly acquired skills using VM and reflected an improvement in students' achievements after using VM. Ordi et al. also state that VM can effectively replace LM in teaching and learning pathology in undergraduate courses in medical schools [7]. The universal feedback from the students had been positive and 70% of them considered VM easier to use than LM. Krippendorf et al. assessed the rapid and complete transition from LM to VM in teaching medical histology [3]. The authors found that the students positively received the modality, moreover, the data from their examinations and evaluation surveys suggest that VM may improve the students' performance and learning efficacy. Alotaibi et al. measured the preference of VM and LM in dental students as a tool in teaching oral histology and pathology, concluding that VM was the highly preferred substitute [8]. Tian et al. reported similar results, with VM being preferred by 95% of students, yet as they emphasize, students taught only with VM, may have difficulties using conventional light microscopy later in their practice [9]. In a substantial study done by Bloodgood et al. about the trends in histology laboratory teaching in United States medical schools, they analyzed data from 82 medical schools, representing slightly less than two–thirds of U.S. medical schools [10]. When asked about plans for histology teaching, 50% indicated that they plan to use VM, 65.9% responded that they will use LM and glass slides. However, only 12.2% indicated they will be using only virtual slides, and 6.1% indicated that they will use only microscopes and glass slides. Their respondents emphasize that students need to learn "to interpret the entire specimen" and "learn better and remember more if they have to search for things." Traditionalists agree that students must have microscope skills, independent of the use of virtual slides. It is evident that during the past years many medical and dental schools around the world have implemented the use of virtual slides and virtual microscopy to some extent, yet this process has been accelerated during the past year due to the Covid-19 pandemic. An article by Caruso about VM and other technologies used for teaching histology during the Covid-19 pandemic, it is pointed out that although necessary, histological sciences should not be delivered entirely online [11]. In a nationwide survey in 78 medical schools in China, conducted in early 2020, 65% of medical schools increased the use of VM during practical sessions [12]. Although a useful tool, students' considered that there is more effective communication in didactic practical sessions than online. Similar results were reported by Sharma et al. received from a survey conducted in San Antonio with 75% of respondents felt that remote learning adversely affected their ability to participate in the curriculum [13]. As Hande et al. point out, the merits of conventional microscopy cannot be overruled, VM still found an important role as a teaching tool and learning method [14]. Darici et al. reported that although there is a general satisfactory response of VM and it is suitable for the situation, the need for interaction is pointed out, with students' taking a passive approach during practical sessions online [15]. Our results, as discussed above show a general satisfaction with the use of VM in practical sessions during the Covid-19 pandemic, and 65.8% of students passed the practical exam. VM was used by the students' at home and they consider it a valuable tool for learning histology. Furthermore, they don't consider the lack of LM meaning less knowledge, as mentioned earlier 68.1% consider that VM affirmed their knowledge. Concerning which method they consider to be more effective to gain knowledge, the majority, 71.6% said both methods, but 35.8% find VM more inspiring, compared to 17.5% who preferred LM. Yet more than half of the students, 52.9% answered that they strongly disagree/disagree with the complete elimination of LM and replacement with VM. Having a point of comparison, 64.4% of students' still preferred the use of LM, in concordance with previous answers that LM should not be eliminated. Taking all this into account, on the statement which modality they preferred or combination of methods, students predominantly think that conventional light microscopy should stay as the basis of the practical sessions with additional use of VM.

CONCLUSION

Our result indicated that VM is a practical tool, effective for teaching and learning histology, especially in such conditions, and it maintains the student's performance during the distance learning process. It was well accepted by students, used by the majority of them and its use didn't affect the students' results and it has proven to be a reliable method. Our students, are still not keen on eliminating LM. They consider the combination of methods, with the basis remaining LM and additional VM, to be the most suitable modality to achieve the most knowledge in the subject. We recommend the use of VM as it is proven to be a helpful and educational tool, for now as a substitutional tool during the pandemic, later as an additional tool to the conventional light microscopy in concordance with the preference of the students.

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CASE REPORT

GENETIC TESTING ESSENTIAL IN EARLY DIAGNOSIS AND TREATMENT IN TUBEROUS SCLEROSIS COMPLEX

Danilo Nonkulovski, Duma Filip, Muaremoska Kanzoska Lejla, Adili Ademi Learta, Cilevska Mladenovska Sanja, Cilevska Sandra, Serafimova Emilija, Kirovski Ilija, Pandovska Bisera

University Clinic for Paediatric Diseases, Skopje, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: Tuberous sclerosis complex (TSC) is rare genetic neuro cutaneous disease. It is characterized by cutaneous changes and formation of hamartomas in multiple organs with consecutive, mostly neurologic conditions that lead to morbidity and mortality. The affected genes are TSC complex subunit 1 and TSC complex subunit 2, encoding hamartin and tuberin respectively. Failure in formation of TSC1:TSC2 complex is culminating in loss of tonic inhibition of the mechanistic target of rapamycin (mTOR) pathway that leads to deregulation of protein synthesis and cell growing. The mTOR inhibitors, rapamycin (sirolimus) and everolimus improve pulmonary function in patients with TSC and reduce the size of renal and brain lesions.

Case presentation: Here, we present a case report of an early diagnosed 3 months old male infant with characteristic clinical, radiological, and genetic findings of tuberous sclerosis complex. The physical examination revealed conscious and alert infant with normal growth and development, with irregular heartbeat and 4 hypo melanotic macules, three on the back and one on the gluteal zone. Cardiac ultrasound was with normal findings and electrocardiogram revealed sinus arrhythmia.Because of the presence of more than three hypo melanotic macules, the diagnosis of possible TSC was made and parents got recommendation for genetic tests. Molecular genetic tests revealed pathogenic mutation c.1832G>A; p.(Arg611Gln) in TSC 2 gene, that wasn't found in infant parents, which indicates "de novo" occurred mutation.

Conclusion: Early age at diagnosis will open new avenues to new multidisciplinary therapeutic interventions, like earlier use of mTOR inhibitors and earlier start of occupational and neurobehavioral therapy, thus further modifying the clinical trajectory and phenotype in affected children.

Keywords: TSC, seizures, hypomelanotic macules, subependymal nodules, sirolimus

INTRODUCTION

Tuberous sclerosis complex (TSC) is a an autosomal-dominant or sporadic, rare neurocutaneous multisystem disorder associated with hamartomas or benign tumor growths in the brain, heart, lung, eye, or kidney [1]. TSC is estimated to affect one in 6,000 to one in 10,000 live births across all ethnic demographics [2].

Patients with TSC have inherited autosomal dominant or sporadic mutations in either TSC or TSC2 gene. Two out of three TSC cases are sporadic mutations that are not linked to a family pedigree, but arise de novo at the initial time of zygote formation [3]. Mutations in TSC1 are often small insertions or deletions, whereas TSC2 mutations include large deletions, indels, nonsense, and missense mutations [4].

Each mutation type functionally inactivates, either TSC1 or TSC2 or prevents formation of TSC1:TSC2 complex, leading to loss of tonic inhibition of the mTOR pathway. The mTOR pathway is a primary regulator of cell growth and proliferation in response to growth factor stimulation and cellular nutrition. TSC1 and TSC2 are genes that encode proteins hamartin and

tuberin, the proteins that form the TSC1 and TSC2 complex (TSC1:TSC2). The complex is negative regulator of mTOR through GAP (GTPase-activating protein), acting on Rheb (Ras-homolog enriched in the brain). In the absence of TSC1:TSC2 complex, the levels of Rheb-GTP increase, leading to the activation of the mTOR–Raptor pathway and constitutive deregulation of protein synthesis and cell growth [5].

Tuberous sclerosis complex has highly variable clinical presentation. In 2012, diagnostic criteria were updated, two types of TSC diagnosis can be made - definite and possible, predicated on the detection of "major" and "minor" diagnostic findings (Table 1). Individuals with two major features or one major feature with two minor features meet criteria for definite TSC. Patients with only one major feature, one major and one minor feature, or two or more minor features meet criteria for possible TSC. Also, the identification of pathologic mutation at TSC1 or TSC 2 gene became sufficient to make a definite diagnosis of tuberous sclerosis complex, without any clinical diagnostic criteria. We have to note here, that 10-25 % of TSC patients have no mutations identified by conventional genetic testing, so normal result does not exclude TSC diagnosis [2].

