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**ORIGINAL PAPER**

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# Lycopene activity on lung and kidney cancer cells by $T_2$ relaxation time $^1$H Magnetic Resonance Imaging *in vitro*

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**ABSTRACT**

**Introduction.** The paper presents the results of a study of cell cultures of lung cancer and kidney cancer using lycopene performed using clinical magnetic resonance imaging.

**Aim.** The aim of the study was to evaluate lycopene activity on tumor cell cultures.

**Material and methods**. For this purpose, MR tests were performed using the technique of determining transverse relaxation.

**Results.** Described here studies demonstrated that lycopene may inhibit the growth of A549 and ACHN cell lines.

**Conclusion.** We determine changes in spin lattice relaxavity $T_2$ to monitor treatment of lung cancer cell line A549 and kidney cancer cell line ACHN cells treatment with lycopene.

**Keywords**. cell cultures, lung cancer, lycopene, kidney cancer, magnetic resonance imaging, relaxation times

## Introduction

Lycopene is an antioxidant from the carotene group, containing unsaturated hydrocarbon. It has many health-promoting properties, primarily it helps in inhibiting the development of tumors, reduces the risk of heart attack and improves resistance to infection.[1] It is a carotenoid (a natural dye) in blood serum. We find it in fruits and vegetables, the most popular source are tomatoes and preserves. Studies show that the highest content is found in red extracts, but we can also find it in green tomato extracts.[2] The so-called oxidative stress in the body leads to the development of various types of diseases, including cancer, hence the need to use substances with a strong antioxidant effect. Such health-promoting properties result from the use of lycopene. It has the ability to neutralize free radicals, and also has the ability to regenerate antioxidants such as, for example, lutein. In addition, it removes free radicals from the body responsible for cancer development and reduces the risk of cancer.[3-6] Numerous scientific

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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studies present the use of lycopene in scientific studies using methods to determine relaxation times. The transverse relaxation time T2 is the time after which they reach a certain defined value. In the case of T2 it will be about 37% of the total magnetization value. There are many reports about the beneficial effects of lycopene, one of them is the work of Arab et al. on the content of antioxidants in the lining of the lung epithelium, which may have a protective effect.[7] Lian et al. in 2007, he presented the effects of apo-10'-lycopenoic acid in inhibiting the growth of bronchial cancer cells and A549 non-small cell lung cancer cells in both in vivo and in vitro studies.[8,9] Muzandu et al. presented the effect of lycopene and beta-carotene on cellular modifications that may contribute to the capture of reactive oxygen (ROS).[10] In addition, lycopene has the ability to inhibit the expression and growth of prostate, colon and lung cancer cells.11 In addition, lycopene may reduce the incidence of lung cancer.[12-13] In his clinical trials, Talwar et al. in 1997 they presented the effect of inflammation on the content of antioxidants, among others, lycopene in patients with non-small cell lung cancer (NSCLC), concentrations were much lower than in the control group.[14-16]Aizawa et al. in 2016 conducted research on the effects of smoking and alleviation of these effects with the help of lycopene supplementation, ferrets in which lung cancer was induced, among others, were examined.[17] In addition, Satia et al. in their work from 2009 they describe the long-term use of supplements, including lycopene and the assessment of lung cancer risk.[18] Huang et al. and Chow in their studies have shown that lycopene can contribute to the inhibition of tumor metastasis in in vitro studies.[19-20] While Shareck et al. showed an inverse relationship between the intake of selected carotenoids and vitamins and the risk of lung cancer.[21]

## Aim

The aim of the study was to evaluate lycopene activity on tumor cell cultures.