Table. 1: Major and Minor Criteria of tuberous sclerosis complex

Genetic diagnostic criteria	-Pathologic mutation in TSC1 or TSC 2 gene
Major diagnostic criteria	-Hypomelanotic macules (≥ 3, at least 5 mm diameter) -Angiofibromas (≥3) or fibrous cephalic plaque -Ungual fibromas (≥ 2) -Shagreen patch -Multiple renal hamartomas -Cortical dysplasias (≥3), includes tubers and white matter radial migration lines) -Subependymal nodules (≥ 2) -Subependymal giant cell astrocytoma -Cardiac rhabdomyoma -Lymphangioleiomyomatosis(LAM) AMLs, -Angiomyolipomas(AMLs) (≥ 2)
Minor diagnostic criteria	-Confetti skin lesions -Dental enamel pits (≥ 3) -Intraoral fibromas (≥ 2) -Retinal achromatic patch -Multiple renal cysts -Nonrenal hamartomas

CASE REPORT

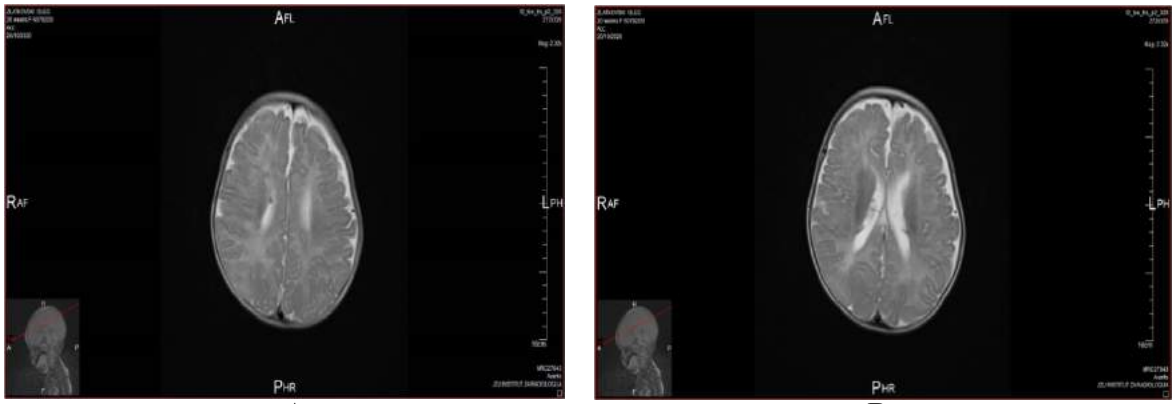
A 3 month old male infant was first referred to our clinic due to the appearance of hypo melanotic macules in the last three weeks. He had unremarkable antenatal, perinatal and postnatal medical history. Parents and close relatives didn't have history of skin conditions or neurological diseases, and there was no consanguinity.

The physical examination revealed conscious and alert infant with normal growth and development, with irregular heartbeat and 4 hypo melanotic macules, three on the back and one on the gluteal zone.

Cardiac ultrasound was with normal findings and electrocardiogram revealed sinus arrhythmia. Because of the presence of more than three hypo melanotic macules, the diagnosis of possible TSC was made and parents got recommendation for genetic tests. A month and a half later the infant referred again to our clinic due to appearance of short seizures in the last three days, in form of up rolling eyes and stiffening of arms.

Investigations

EEG had normal background for the age, with right temporal-parietal focus of spike-wave complexes. Rare generalized paroxysms of spike-wave complexes (Fig.2). Brain MR showed subependymal nodules in lateral ventricles, bilaterally, more prominent right with diameter up to 7 mm, and multiple subcortical and cortical tubers (Fig.1a and 1b). Cardiac and abdominal ultrasound were with normal findings but sinus arrythmia and supraventricular extrasystoles were noted. Other investigations such as hemoglobin, complete blood count, renal and liver function tests were in referent ranges. Molecular genetic tests revealed pathogenic mutation c.1832G>A; p.(Arg611Gln) in TSC 2 gene, that wasn't found in infant parents, which indicates "de novo" occurred mutation.



A **B**

Fig. 1. Subependymal nodules in lateral ventricles, bilaterally, more prominent right with diameter up to 7 mm, and multiple subcortical and cortical tubers

During the hospital stay, antiepileptic drugs were administered- phenobarbital and sodium valproate, through he still had seizures. Therefore, Vigabatrin was included in therapy and after few days observation the patient was seizure free and discharged in good condition. In the 3 months following period he continues to be seizure free, and the EEG shows only mildly slower brain activity without any focal or generalized paroxysms. (Fig.3)

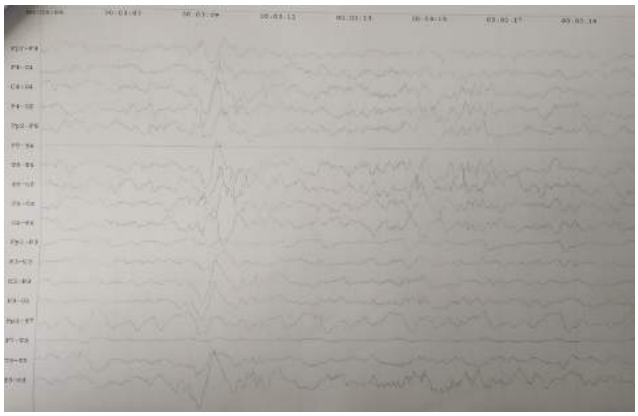


Fig. 2. EEG of the patient: generalized paroxysms of spike-wave complexes

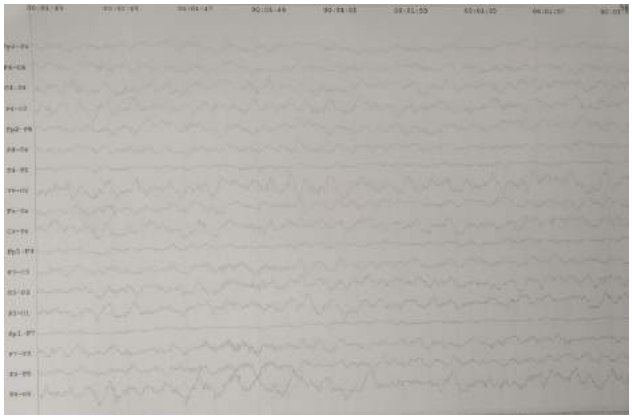


Fig. 3. Follow up EEG: mildly slower brain activity without any focal or generalized paroxysms

DISCUSSION

Tuberous sclerosis complex is genetic disease with great phenotypic variability. Diagnostic criteria defined by the Tuberous Sclerosis Complex Consensus Conference in 2012, are used for the diagnosis of TSC. There are 2 diagnostic criteria; genetic and clinical (major and minor) and two types of TSC diagnosis can be made - definite and possible [2]. The most common antenatal features that may lead to diagnosis of possible TSC are cardiac rhabdomyoma detected by antenatal ultrasound. Routine antenatal ultrasound examination performed at a gestational age of 19–22 weeks may possibly miss a certain percentage of late onset cardiac rhabdomyoma [6]. After birth, clinical findings seem to be age related, so in early infancy the most common clinical feature is central nervous system (CNS) involvement, mostly seizures. Other common clinical feature in early infancy are cutaneous lesions, mostly hypo melanotic macules. Cutaneous lesions other than hypo melanotic macules (angiofibroma, shagreen patches) is a recognized manifestation, particularly in adult cases [7]. In the long term follow up, the patients with TSC may progress intellectual disability, autism spectrum disorder, attention deficit hyperactivity syndrome, epilepsy, learning and behavior problems [8].Prospective, national surveillance study was made in Germany over a 2-year-period (03/2015–02/2017) using current revised criteria for TSC. Age range of diagnosis, definite or possible TSC, was from 5 months before birth to 197 months, median age- 6 months. When excluding prenatally diagnosed patients, median age at diagnosis (definite or possible TSC) was 11 months with a range from 0 to 197 months. Of note, age at first diagnosis in this study was substantially lower than in previous epidemiological reports, that may be due to reviewed diagnostic criteria [6]. Our patient at age of three months got possible TSC diagnosis and parents got recommendation for genetic tests that weren't performed. A month later due to the occurrence of seizures and radiological findings of subependymal nodules and cortical tubers the definite diagnose of TSC was made. Still molecular genetic test were performed, for proper genetic counseling and legal insurance procedures for early treatment with mTOR inhibitor-Eurolimus. A large number of antiepileptic drugs (AEDs) are available for seizure treatment. In 2012 the TSC expert panel specifically recommended vigabatrin as first-line intervention for infants and AEDs with c-aminobutyric acid (GABA) ergic mechanisms for older children [13]. In addition of that, our patient became seizure free after Vigabatrin was introduced in therapy. Inhibition of mTOR can be achieved pharmacologically by rapamycin (sirolimus), which causes dissociation of mTORC1 from its binding partner Raptor, thus successfully inactivating it. The results of several small reports suggest that sirolimus may be effective for the treatment of TSC-associated seizures [11].

Rapalogs (rapamycin derivatives) are also effective in inhibiting mTORC1 and are therefore used in treatment of different cancer malignancies in addition to TSC [9]. Everolimus, a derivative of sirolimus, has been successfully used to treat subependymal giant cell

astrocytoma [10]. There are concerns about effects on patient growth and sexual maturation of long term exposure on mTOR inhibitors in youth . Although data are limited, the EXIST-1 study reported that everolimus had no significant effect on puberty or development in patients with TSC after median exposure of 47 months [12]. In the following period, after all procedures are being done, it's planned for our patient to receive Everolimus in therapy.

CONCLUSION

TSC is lifelong condition and one of the neuro cutaneous syndromes affecting almost all organs. The quality of life depends on the neurological manifestation like seizures and cognitive and behavioral impairment, which is improved by multidisciplinary approach and symptomatic organ specific treatment. Clinical diagnosis complementing with DNA testing allows precise genetic counseling and lower age at definite diagnosis.

Early age at diagnosis will open new avenues to new multidisciplinary therapeutic interventions, like earlier use of mTOR inhibitors and earlier start of occupational and neurobehavioral therapy, thus further modifying the clinical trajectory and phenotype in affected children.

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CASE REPORT

MALE GONOCOCCAL URETHRITIS – A CASE REPORT

Branka Petrovska Basovska[1], Osmani Dugagjin[1], Kakaraskoska Boceska Biljana[1], Kostovski Marko[2], Cekov Vesna[3], Memeti Shaban[1]

[1]Institute of Public Health of the Republic of North Macedonia – Skopje, Skopje, Republic of North Macedonia

[2]Institute of Microbiology and Parasitology, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

[3]University Clinic for Gynecology and Obstetrics, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: Sexually transmitted infections of the urethra are mostly consequence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Gonococcal urethritis in men has clinical representation of purulent urethral discharge and dysuria, but still 10% of cases may remain asymptomatic. Due to the authentic symptoms, this infection is often treated clinically, without microbiological confirmation.

Case presentation: A 29-year-old male patient went to general practitioner's office for burning sensation during urination and purulent urethral discharge, one day after heterosexual intercourse. Urethral swab was sent to the Institute of Public Health for microbiological examination. Following the microbiological, microaerophilic atmosphere incubation period, grayish-white, oxidase and catalase-positive colonies, were observed on Thayer-Martin agar. The result (*Neisseria gonorrhoeae*) was confirmed by Polymerase Chain Reaction. Antibiotic susceptibility testing was performed with the Minimum Inhibitory Concentration and the strain was susceptible to ceftriaxone and ciprofloxacin. The patient had been immediately managed with cephalosporin (cefuroxime) after the urethral swabbing.

Conclusion: Gonococcal infection was immediately recognized, microbiologically detected and treated according to antibiotic susceptibility testing. The antibiotic treatment was successful without further complications.

Keywords: urethritis, *Neisseria gonorrhoeae*, male, treatment

INTRODUCTION

Gonorrhea and *Chlamydia trachomatis* infections are sexually transmitted infections which cause urethritis in men. The role of sexual behavior and clinical presentation of urethritis is crucial. Gonorrhea is one of the most common sexually transmitted diseases. This genital infection is recognized as an acute bacterial infection, but the patients may be often asymptomatic. Symptoms of urethritis in men typically include purulent urethral discharge, penile itching or dysuria [1, 2].

Gonorrhea is often asymptomatic in females. A symptomatic gonococcal infection in women is represented by the clinical presentation which includes vaginal discharge, dysuria, abnormal uterine bleeding, lower abdominal or rectal pain. Untreated *Neisseria gonorrhoeae* infection can lead to serious complications like pelvic inflammatory disease, ectopic pregnancy and most important infertility in females. In males it can cause epididymitis, reactive arthritis and infertility, but in rare cases. In both genders, this infection can be complicated to disseminated disease. Gonorrhea is the second most commonly reported sexually-transmitted infection in the United States and rates are higher among women than men. Routine genital screening is recommended annually for all sexually active women at risk for infection [2-4].

Gonococcal infections are an urgent problem because *Neisseria gonorrhoeae* is developing resistance to multiple antibiotic classes [4]. Gonococcal infections are usually treated with

single-dose therapy with an efficiency of 95% of cases. In the last decade *Neisseria gonorrhoeae* has developed resistance not only to less expensive antimicrobials such as penicillin but also to fluoroquinolones [5-6].

OBJECTIVE

This case report presents the first isolated strain of *Neisseria gonorrhoeae* in the Department for Bacteriology and Antimicrobial Resistance at the Institute of Public Health. This was the first microbiological confirmed case of gonococcal urethritis in men at the Institute.

CASE PRESENTATION

A 29-year-old male patient went to primary care physician's office for burning sensation during urination and purulent urethral discharge. The symptoms started one day after heterosexual intercourse. After the physical examination, the general practitioner directed the patient (with diagnosis: N39.0 - Urinary tract infection, site not specified) to a microbiological laboratory for bacteriological examination of urethral swab, urine and sperm culture. The materials were sent to Institute of Public Health for microbiological examination. The urine and sperm samples were cultivated on Blood agar with the standard semi-quantitative microbiological method and incubated in aerobic conditions (at 37°C for 24 hours for urine and 48 hours for the sperm sample). The sperm was also cultivated on Schaedler agar with the standard semi-quantitative microbiological method and set on an anaerobic incubation (at 37°C for 48 hours). The collected specimens (urine and sperm) were both sterile. The urethral swab was used for culture and Gram stain. The urethral swab was streaked on Blood agar, Thayer Martin agar and Sabouraud agar and slide for Gram-straining. The Blood agar and Sabouraud agar were set on an aerobic incubation (at 37°C for 48 hours) and the Thayer Martin agar was incubated in microaerophilic atmosphere (presence of 5% CO_2) at 37°C for 48 hours.

Growth of pathogenic bacteria on Blood agar and fungi on Sabouraud agar was not found. Following the incubation period, grayish-white, oxidase and catalase-positive colonies, were observed on Thayer-Martin agar.

After Gram-staining from the colonies, Gram-negative diplococci were detected. Gram-negative cocci were detected on a direct urethral Gram-stain smear with unusual absence of leukocytes.

The result led for the presumptive identification of *Neisseria gonorrhoeae*. The result was confirmed by Polymerase Chain Reaction **(**PCR). Antibiotic susceptibility testing was performed with the Minimum Inhibitory Concentration Method (MIC) with E-tests for benzylpenicillin, ceftriaxone, ciprofloxacin and azithromycin. *Neisseria gonorrhoeae* strain was resistant to azithromycin, intermediate to benzylpenicillin, and susceptible to ceftriaxone and ciprofloxacin.

DISCUSSION

The patient had been managed with cephalosporin (cefuroxime) after the first urethral swabbing at early stage. On the eighth day of treatment, swab was taken again and proved negative which contributed to a negative result. A positive therapeutic point is that the therapy is effective, because in previous periods, patients were treated with sulfonamides and penicillin and this strain is intermediate sensitive to penicillin. The sensitivity of this *Neisseria gonorrhoeae* strain to cephalosporin is an additional positive contribution to the therapy, because there is a wide distribution of super-gonorrhea strains which secrete beta-lactamases and they are resistant to this group of antibiotics [7].

The *Neisseria gonorrhoeae* strain shows resistance to azithromycin, which is increasingly common worldwide. This result is extremely important because the patient was not sent for a

microbiological examination for *Chlamydia trachomatis*. Gonococcal infections are often accompanied by co-infection caused by *Chlamydia trachomatis*, and azithromycin is the primary therapy for chlamydial infections. This moment highlights the importance and need for examination and *Chlamydia trachomatis* detection with the other microbiological tests of urethral swabs [1, 8].

The general practitioner prescribed oral fluroquinolone (ciprofloxacin) for 7 more days. This was good antibiotic management, because fluoroquinolone would also cover any possible chlamydial infection.

The antibiotic treatment was successful and there was an absence of further complications.

CONCLUSION

This bacterium quickly develops resistance worldwide, therefore microbiological determination of antibiotic susceptibility is necessary [9-10]. Empirical therapy based on the clinical representation should be avoided: unsuccessful treatment, relapses which lead to complications. Prescribing antibiotics without microbiological confirmation can contribute to development of antibiotic resistance.

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CASE REPORT

PRIMARY BREAST LYMPHOMA – CASE REPORT

Tatjana Sotirova[1], Stojanoski Zlate[1], Amzai Gazmend[1], Spasovski Dejan[2]
[1]University Clinic for Hematology, Skopje, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2]University Clinic for Rheumatology, Skopje, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia

ABSTRACT

Introduction: The entity "Primary breast lymphoma" (PBL) refers to a malignant lymphoma occurring primarily in the breast, without the prior presence of lymphoma in the lymph nodes.

Case presentation: We present a case of a 40-year-old woman with PBL of the right breast and an enlarged right axillary node and thrombosis of the right arm. The pathological diagnosis (made in the Institute of Pathology, "Ss. Cyril and Methodius" University, Skopje, Republic of Macedonia) was Non-Hodgkin's lymphoma (aggressive B-cell type). According to the Ann Arbor staging system, the stage was IIE. The patient was treated in the University Clinic of Hematology, "Ss. Cyril and Methodius" University, Skopje, Republic of Macedonia). She received 8 cycles of CHOP protocol. (Rituximab was ceased due to a very severe allergic reaction). After finishing with chemotherapy she was in remission with almost complete restitution of the breast and arm. At a follow-up period of 5 years, the patient has survived with no evidence of disease.

Conclusion: This strongly suggests that central nervous system (CNS) prophylaxis should be associated with systemic chemotherapy in localized PBL.