## Material and mehods

### *Cell culture*

In the experiment we used A549 lung cancer cell line (American Type Culture Collection, VA, USA) and ACHN renal cancer cell line (American Type Culture Collection, VA, USA). Cells were cultured in standard conditions: temperature 37°C, 5% CO2 and 95% humidity. Culture medium consisted of Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, MO, USA), Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham (Sigma-Aldrich, MO, USA), Fetal Bovine Serum (Biochrom, Germany) and Penicillin-Streptomycin-Neomycin Solution Stabilized (Sigma-Aldrich, MO, USA). The culture of lung cancer cells was passaged in 3rd day with the use of Accutase Cell Detachment Solution (Corning, NY, USA) to five 70 ml Tissue Culture Flasks (ThermoFisher Scientific, MA, USA). In 7th day different doses of Lycopene ≥ 90% from tomato (Sigma-Aldrich, MO, USA). Cells were treated with lycopene (2.5 µL/mL, 5.0 µL/mL and 25 µL/mL). Two another cell culture were negative controls – one with addition of 5 µl Dimethyl sulfoxide (VWR, PA, USA) per 1 ml of culture medium and the second one without any supplements. 24 hours after supplementation with Lycopene (Sigma-Aldrich, MO, USA) cells were treated with Accutase Cell Detachment Solution (Corning, NY, USA) and washed in Phosphate Buffered Saline Dulbecco without $Mg^{2+}$ and $Ca^{2+}$ (Biochrom, Germany). Cells were counted with the use of Muse Cell Analyzer (Merck Millipore, MA, USA). In the next step samples were centrifuged (5 min., 250xg in room temperature) in 1,5 ml Eppendorf tubes (Eppendorf, Germany) and supernatant from samples was discarded.

ACHN renal cancer cell line was cultured also in five 70 ml Tissue Culture Flasks (ThermoFisher Scientific, MA, USA). Cells were treated with lycopene (2.5 µL/mL ,5.0 µL/mL, and 25 µL/mL). After 24 hours incubation with Lycopene (Sigma-Aldrich, MO, USA) cultures were also treated with Accutase Cell Detachment Solution (Corning, NY, USA) and cells were counted with the use of Muse Cell Analyzer (Merck Millipore, MA, USA).

### *MRI quantitative technique*

All MR scans were performed with Optima MR360 magnetic resonance from General Electric Healthcare (Milwaukee, Wisconsin, USA). The camera was supported in the SV23 software version. The prepared samples were placed in an MR tunnel and then a series of measurements was made to determine the T2 relaxation time. The lung cancer cells in the vials were placed on the FLEX Small transceiver coil. To perform the measurements, the Fast Spin Echo (FSE) sequence was used with the following parameters: FOV field of view=10x10 [cm]; Matrix=320x224; NEX=2.0; Slice Thickness=1.0 [mm]; Spacing=0.5). TE time varied in the range of 1÷170 [ms] (1, 5, 10, 15, 20, 30, 40, 50, 70, 100, 120, 150, 170ms). TE time was 15,000 m.

## Results

Measurements were made to determine the T2 relaxation time in tomatoes (*Lycopersicon esculentum L.*). Figure 1 below shows sample DICOM images.

The next stage of the study were as follow:

- measurements of T2 relaxation time in lung cancer cells A549
- measurements of T2 relaxation time in lung cancer cells A549 in DMSO