Keywords: primary breast lymphoma, chemotherapy, chop

INTRODUCTION

Primary breast lymphoma (PBL) is a rare form of extranodal lymphoma, defined by the presence of a primary lesion within the breast with or without regional nodal involvement, but no other extra-mammary sites of involvement [1]. PBL was described for the first time in 1959 by Dobrotina et al. [2,3]. It accounts for about 0.04%–0.74% of all malignant breast tumors, and 0.7% of extranodal non-Hodgkin's lymphomas (NHLs) [4]. Domchek et al. [5] showed in their report that PBL was only 0.05%–0.53% of all malignant breast tumors, and 2.2% of primary extranodal lymphomas. PBL is mainly found in female patients, accounting for 95%–100% of all the PBL patients [6]. It is very rare in men and only a few cases have been reported in the literature so far. [6,7]. The age distribution of the PBL patients ranges widely from 17 to 95 years. In western countries, the age of patients varies from 55 to 62 years [8,9]. PBL is commonly found in only one breast, i.e. the right breast, and predominantly in its upper quadrant. There are also cases with bilateral breast lymphomas [10], which account for 1%–14% of all PBL [11]. PBL has been divided into 2 groups [12]: unilateral (the onset age of PBL is the same as that of breast cancer) and bilateral (mostly occur in the period of childbearing, pregnancy, or lactation). PBL is defined as stage IE and IIE non-Hodgkin's lymphoma (NHL). The staging of bilateral tumors is controversial [13]. This rare situation is especially observed during pregnancy or postpartum, suggesting that tumor growth is influenced by hormonal stimulation.

The majority of breast malignancies are adenocarcinoma with the rare occurrence of other histologic subtypes such as soft tissue sarcoma [14]. Domchek et al reported that more than

90% of patients with breast lymphoma presented with a palpable mass [5]. The origin of PBL cells is from mucosa-associated lymphoid tissue (MALT) [15]. PBL could derive also from lymphatic tissue in the breast next to ducts and lobules, or intramammary lymph nodes [16, 17].

More than 80% of PBL are B-cell lymphomas, mostly CD20+. The most frequent histopathologic types are diffuse large B-cell lymphoma (DLBCL) which accounts for up to 50% of all PBL, follicular lymphoma (FL) – 15%, MALT lymphoma – 12.2%, Burkitt's lymphoma (BL), and Burkitt-like lymphoma – 10.3% [18]. Other histological types of PBL include marginal zone lymphoma (MZL), small lymphocytic lymphoma (SLL), and anaplastic large cell lymphoma (ALCL). Diffuse large B-cell lymphoma (DLBCL) is the most common histological type. These lymphomas are of a non-germinal center B-cell phenotype with a high proliferation index and are thought to be associated with a poor outcome [19]. There is a close relation between ALCL and silicone breast implants [20, 21]. The most frequent presentation of PBL is a painless mass present roughly in 61% of cases, predominantly in the external superior quadrant (48%).

Other symptoms and signs are the following: local pain – 12%, local inflammation – 11%, palpable lymph nodes – 25%, incidental mammography finding – 12% [22]. Mammographic findings are nonspecific. Most of the lesions are oval-shaped (71%) and high-density (90.9%) masses. The masses revealed by ultrasound examination are single (75%), circumscribed (50%), microlobulated (37.5%), and oval (50%). The ultrasound finding of the mass is generally hypoechoic (87%). No masses have spiculated margins or calcifications [23]. The usual methods for diagnosis of breast and axillary lymph nodes are fine needle aspiration, core biopsy, and excisional biopsy. However, histological immunohistochemistry and, sometimes, genetic studies are necessary for establishing the diagnosis. The initial description of clinical criteria for defining primary breast lymphoma was reported by Wiseman and Liao in 1972 [24]. They outlined the criteria for the diagnosis of primary breast lymphoma, which are: 1. having an adequate specimen for the diagnosis, 2. having mammary tissues and lymphomatous infiltrates close to each other, 3. showing no evidence of concurrent widespread disease, and 4. having had no prior diagnosis of extramammary lymphoma. WHO classifies malignant lymphomas of the breast as diffuse large B-cell lymphoma, Burkitt lymphoma, extranodal marginal-zone-B-cell lymphoma of MALT type, and follicular type [25].

CASE PRESENTATION

A 40-year-old female was referred to the University Clinic of Hematology, "Ss. Cyril and Methodius" University, Skopje, Republic of Macedonia, with complaints of painless progressive swelling in entire right breast discovered one month before hospitalization. Her medical history was unremarkable and the review of symptoms was negative for night sweats, weight loss, or fever. The woman had no history of lymphoma. She had a positive family history of malignant diseases. Her mother had uterine carcinoma.

The involved breast appeared diffusely swollen on the inspection with propagation to the right axilla. Almost the whole right breast was purple-reddish. The overlying skin was shiny and few prominent vessels could be seen over it. The nipple was not retracted, with yellowish secretion. The skin around the nipple was with crusts. On palpation, the entire right breast was firm and not tender. The contralateral breast was normal. An enlarged right axillary node was also seen and edema of the right arm. CT scan of the chest showed thrombosis of the axillary and brachial veins and a massive tumor in the right breast with the involvement of the right upper anterior pleura. CT scan of the abdomen did not demonstrate any mass lesion.

A core biopsy of the enlarged axillary node was made. The histopathological finding showed lymphoproliferative disease. After that, a core biopsy of the right breast was performed. The histopathological finding was malignant lymphoma – aggressive B – cell type.

Both diagnoses were made in the Institute of Pathology, "Ss. Cyril and Methodius" University, Skopje, Republic of Macedonia
Microscopically there was tissue with predominantly lymphoid differentiation of polymorphic type. In part of the tissue dominated large lymphoid cells with blastic morphology and mitoses, surrounded with small lymphocytes.

On immunohistochemistry, there was a high proliferative rate, in parts with lymphoid cells CD20+, CD10+, and Kappa light chains +.
The rest of the lymphoid population was from T cell origin: CD3+, Bcl2+, while BCL1, Lambda, and CD25 were negative such as markers for epithelial differentiation (E-cadherin, CKWS, and CK7). According to the leukocyte common antigen-positive and CD20-positive immunohistochemistry, the pathological diagnosis was Non-Hodgkin's lymphoma (aggressive B-cell type).

A complete blood count test showed platelet elevation – 579x109/L. Biochemical analyses showed elevation of Lactate dehydrogenase (LDH) – 428 U/L, which is usual in lymphomas. Echocardiography showed EF>65%. Viral markers HBs Ag, Anti-HBs, Anti-HBc, and Anti-HCV were negative.

The patient was planned to receive 8 courses of R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone plus rituximab) chemotherapy (CTx). But, she received 8 cycles of CHOP protocol. (Rituximab was ceased due to a very severe allergic reaction and was not given afterward). Also, she started with low-dose heparin and continued with oral anticoagulant therapy for the treatment of right arm thrombosis. After finishing with chemotherapy she was in remission with almost complete restitution of the breast and arm.

At a follow-up period of 5 years, the patient has survived with no evidence of disease. Medical history, physical examination, and blood tests were made every 3 months for the first two years, then every six months for the following three years. CT scan is not mandatory, only if needed according to NCCN recommendations. No morbidities occurred in this patient during the follow-up period.

DISCUSSION

The behavior of primary lymphoma of the breast is thought to be similar to that of lymphomas of the same histological types and stages arising at other sites.
The lifetime risk of developing non-Hodgkin lymphoma for a woman is approximately 1.8%. The rarity of this cancer is because the breast contains less lymphoid tissue than other organs, such as the intestines and lungs, where primary lymphomas are more common [26].

The CTx, especially with an anthracycline-based regimen that showed positive effects on overall survival is the main treatment component of PBL patients with DLBCL [27]. The addition of rituximab to the CHOP regimen was also evaluated by several studies [28, 29]. Avilés et al. [28] reported that there were no CNS relapses in patients treated with rituximab, whereas a relapse rate of 11% occurred in patients treated without rituximab. Outcomes in patients of these studies are comparable with our patients. We should note that allergic reactions to monoclonal antibodies (like Rituximab) are very frequent and if intense, therapy should be ceased.

The ultimate goal of radiotherapy (RT) is to consolidate the primary lesions after CTx. The CTx followed by RT has shown a significantly improved survival benefit compared to CTx or RT alone [27, 29]. Studies published lately have revealed that the treatment of aggressive B-cell lymphomas should be made with chemotherapy alone or combined with radiotherapy.

The most effective combination reported in the literature is radiotherapy and 3 to 10 cycles of treatment with CHOP [30].

According to the NCCN Guidelines for Diffuse Large B cell lymphomas Stage I, II small or large, first-line therapy is R-CHOP protocol for 6 cycles ± Involved Site Radiation Therapy (ISRT).

Jennings et al compiled a database of patient information from all published primary breast lymphoma reports during the previous 33 years where individual patient treatment and outcome data were included in the publication. A total of 465 patients were identified that met these criteria. This study found that mastectomy offered no benefit in the treatment of primary breast lymphoma. Treatment that included radiation therapy in stage I patients (node-negative) showed benefit in both survival (P = 0.002) and recurrence rates. Treatment that included chemotherapy in stage II patients (node-positive) showed benefit in both survivals (P = 0.001) and recurrence rates. Data regarding combined radiation and chemotherapy were equivocal due to small sample sizes, but together they likely had the same survival and recurrence advantages as single treatment options inappropriate node status groups. Comparison of survival characteristics that do not account for node status may lead to erroneous conclusions. Histologic tumor grade predicted survival [18].

Gholam et al. [31] analyzed 34 cases of PBL over 25 years. They observed a high incidence of CNS relapse in this group of localized extranodal lymphoma.

CONCLUSION

This strongly suggests that CNS prophylaxis should be associated with systemic chemotherapy in localized PBL.

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REVIEW ARTICLE

PROMISING TREATMENT FOR BLINDNESS – BIONIC DEVICES

Emilija Gjosevska Dashtevska[1], Ivanova Cekov Maja[2], Trpevska Shekerinov Natasha[1]
[1]University Clinic for Eye Diseases, Faculty of Medicine,
University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2] University Clinic for Eye Diseases, Skopje, Republic of North Macedonia

ABSTRACT

More than 39 million people worldwide suffer from total blindness. Neurobionics is a new science that connects electrical devices and the nervous system and is promising to solve this global health problem. Currently, there are a number of research groups, internationally, that are engaged in the development of visual prostheses. These teams work on almost all target regions of the visual pathway, from the cornea to the geniculate nucleus.

In this review paper we have shown the most important insights from the literature. We searched the database of medical publications, PubMed Database. We entered the following keywords: neurobionics, bionic eye, retinal prostheses, electrical stimulation, Argus II. We have received 75 data for published articles in the past 5 years. We also surveyed LWW where 31 items were available from 2016. However, research has shown that Google search and Google Scholar offer us many more articles than PubMed.

Bionic devices: There are nearly 20 research teams working on implants with different locations on the visual pathway. These groups are based in Australia, the United States, Germany, France, and Japan and report advances in retinal visible prostheses, both experimental and clinical. The most famous of these implants is Argus II, which in 2013 was approved for human use by the FDA. The implantation of Argus II is not very complicated, the implant is durable and studies so far have shown that it gives a small number of complications. The principle of retinal implants is to replace photoreceptor function in patients with degeneration of the outer layers of the retina. The results so far in this field show that the plasticity of the brain is greater than initially assumed and electrical stimulation can restore visual functions. Proper selection of candidates for this implant surgery is required, as well as good postoperative rehabilitation.

Bionic devices are revolutionary and promising for one of the most challenging public health problems in the world, blindness. These devices will significantly improve the quality of life of millions of people.

Keywords: neurobionics, bionic eye, retinal prostheses, electrical stimulation, Argus II

INTRODUCTION

More than 39 million people worldwide suffer from total blindness. Neurobionics is a new science that promises to do something about this global problem. Potential solutions are bionic devices that use electrical stimulation of the visual pathways.

Retinal stimulation can restore vision in patients with retinitis pigmentosa, but ganglion cell loss can be a problem. The optic nerve, the lateral geniculate nucleus as well as the visual cortex are also targets for stimulation, and a cortical device capable of working with hundreds of electrodes is currently being developed. Neurobionics is the science that connects electrical devices and the nervous system. This connection is used to make single or multiple stimuli to the nervous system.

This connection has been demonstrated through the production of prostheses, which stimulate the motor cortex and allow movements of artificial organs, such as the movement of

an artificial hand or arm. Work is also being done on improving sensory feedback, such as mechanical sensations. This has already been demonstrated in macaques through microstimulation of the somatosensory cortex [1-3].

Analyzes examining the global increase in blindness and visual impairment show that the total number of visually impaired people in 2010 was estimated at 191 million and 285 million globally, and the number of legally blind people is estimated at 39 million. Recent studies have found that the most common causes of blindness are cataracts (33%), uncorrected refractive error (21%), and macular degeneration (7%). There is considerable regional variation in these figures; in high-income regions, such as Western Europe, Australia, Asia-Pacific, and North America, the most common causes are macular degeneration (16.1–19.5%), uncorrected refractive error (14–14.1%), and cataracts (12.7–14.5%), followed by glaucoma and diabetic retinopathy, consisting of an additional 14.5–16% [4].

Retinal degenerations and dystrophies are a large group of inherited eye disorders that result in permanent vision loss. Retinitis pigmentosa, a heterogeneous inherited degenerative disease, is characterized by loss of rods and impaired cone function. It usually occurs between the ages of 40 and 50 [5,6].

So far, these therapies have been investigated: gene replacement therapy, neurotrophic factors to stimulate the growth of photoreceptors, retinal cell replacement, and prostheses, which collect light and transmit electrical signals through neurons to the brain. Several of them are being tested on patients and have shown safety and efficacy of treatment. A combination of gene replacement and cell replacement may be of optimal benefit [6].

MATERIAL AND METHODS

In this review paper, we have the most important insights from the literature. We searched the database of medical publications, PubMed Database. We entered the following keywords: neurobionics, bionic eye, retinal prosthesis, electrical stimulation, Argus II. We have received 75 data for published articles in the past 5 years. We also surveyed LWW where 31 items were available from 2016. However, research has shown that Google search and Google Scholar offer us many more articles than PubMed.

Bionic devices

Prostheses for the treatment of patients with retinitis pigmentosa are miniature arrays of electrodes that receive light and emit electrical signals that are transmitted through nerve cells to the inner retinal layers. These include retinal ganglion cells, bipolar, horizontal, and amacrine cells, as well as the retinal nerve fiber layer. Nerve fibers are relatively intact in patients with dystrophy [6]. ASR-artificial silicon retina microchip is one such prosthesis, which consists of a series of semiconductors with micro-photodiode, whose diameter is 2 mm and thickness of 25 microns. It is designed for subretinal space implantation. 5000 micro-photodiodes are stimulated by natural light [6].

Light stimulation occurs when the electrical charge from this microchip changes the membrane potentials of the neurons to which it connects. ASR microchips showed a retinal and possible cortical response in animals. A pilot clinical trial of six patients with retinitis pigmentosa was performed to test the safety and efficacy of the chip. Patients in this study did not experience any side effects such as inflammation, discomfort or toxicity and showed improvement in vision. Vision improvement was in terms of: visual acuity, color perception and field of view. They also reported an improvement in the general quality of life. These results were evident from a few weeks after implantation to a year after [6, 7]

These improvements can be said to be partly due to the general neurotrophic effect on the retina by electrical stimulation.

Argus I is a 16-electrode prosthesis and was the first permanently implanted prosthesis. Since 2002, two phases of clinical trials for this prosthesis have been started in six subjects. A newer version is Argus II, a 60-electrode implant also called a bionic eye. It has platinum-coated electrodes with a diameter of 200 microns. Contains: a device for receiving and transmitting, which is placed on the sclera, and an electrode array (of 60 electrodes), which is surgically placed on the surface of the retina [6].

The Argus II system also has a camera and transmitter, which are mounted in glasses and a video processor and battery, which can be worn on the belt or as a shoulder strap.

Argus II was tested in a multicenter examination of 30 patients with external retinal degeneration, in the final stage, retinitis pigmentosa for example.

There were improvements in the vision of the respondents who did not even have a perception of light at the beginning. Respondents had much better orientation and mobility in space, as well as higher contrast vision.

Side effects from implants have been reported: endophthalmitis, conjunctival erosion, and conjunctival dehiscence. However, the safety profile has been shown to be more acceptable than other intraocular devices and has been approved by the FDA on the basis of safety and potential benefit. It is also commercially available [6, 7].

Since the 1950s, vision researchers have been working ambitiously to restore the functional level of vision in blind people through electrical stimulation of the visual pathways. Groups based in Australia, USA, Germany, France and Japan report on progress in the development of retinal visible prosthesis, both in experimental and clinical domains. Two retinal visual prostheses have recently received regulatory approval for clinical use [8].

No matter what the structure of the visual pathway is targeted, light perceptions, called phosphons, are expected in response to electrical stimulation, which, if converted to appropriate numbers, can be used to provide blind people with useful information about their environment.

This is fundamental for those who are currently making efforts to develop visible prostheses [9].

Argus II (Second Sight Medical Products, Inc., Silmar, California, USA) and Alpha IMS (Retina Implant AG, Reutlingen, Germany) received regulatory approval for clinical use in the European Union in 2011 and 2013, respectively. The FDA also approved Argus II for use in 2013 under the „Humanitarian device exemption program“. Clinical studies of these devices show improvements in visual acuity and / or the ability to perform activities of daily living [8, 9].

In terms of cortical prostheses, 4 groups are known so far that are working on the first trials and have clinical trials for them, namely Australia, USA, Canada and Spain.

Two of the groups report the development of bionic electrode-based vision devices that are implanted in or around the optic nerve. The other two describe the implantation of electrodes in the lateral geniculate nucleus of inhuman primates and rats. These groups work to develop a visual prosthesis based on the stimulation of these structures [10].

Retinal implants

There is a certain similarity between the architecture of the electrical visible prostheses, whether they are retinal or cortical. A common feature is the capture of digital images with a camera, usually embedded in glasses.

Before generating the electrical impulse and transmitting it through the electrode, the images are processed, the objects are highlighted, as well as the floor surfaces or the printed text or the contrast is improved [10].

The data from the image are transmitted to a combined series of photodiodes / electrodes, with a miniature mounted infrared projector with glasses, where the light is converted into electricity, which serves to power the electrodes [11, 12].

In any case, the principle of all retinal implants is to replace photoreceptor function in patients with degeneration of the outer layers of the retina. Two fundamentally different approaches have been developed in this area: 1. Epiretinal implantation of electrode strings that connect to retinal ganglion cells, and 2. Subretinal implantation of microchips under the transparent retina to replace degenerated photoreceptors [12].