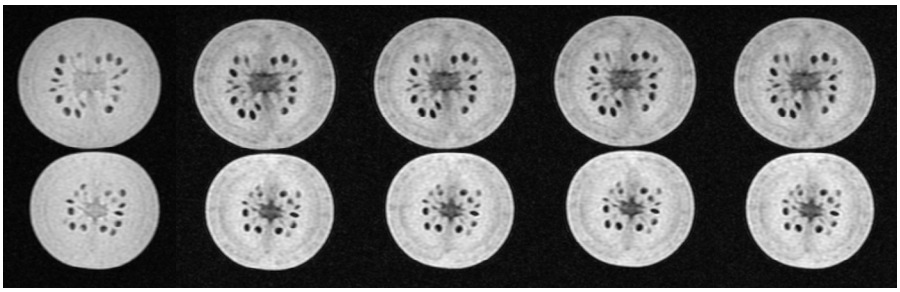

**Fig. 1.** Sample images of T2 FSE DICOM for TR = 12000 and TE 10, 500, 1000, 15000, 2000 ms, respectively

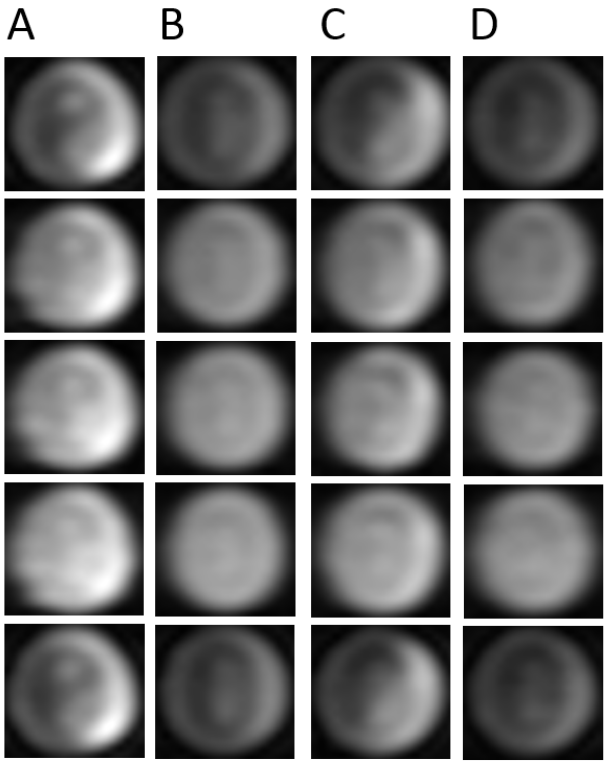


**Fig. 2.** Sample images from the determination of T2 relaxation times (A) control sample, lung cancer cells, (B) lung cancer cells+2.5μL Lycopene, (C) lung cancer cells+5μL Lycopene, (D) lung cancer cells+20μL Lycopene

– measurements of T2 relaxation time in lung cancer cells A549 treated with lycopene in different concentrations

Columns from AD in Fig. 2 show examples of DICOM images for various samples: (A) control sample, lung cancer cells, (B) lung cancer cells+2.5μL Lycopene, (C) lung cancer cells+5μL Lycopene, (D) lung cancer cells+20μL Lycopene. The viability of cells treated with Lycopene was 96.2%, 94%, 92 and 87%, after 24 h treatment with 0 μl, 2.5 μl, 5 μl and 20 μl of Lycopene respectively. Images were taken for the following echo and repetition times: TE = 5; 50, 100, 150, 200 ms for constant TR = 12000 ms. T2 relaxation curves for lung cancer cells and the same cells in DMSO and with lycopene at various concentrations are shown in the figure below.

**Table 1.** Relaxation times of lung cancer cells with the addition of lycopene and DMSO

| Sample | A549 | A549+2.5 μl lycopene | A549+5 μl lycopene | A549+25 μl lycopene | A549+DMSO |
|---|---|---|---|---|---|
| $T_2$ [ms] | 77 | 77 | 76 | 57 | 142 |

The columns from AD in Fig. 3 show examples of DICOM images for various samples: (A) control sample, kidney cancer cells, (B) kidney cancer cells+2.5 μL Lycopene, (C) kidney cancer cells+5 μL Lycopene, (D) kidney cancer cells+20 μL Lycopene, the viability was 98, 94, 92 and 87% respectively. Images were taken for the following echo and repetition times: TE=5; 50, 100, 150, 200 ms for constant TR = 12000 ms.

Analyzing the results obtained, it can be seen that in the case of the A549 lung cancer cell line, based on the