These implants that are used today are:

1. Epiretinal – Argus II – which is approved by FDA and CE. It was approved by the European Union in March 2001, and by the FDA on February 14, 2013, after more than two decades of research and development. The implantation of these implants is simple, these implants are permanent and studies have shown that they give a small number of postoperative complications.

2. Subretinal – Alpha IME – Their implantation is difficult, it must be in cooperation with a neurosurgeon, they are not permanent, ie they need to be removed after 5 years [5, 9, 12].

Principle of operation

The idea of neuroprostheses is to artificially replace the function of the damaged parts of the visual pathway. The micro-electrical stimulation is transmitted through a series of small microelectrodes to obtain light perception. Electrical stimulation of these remaining functional visible neuronal elements causes a subjective sensation in the form of discrete points of light (called "phosphenes") [9].

Thus, by delivering more such light stimuli, a geometric visual figure and perception of a visual image can be created. The image is obtained with a camera or created in the optics of the eye itself.

This is a simplistic explanation of the principle. Of course there are attributes that are difficult to capture, such as color, movement and shape, but the idea of these prostheses is to convey at least one of the essential components of sight [12, 13].

Methods of implantation

Current research focuses mainly on retinal implants (epiretinal, subretinal, suprachoroidal), and cortical implants.

A series of microelectrodes are placed in the subretinal space in order to act as a photo-conductor and are in a more natural position, as an artificial layer of photoreceptor, but there are surgical and technical challenges. As the name implies, they can be placed also epiretinal or supra choroidal (over choroid). In all cases, a pars plana vitrectomy and an experienced surgeon are required.

Cortical implants are placed in the primary visual cortex and they are examined as an option for patients who have completely lost their sight for various reasons [14].

- Subretinal implantation

The structure of this prosthesis is made of easily sensitive microphotodiodes. The micro-diode array is inserted under the retina, between the retinal pigment epithelium (RPE) and the degenerate layer of photoreceptors. The implant can be inserted into the subretinal space either externally through a scleral incision or internally through vitrectomy and retinotomy [14].

The array has thousands of small, light-sensitive units. The units contain: diode, amplifier and microelectrode components, all of which are embedded in a silicon matrix. They are made of titanium nitrite and gold.

The sequence processes and amplifies the signal and stimulates nerve cells. The process of photo-voltage that occurs when a crystal is exposed to natural light creates electricity that directly and precisely stimulates degenerated photoreceptors and the bipolar cells to which it

connects. A small threshold is sufficient to generate a visual response. However, natural light can not provide enough energy for the whole array, so the power supply must be from external electronics [6, 14, 15].

- Epiretinal prosthesis Argus II

The Argus II system (Figure 1) uses an epiretinal approach, in which a series of microelectrodes are implanted on the inner surface of the retina (the macula). In this way the implant is very close to the layer of nerve fibers [14, 15, 16]. In this way electrical stimulation affects the partially functional inner retinal nerve layer, ganglion cells (RGC) and / or bipolar cells (BC). The action potential formed by the nerve layer travels to the visual cortex, through the optic nerve and optic tract, and generates a basic visual perception called phosphene [14]. External aids are: glasses, video processing unit (VPU) and cable. The video portable unit can be attached to a belt or carried in a pocket. When the patient puts on glasses and turns on the system, the video microcamera receives images while the patient scans through head movements and they are sent to the VPU via cable. The image processor reduces the resolution of the images and converts them into real-time electrical signals to produce digital stimuli [14, 15].

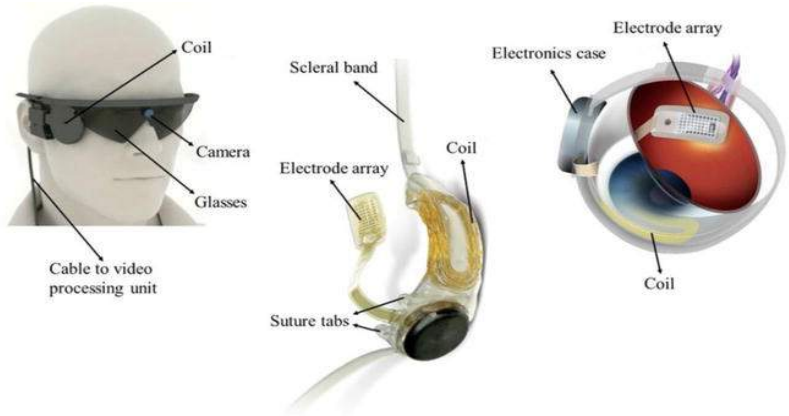

Fig. 1. Parts of the Argus II retinal prosthesis

(Edward Bloch and Lyndon da Cruz (April 3rd 2019). The Argus II Retinal Prosthesis System, Prosthesis, Ramana Vinjamuri, IntechOpen) [16]

Available from: https://www.intechopen.com/books/prosthesis/the-argus-ii-retinal-prosthesis-system

Indications and contraindications

Preoperatively in all patients should be done: detailed clinical examination, OCT, ultrasound and biometrics. *Indications* for implantation are: age over 25 years, minimum light projection, if there is no perception it is necessary to do a flash light test and VEP, to have visual memory, and axial length of the bulge between 20.5 and 26 mm [5]. *Contraindications* are: optic nerve diseases, central vascular occlusions, strabismus with a large angle of deviation, amblyopia, trauma, retinal ablation, corneal diseases (which prevent visualization of the posterior segment), cataracts (which should be treated in advance), predisposition to rubbing the eyes, assessment of the patient's general health and the patient's ability to tolerate general anesthesia of about 4 hours.

Patients with implanted metal or similar devices in the head, including cochlear implants, are not candidates as they will interfere with the functionality of the Argus II device [5].

The surgical technique is similar to buckling, for implantation of the outer part of the sclera, and the implant is inserted preretinally through the PPV. [17, 18, 19]

It is implanted in the weaker eye, and if both eyes are equal, the patient decides which eye will be operated. Electrical stimuli can restore visual functions. Obviously the plasticity of the brain is greater than previously thought [5].

Postoperative follow-up is recommeded on the first day, after one and two weeks, the first, second and sixth months and then annually. One week after the operation, the implant is connected to a glasses system, the electrodes are tested and the stimulus limit is defined. The device must be programmed before the camera can be turned on. The electrical stimuli, ie the level of stimulations from the device are adjusted during several sessions. Then, in fact, the patient begins to learn how to use this device and gets a new "sight", begins to recognize objects and move around.

Visual rehabilitation is with a coach assisted 50 postoperative sessions. This rehabilitation is necessary to the patient's independence, because they often need encouragement and motivation [5].

DISSCUSION

Loss of vision can have a devastating negative impact on a person's quality of life. Technological advances have inspired numerous multidisciplinary groups around the world to develop neuro-prosthetic devices that could potentially provide useful vision and improve the quality of life of blind people [13].

This goal, although courageous, faces enormous challenges. But there is reason to be optimistic and to believe that the goal can be achieved. It is also important to understand that the rehabilitation of the blind is a very complex problem, which requires long and close cooperation between scientists, engineers, clinicians, educators and rehabilitation experts [13].

In one study, eleven prosthetic devices were analyzed, according to certain inclusion criteria, one of which is the target stimulation location (part of the visual stimulus target path). The study said that four prosthetic researchers had recently completed human trials, three had conducted multi- or single-center trials on humans, and three were in the pre-clinical animal testing phase.

The same study states that the FDA has approved Argus II (FDA 2013, CE 2011) and Alpha-IMS (CE 2013). Both devices tested the best corrected visual acuity with the Landolt-C test. The same study mentions that new approaches to prosthetics will be introduced, including: alternating magnetic fields, low-intensity ultrasound, optogenetics, the implementation of ion gradients on nerve cell membranes, or the influence of neurotransmitter levels [20].

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The system has so far been tested in patients with pigmented retinopathy and choroidemia. It is also seen as an encouraging therapeutic option in AMD (geographical atrophy or dry form). Examinations so far indicate that there are no significant postoperative complications and that there is a very good electrical threshold [5].

Electronic prostheses serve to replace the function of photoreceptor cells, so that secondary retinal neurons receive a signal that simulates an external visual image. The device has a miniature video camera built into the glasses, which captures images and transmits them to a microprocessor that converts the data into an electronic signal [20]. This signal is further

transmitted to a series of electrodes placed on the surface of the retina that transmit the signal to the functional secondary neurons. These neurons (ganglion, bipolar cells) begin to process the signal and transmit it through the optic nerve to the brain for final integration into the visual image [14, 20, 21].

Electrical prosthetic devices appear to offer hope of replacing the function of degenerate or dead photoreceptor neurons. Devices with new, improved design and increased number of electrodes can enable long-term restoration of functional vision in patients. This is made possible by improving object recognition, improving mobility, enabling a degree of independent living and of course improving the overall quality of life [21].

The Argus II implant, also called the bionic eye, is a commercially available visual prosthesis developed by Second Sight Medical Products. It is implanted in over 125 patients with external retinal dystrophies, such as retinitis pigmentosa. The system has received regulatory approval in both the United States and Europe and aims to restore vision by electrically stimulating nerve cells in the inner retina [22].

Teams are working on improving the system in order to simplify the operation of this system. They are also working on improving: digital HD image, software and camera, designing different chips for RPE and AMD, wider field of view, color vision and letter reading [5].

Based on the artificial bionic eye and the mechanism of foveolar vision, researchers have worked to improve peripheral vision by increasing its width and resolution [23].

A study conducted in China at a laboratory for biomimetic robots and systems presented a hybrid bionic image sensor, and also explained the mechanism and the advantages and disadvantages [23].

Artificial eyes are said to have a wider field of view, infinite depth and high resolution, low aberrations, good motion tracking and can be used in motion sensors, machine eyes, etc. There are two types: planar artificial compound eyes (PACE) and curved artificial compound eyes (CACE). Foveolar vision is inspired by the human eye [23].

In order to achieve a large expansion of the foveolar vision and to obtain high resolution images at the same time, HBIS (hybrid bionic image sensor) is proposed which combines several approaches (CACE, PACE and foveolar vision) [23, 24].

The simulations showed that using a series of cameras, the foveolar vision could be expanded 2.9 times [23, 24].

Improvements to artificial eyes are also made using new lenses. Thus, the Optical Society of America proposes a method for self-adapting the focus of the artificial eye and the hybrid eye with a series of micro-lenses (NUMLA) to reduce defocused aberrations using a liquid lens. The models are derived and validated through simulations. With this method the artificial eye can independently adjust the focal length according to the distance of the object and the distance of the image [25].

CONCLUSION

Bionic devices and their improvement are promising for patients who have lost their sight. Neurobionics is definitely a science that will evolve. Of the bionic devices researched so far, only a few have been approved by the Food and Drug Administration (FDA), and one of them is Argus 2, which is also available for commercial use and is most commonly used in retinal degenerations, especially retinitis pigmentosa.

Scientists are even working on improving these implants in order to obtain better sharpness, higher image resolution, wider field of view, so that artificial vision would be as close as possible to natural vision.

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REVIEW ARTICLE

ODONTOGENIC ORBITAL CELLULITIS–ETIOLOGY, DIAGNOSIS, TREATMENT AND COMPLICATIONS

Emilija Gjosevska Dashtevska[1], Bajraktarova Misevska Cvetanka[2], Ivanova Cekov Maja[3], Trpevska Shekerinov Natasha[1], Shekerinov Dejan[4]
[1]University Clinic for Eye Diseases, Faculty of Medicine, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[2]Faculty of Dentistry, University Ss. Cyril and Methodius in Skopje, Skopje, Republic of North Macedonia
[3]University Clinic for Eye Diseases, Skopje, Republic of North Macedonia
[4]City General Hospital „8th September", Skopje, Republic of North Macedonia

ABSTRACT

Dental infections as a cause of ocular diseases have been known for a long time. The use of antibiotics has reduced their frequency.

Today, dental foci are most commonly thought to cause orbital cellulitis and orbital abscess, cavernous sinus thrombosis, episcleritis, and less commonly, uveitis.

Early diagnosis and focus detection are extremely important. Computed tomography is the gold standard for diagnosis. Timely assessment for treatment is necessary to prevent possible intracranial complications, as well as to preserve visual acuity.

The aim of the paper is to emphasize the importance of dental foci as a cause of orbital diseases, as well as the need for timely diagnosis and treatment.

In this review paper we showed the most important observations from the literature, as well as our observations from the research in this direction.

Odontogenic orbital cellulites are rare but devastating infections that can lead to vision loss and neurological complications. Collaboration between ophthalmologists and dentists / maxillofacial surgeons as well as radiologists is necessary in order to timely detect the infectious focus and start therapy. In some cases, timely assessment for surgical treatment is required, which is which is of great importance for preserving patient's life and vitality".

Keywords: dental foci, orbital cellulitis, odontogenic infections, computed tomography, antibiotic treatment, drainage

INTRODUCTION

Dental infections have been recognized as the cause of orbital cellulitis since the time of Hippocrates, but over the years and the frequent use of antibiotics, they have become less common. The correlation between ocular and dental diseases is estimated to be 3 to 5%, including allergic pathogenesis (adnexal ocular diseases).

The most common causes are: radicular focal diseases, periodontitis after prosthetic preparation, caries and chronic dental maxillary osteitis [1-3].

Dental infections can cause preseptal cellulitis, orbital cellulitis, orbital abscess, subperiosteal abscess, and cavernous sinus thrombosis. Acute odontogenic infections can spread through tissue or venous canals. In the past, these infectious foci were considered the leading cause of uveitis.

Infection of the maxillary incisors or canines can spread locally through the soft tissue structures of the maxilla, resulting in swelling of the upper lip, canine fossa, and periorbital tissue. Retrograde expansion into orbit, due to the absence of valves, may occur through the anterior facial or angular ophthalmic veins [1, 2]. The interval from tooth extraction to the onset of orbital symptoms varies from 2 hours to 13 days [3, 4].

The roots of the maxillary premolars and molars are located near the maxillary sinus. Sinusitis may occur due the perforation of the floor of the maxillary sinus during the extraction of infected teeth. The infectious agent further spreads into orbit [1, 2].

Infection of the teeth in the frontal region of the maxilla may result in a subperiosteal abscess. On the other hand, infection of the teeth in the posterior region of the upper jaw, usually the third molar, may result in the infection spreading to the pterygopalatal and infratemporal fossa, and is further transmitted into the orbit through the lower orbital fissure. [1, 2].

Predisposing factors are upper respiratory infection, heroin addiction and nephrotic syndrome. Patients have increased leucocytes and radiological evidence of ipsilateral paranasal sinus infection. Most patients have a fever, however meningitis is rare [4].

Microorganisms from odontogenic infection can enter the orbit locally through the soft tissue structures, hematogenous spread or through the paranasal sinuses [3, 4]. Fortunately, due to the use of antibiotics, this is an increasingly rare occurrence, and on the other hand, the general health condition of the patient is very important.

In addition to orbital cellulitis, cases of fibrinous pan uveitis because of abscesses of the teeth that support a fixed prosthetic structure (bridge) have been described [5]. Cases of cavernous sinus thrombosis have also been described [6].

In the 20th century, there was a tendency for uveitis to be attributed to focal sepsis, usually if no other cause could be found or if uveitis has cured after sepsis treatment. Infection commonly spread through the sinuses , blood, focal irritation, or may occur as allergenic reaction to the bacterial products [7].

Systemic immune diseases are considered to be the leading cause of scleritis, although the dental focus may also provoke the inflammation. A case of a patient with periodontitis and diffuse scleritis has been described in the literature. The reason was considered to be the increased systemic inflammation associated with periodontitis, which can initiate scleritis. Periodontitis is a chronic inflammatory disease with constantly increased CRPs and interleukins (which have decreased after tooth extraction and proper oral hygiene).

If the cause of scleritis cannot be found and the patient does not have a systemic or immune mediated disease, such as rheumatoid arthritis or Wegener, a possible cause of this inflammation may be periodontal inflammation [8].

MATERIAL AND METHODS

We searched the largest database for medical publications Medline database, by entering the keywords: dental foci, orbital cellulitis, odontogenic infections, computed tomography, antibiotic treatment and drainage. We have received data for 99 published articles in thepast 5 years, however, the research shows us that Google search and Google Scholar have to offer us as much more articles than Medline.

In this review paper we showed the most important observations from the literature, as well as our observations from the research in this direction.

Odontogenic orbital cellulitis and abscess

Orbital cellulitis and dental abscess usually occur in pediatric patients as a complication of acute sinusitis. If left untreated, it can lead to vision loss and life-threatening intracranial complications [9].

Orbital complications according to Chandler are urgent care medical conditions. Early diagnosis and treatment are required. The stage and origin may be evaluated by rhinoendoscopy, ophthalmic examination, and CT of the orbit and paranasal sinuses. Periorbital cellulitis and early stage orbital cellulitis can be successfully treated conservatively with intravenous antibiotics. In cases without improvement for a period of 24-48 hours, surgical

treatment is indicated. Most patients are young people, in their 3-4th decade of life. The most common cause is ethmoiditis, followed by maxillary and frontal sinusitis, and a common finding is polysinusitis [10].

Odontogenic orbital abscess is a rare but well-known complication of sinusitis and infections that spread from dental apical foci.

In the case described in the paper of Procacciet al., 2016, it is stated thatmost patients have a positive history of a dental infection. In their case it is about a periapical infection of the maxillary left second premolar, which has spread to the maxillary sinus [11].

Etiology

Orbital abscess is a rare disease that occurs as a complication of paranasal, ethmoid, frontal, or maxillary sinus infection. Maxillary sinusitis can be caused by a dental infection, and the maxillary teeth are the most common source of odontogenic infections, although the literature has reported cases of spreading infection from mandibular teeth [12]. Infections occur by direct spread through the pterygopalatinal and infratemporal fossa, through the inferior orbital fissure.

Odontogenic infections usually result from infection of the maxillary premolars or molars, periapical infection, extraction of the maxillary molars, surgery of the maxillary third molars, treatment of the root canals, or infected periodontal pockets [12].

Predisposing factors are contagious foci, such as paranasal sinusitis, otitis media, or dental abscess. The etiologic agent is usually polymicrobial (>50-60%). Last can be detected if brain bacteria are cultured on an anaerobic culture medium [13].