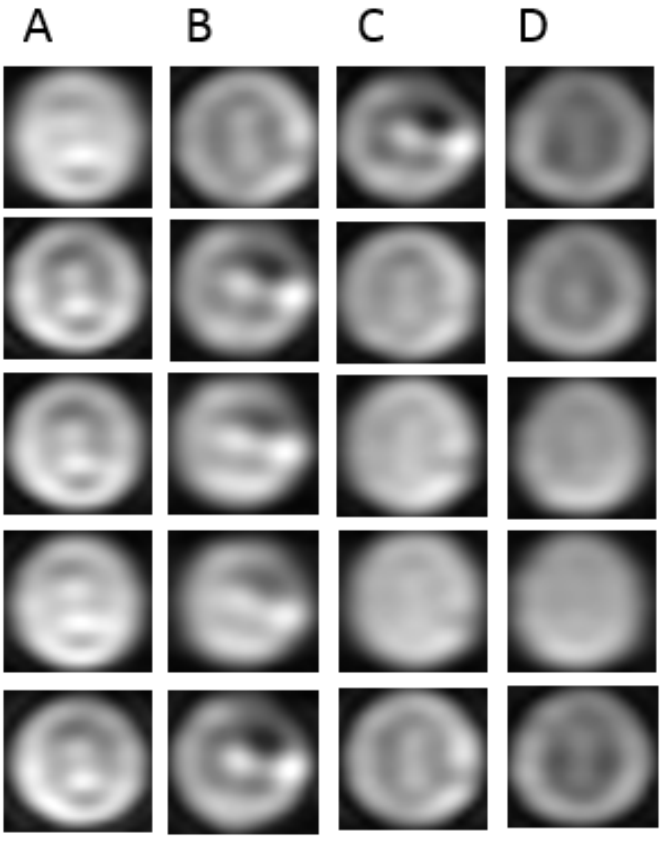


**Fig. 3.** Sample images from the determination of T2 relaxation times, (A) control sample, kidney cancer cells, (B) kidney cancer cells+2.5μL Lycopene, (C) kidney cancer cells+5μL Lycopene, (D) kidney cancer cells + 20μL Lycopene

results from the T2 relaxation time, we can see a downward trend as the concentration of lycopene increases. The difference between the T2 relaxation time of the control sample with pure A549 cells and the sample with 25 μl of lycopene is 20 ms.

**Table 2.** Relaxation times for cell cultures of kidney cancer, kidney cancer with addition of lycopene and DMSO

| Sample | ACHN | ACHN+2.5 μl lycopene | ACHN+5 μl lycopene | ACHN+20 μl lycopene | ACHN+DMSO |
|---|---|---|---|---|---|
| $T_2$ [ms] | 92 | 83 | 77 | 76 | 68 |

However, in the case of the ACHN kidney cancer cell line, it can be seen that the difference between the T2 relaxation time of the clean cell control sample and the sample with 20μl of lycopene is 16 ms.

## Discussion

Lycopene is found mainly in tomatoes, and in smaller amounts in guava, pink grapefruit, watermelon and papaya. In tomatoes with intense red color, the average lycopene content is 5.6 mg/100g and in lighter tomatoes, available in the spring and autumn only 2.6 mg/100g. Lycopene does not dissolve in water, but in fat, which is why fats are necessary for its absorption by the body. Its bioavailability is also increased by heat treatment.

In work presented here we used lycopene (2,5 μl, 5 μl and 25 μl) to treat lung and kidney cancer cell cultures. With increasing concentrations of lycopene we observed lower values of T2 due to cell killing process. T2 values decreased much faster in lung cancer cells than kidney cancer cells. The health-promoting effect of lycopene is mainly due to its strong antioxidant properties. Lycopene is the most active antioxidant in the carotenoids group and significantly exceeds beta-carotene and alpha-tocopherol in this respect.

## Conclusion

The trend in T2 relaxation time may indicate damage to cancer cell nuclei.

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**ORIGINAL PAPER**

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# Depressive symptoms during pregnancy and their risk factors – a cross-sectional study

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**ABSTRACT**

**Introduction.** It was established that intragestational depression is a common disease, with the estimated average prevalence of 10–25% in all expectant mothers worldwide.

**Aim of the study**. The aim of the study was to evaluate the frequency of depressive symptoms in pregnant women in Poland and to identify which factors may be related to a higher risk of depressive symptoms during pregnancy.

**Material and methods**. A prospective cross-sectional study was performed. Depressive symptoms were assessed with the validated Edinburgh Postnatal Depression Scale (EPDS). 346 women were enrolled in the study.

**Results**. 130 women (37.6%) scored 13 or more points and were considered as presenting with depressive symptoms. Independent risk factors of depressive symptoms during pregnancy including mood disorders diagnosed before the current pregnancy (aOR=2.68, 95%CI 1.37-5.22), mental disorders confirmed in family members (aOR=2.72, 95%CI 1.24-5.98), unhappiness in their current relationship (aOR=4.0, 95%CI 1.77-9.01), lack of support from family members (aOR=2.73, 95%CI 1.51-4.96) increased the risk of DS and good financial status decreased the risk of DS occurrence (aOR=0.45, 95%CI: 0.25-0.80).