Sinus infections can result in ocular infections, and in later stages, infection of the brain parenchyma [5].

Iatrogenic causes of sinusitis and cellulitis have been described in misplaced dental implant, communication between the sinus and maxillary and alveolar processes after extraction, as well as third molar root pushed into the sinus, have been reported as iatrogenic causes of sinusitis and cellulitis [14].

Orbital cellulitis and cavernous sinus thrombosis as consecutive diseases occurred in a 19-year-old boy with an apical abscess [6].

Orbital abscess caused by a direct spread via the maxillary sinus of a dento-alveolar abscess of the maxillary first premolar, resulted in an eye loss in a HIV seropositive woman [15].

The most common causes of odontogenic infections after tooth extraction are: antihemolytic (gamma type) streptococcus, viridians (gamma) and as a rarity - hemolytic (beta) streptococcus, staphylococci, gram-negative organisms, and anaerobic organisms [13, 16].

Clinical manifestations

Periorbital edema, proptosis, chemosis, facial edema, ocular pain, ophthalmoplegia, diplopia, glaucoma and initial visual symptoms are the most common clinical manifestations. Staphylococcus aureus and epidermidis are isolated in half of the cases [10, 11].

A case of a 30-year-old man with acute proptosis of the left eyeball, pain, and decreased vision has been reported, caused bya periapical process of the left maxillary second premolar [17].

In a case of 22-year-old female, sinusitis with a history of previous treatment of a second molar, complicated by orbital cellulitis, epidural empyema, and intracerebral abscess has been reported; ipsilateral exophthalmos, limited motility in all directions, conjunctival congestion, and chemosis were observed, while visual acuity was normal [13].

Sometimes the only symptom of orbital cellulitis may be eyelid swelling [18].

A case report presents periapical infection as a cause of unilateral maxillary sinusitis and ipsilateral cellulitis of the lower eyelid [18].

A periapical process of the maxillary left second premolar was the reason for an acute proptosis of the left eyeball, pain, and decreased vision in a 30-year-old man.

An abscess in the left orbit was confirmed by CT. Intravenous therapy has not been shown to be effective, so surgical abscess drainage and dental surgery were performed [17].

Pan tomograms and intraoral X-rays are used to diagnose the presence of a periapical radiolucency, radix cysts, deep caries, periodontal pockets and periodontal disease [14].

Diagnosis, treatment and complications

Contrast computed tomography is the optimal diagnostic method for orbital inflammation. [9].

A case of a 61-year-old man having a dental bridge of the right maxillary canine, hypertensive fibrinous pan- uveitis (endogenous endophthalmitis), increased CRP and antistreptolytic antibodies, and abscess of the tooth carrier of the fixed prosthesis, have been reported. Treatment procedures covered: a removal of the bridge, systemic and intravitreal application of antibiotics, followed by vitrectomy and phacoemulsification. No recurrent infections were reported after the treatment [5].

In a case of pansinusitis complicated by orbital cellulitis, epidural empyema and intracerebral abscess of fronto-basal region drainage of the collection was performed, through middle maxillary anthropostomy and anterior-posterior ethmoidectomy, ablation of the brain abscess, and postoperatively prolonged antibiotic therapy [13].

As a required treatment, surgery, abscess drainage and revision of the dental lesion and maxillary sinus are described [11].

When ordering antibiotics, the third generation of cephalosporins and metronidazole proved to be effective, due to the broad spectrum of activity and due to the good penetration through the blood-brain barrier and into the abscess. If osteomyelitis is present, prolonged intravenous therapy (6 to 8 weeks) is also recommended [13].

Odontogenic orbital cellulites are rare but devastating infections that can lead to vision loss due to progressive tension orbit syndrome and life-threatening intracranial complications [9].

DISCUSSION

In the 20th century, there was a tendency to think that focal sepsis was the cause of uveitis, usually if no other cause was found or if the clinical picture of uveitis was improved after sepsis was cured [7].

It is believed that the mechanism of transmission is direct through the sinuses, through focal irritation, through the bloodstream directly into the uveal tract, or as an allergic reaction to bacterial products [7].

In some animal studies, iritis was caused by injecting streptococcus from a dental focus in the uveal tract. There were opposing opinions among authors, some believing that only close foci of pulp or periapical tissue could be responsible for ocular manifestations, while periodontal infections were not considered significant [7].

From researches it is estimated that the correlation between uveitis and dental disease is 1.2 -1.8% in hospitalized patients, and according to other authors it is 3-5%. Dental diseases associated with uveitis are radicular focal infections, periodontitis, and chronic dental maxillary osteitis [3].

Sinusitis, although a common problem, can be complicated by intracranial or orbital infection. Intracranial abscess is also rare, as a serious neurological disease with high mortality [13].

According to Chandler, the orbital inflammation is classified as preseptal or septal. All cases of pre-septic inflammation are treated with oral antibiotics, while post-septic ones require intravenous antibiotics, and surgical treatment of some abscesses.

Children under 9 years of age respond better to treatment than older patients, and treatment of choice in children is drug therapy followed by surgery. Medial subperiosteal abscesses, which do not respond to drug treatment, are usually drained endoscopically, while lateral and intraconal abscesses require an open method [9].

Two cases of orbital involvement have been described. In the first case a subperiosteal abscess was formed in the posterior lateral orbit from infection of maxillary premolar and molar. The infection was spread through infratemporal and pterygopalatine fossa to the lower orbital fissure. While in the second case, the maxillary tooth infection with consequent pansinusitis and unilateral orbital cellulitis, was described. The importance of computed tomography for the localization of the infection and planning a surgical approach is emphasized [1].

A case of a 41-year-old man admitted for periorbital swelling who had previously been treated for periodontal abscess has been described. It has been diagnosed as odontogenic sinusitis and orbital cellulitis. Emergency sinus drainage was performed, but the next day he had a sudden loss of vision due to ischemic optic neuropathy and occlusion of the central retinal artery. This is a progressive orbital tension syndrome that can lead to blindness. The patient was treated with high doses of corticosteroids, which reduced the swelling in the orbit, but visual acuity did not improve.

Damage usually occurs due to tension orbit syndrome and deformity of the eyeball, due to severe proptosis and traction of the optic nerve (cone shape), but it may also be a direct dissemination of the infectious agent to the optic nerve [19].

In tooth abscess, the spread of infection is possible through the root canals of the tooth, through the blood and lymphatic circulation [20]. The importance of antibiotics was also emphasized [20].

Communication between ophthalmologists, radiologists and dentists is essential in the treatment of orbital diseases [14].

CONCLUSION

Orbital cellulitis, caused by dental foci, is a rare but serious complication. The most common is the direct route of transmission, through the paranasal sinuses, mostly from the ethmoid sinuses.

Ophthalmologists deal with orbital cellulitis in their practice. When dealing with an infectious process in the orbit, dental focus should be considered as the possible cause, appropriate investigations made, and the most appropriate treatment approach should be selected.

Prompt therapy is key to preserving vision. A multidisciplinary approach and cooperation between ophthalmologists, dentists, radiologists, maxillofacial surgeons and otolaryngologists is of a great importance.

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ERRATUM

Erratum to: Procalcitonin as a promising biochemical marker for early detection and treatment of sepsis in neonates at intensive care unit and oncologic patients with febrile neutropenia.

Nonkulovski Danilo[1], Tankoska Maja[1], Pandovska Bisera[1], Sofijanova Aspazija[1], Kimovska Mica[1], Bicevska-Mandzukovska Hristina[1], Voinovska Tamara[1], Martinova Kata[1], Kostadinova Lence[2]

[1]University Clinic for Children Diseases, Faculty of Medicine,
Ss. Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia
[2] University Clinic for Oncology, Faculty of Medicine,
Ss. Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia

Unfortunately, in the previously published article "Nonkulovski D, Tankoska M, Pandovska B, Sofijanova A, Kimovska M, Bicevska-Mandzukovska H, Voinovska T, Martinova K, Kostadinova L. Procalcitonin as a promising biochemical marker for early detection and treatment of sepsis in neonates at intensive care unit and oncologic patients with febrile neutropenia. Acta morphol. 2021;17(2):5-11" the author list was given incorrectly. The Technical Redaction of the Acta Morphologica would like to apologize for the eventual inconvenience originated from this.

The correct form of the article should now state, as follows:

PROCALCITONIN AS A PROMISING BIOCHEMICAL MARKER FOR EARLY DETECTION AND TREATMENT OF SEPSIS IN NEONATES AT INTENSIVE CARE UNIT AND ONCOLOGIC PATIENTS WITH FEBRILE NEUTROPENIA.

Nonkulovski Danilo[1], Tankoska Maja[1], Pandovska Bisera[1], Kimovska Mica[1], Bicevska-Mandzukovska Hristina[1], Voinovska Tamara[1], Martinova Kata[1], Kostadinova Lence[2]

[1]University Clinic for Children Diseases, Faculty of Medicine,
Ss. Cyril and Methodius University in Skopje, Skopje, Republic of North Macedonia
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2. Gisondi P, Altomare G, Ayala F, et al. Italian guidelines on the systemic treatments of moderate-to-severe plaque psoriasis. J Eur Acad Dermatol Venereol. 2017;31(5):774–90.
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