**Conclusions.** Pregnant women commonly report depressive symptoms. The evaluation of relations with the family members, socio-economic status, former depressive symptoms and possible prenatal depression are essential for proper screening of depression in pregnant women.

**Keywords**. depression risk factors, depressive symptoms, Edinburgh Postnatal Depression Scale, perinatal depression, pregnancy

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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## Introduction

It was established that intragestational depression is a common disease, with an estimated average prevalence of 10-25% in all expectant mothers worldwide.[1,2]selective serotonin re-uptake inhibitors (SSRIs According to a frequently cited hypothesis, hormonal changes occurring physiologically during gestation are related to decreased mood, which may further evolve into perinatal depression (PD).[3,4] However, other factors may increase the risk of PD occurrence among pregnant women, with mood and anxiety disorders in a patient's medical history, lack of support from a partner and other family members, significant stress and addictions being mentioned the most commonly. Gestation-related complications or ambivalent feelings towards the pregnancy itself may also raise the risk of PD occurrence.[2]but the burden of MDD attributable to perinatal depression is not yet known. There has been little effort to date to systematically review available literature and produce global estimates of prevalence and incidence of perinatal depression. Enhanced understanding will help to guide resource allocation for screening and treatment. Methods A systematic literature review using the databases PsycINFO and PubMED returned 140 usable prevalence estimates from 96 studies. A random-effects meta-regression was performed to determine sources of heterogeneity in prevalence estimates between studies and to guide a subsequent random-effects meta-analysis. Results The meta-regression explained 31.1% of the variance in prevalence reported between studies. Adjusting for the effects of all other variables in the model, prevalence derived using symptom scales was significantly higher than prevalence derived using diagnostic instruments (odds ratio [OR] 1.6, 95% confidence interval [CI] 1.3–2.0

The prevalence of PD varies around the world.[1,5,6]selective serotonin re-uptake inhibitors (SSRIs According to the literature up to 51% of pregnant women in South Korea may suffer from PD, while only 10% of expectant mothers in the United States fit the diagnostic criteria of perinatal depression.[2]but the burden of MDD attributable to perinatal depression is not yet known. There has been little effort to date to systematically review available literature and produce global estimates of prevalence and incidence of perinatal depression. Enhanced understanding will help to guide resource allocation for screening and treatment. Methods A systematic literature review using the databases PsycINFO and PubMED returned 140 usable prevalence estimates from 96 studies. A random-effects meta-regression was performed to determine sources of heterogeneity in prevalence estimates between studies and to guide a subsequent random-effects meta-analysis. Results The meta-regression explained 31.1% of the variance in prevalence reported between studies. Adjusting for the effects of all other variables in the model, prevalence derived using symptom scales was significantly higher than prevalence derived using diagnostic instruments (odds ratio [OR] 1.6, 95% confidence interval [CI] 1.3–2.0 A general tendency towards a higher frequency of possible PD cases in lower-income countries is observed, which may suggest a significant role of socio-economic factors in its development.

According to a regulation by the Polish Ministry of Health, each pregnant woman should undergo screening for perinatal depression twice during pregnancy - in the first and the third trimester, with two recommended questionnaires being EPDS and Beck's Depression Inventory.[7] A positive screening test or the presence of risk factors should be followed by a more detailed examination of a patient's mental state by either a psychologist or a psychiatrist.

If left untreated, intragestational depression may contribute to a number of complications which may affect the mother, fetus and later the neonate, both during the pregnancy and the postpartum period. Severe postpartum depression is cited most often as a possible complication. However, a higher risk of spontaneous abortion, preterm birth, urgent operative delivery, preeclampsia or restricted fetal growth may also be associated with untreated prenatal depression.[8] Delayed child development was also reported more often in children of mothers who suffered from PD.[9] The aforementioned and other complications may be caused not only by depression itself, but by substance abuse as well, as it is more common among expectant mothers with perinatal depression.[9]

The knowledge of PD risk factors is essential in conducting the proper screening of pregnant women.

## Aim of the study

The aim of the study was to evaluate the frequency of depressive symptoms (DS) among pregnant women and to identify which factors may be related to a higher risk of depressive symptoms during pregnancy.

## Material and Methods

A cross-sectional study was performed. Polish language version of the questionnaire was distributed via internet between November 2017 and March 2018. A total of 346 pregnant women were enrolled in the study.

We used the validated Edinburgh Postnatal Depression Scale (EPDS). The questionnaire consisted of 46 questions regarding maternal characteristics, sociodemographic status, obstetric and psychiatric history and current pregnancy. It contained a Polish translation of Edinburgh Postnatal Depression Scale (EPDS) - a 10-question scale with each answer scored between 0 and 3 points (minimum total score 0, maximum 30), which is commonly used in screening for possible de-

pression after delivery.[10] EPDS was primarily designed to detect postnatal depression, but it was proved to be an accurate tool for assessing the likelihood of intragestational,[11-13] with a score of 13 and more points being directly related to a high risk of depression.[11-13]

The respondents were asked to answer questions concerning their wellbeing over the past 2 weeks.

The study protocol obtained the approval of the Ethics Committee of the Medical University of Warsaw. The committee waived the obligation to gain a written consent to participate in the study as completing the questionnaire was tantamount to giving the consent.

Statistica 13.3 software was used for statistical analysis, with Mann-Whitney U-test being used for continuous variables and chi-squared test for categorical variables. *P*-values <0.05 were considered significant and all tests were two-tailed.

## Results

A total of 386 pregnant women participated in the survey 40 of them were excluded as their questionnaires were completed incorrectly (missing data). Consequently 346 women were enrolled in the study: 182 being in the first trimester of pregnancy (52.6%), 82 in the second (23.7%) and 82 (23.7%) in the third.

216 women (62.4%) scored below 13 points in EPDS. Therefore, they were classified as having no depressive symptoms. 130 women (37.6%) had a score of 13 or more points and were considered as presenting with DS. Basic characteristics of the study group are presented in Table 1.

Lower income was related to a higher occurrence of DS, regardless of the type of the mother's occupation. The highest incidence of DS was reported by women in the first trimester of pregnancy (40.8%), with the rates declining with the progression of pregnancy. Hence, the lowest incidence was observed in the third trimester (27.7%; second trimester - 31.5%).

Women with DS more frequently admitted the current pregnancy had been unplanned (21.5% vs 12.5% in the group without DS, p=0.03). The respondents scoring above 13 points at EPDS significantly more often reported the lack of support from their partners (49.2% vs 17.6%, p<0.001) and family members (44.6% vs 16.2%, p<0.001) as well as unhappiness in their current relationship (28.5% vs 5.1%, p<0.001). Moreover, a larger proportion of those women admitted to having smoked during gestation compared to women with a score below 13 points in the EPDS (31.5% vs 18.1%, p<0.01).

Patients' medical history of mood disorders (49.2% vs 25%, p<0.001) and a history of mental disorders in family members (55.8% vs 44.1%, p<0.001) were more common in the group of women with DS. Except for cervical insufficiency none of the analyzed pregnancy complications were related to the occurrence of DS in our study group. Cervical insufficiency was reported significantly more often in women presenting with DS. However, the rates were very low in both groups (3.9% vs 0.5%, p=0.02). Women with DS used sedatives (16% vs 5%, p<0.001), antidepressants or psychotherapy (12% vs 5%, p<0.01) more often during pregnancy.

Possible risk factors of DS were evaluated with logistic regression analysis. Only five of the analyzed factors were found to have a statistically significant impact on DS occurrence. They are presented in Table 2.

Mood disorders diagnosed before the current pregnancy, confirmed mental disorders in a family member, unhappiness in the current relationship and lack of support from family members increased the risk of DS while good financial status decreased the risk of DS occurrence.

23.8% of women with DS admitted to having reported them to medical staff, with 17.7% subsequently getting diagnosed with prenatal depression. However, only 2.9% of the respondents stated they had undergone any form of depression treatment (either pharmacotherapy or psychotherapy) recommended by a doctor.

## Discussion

Lack of family members support and unhappiness in the relationship seem to be the most relevant risk factors of PD occurrence, because of being relatively indicatable and removable risk factors. A systematic review conducted by Fisher et al. revealed that difficulties in a romantic relationship (a partner who rejected paternity, was unsupportive, uninvolved, critical and quarrelsome or presented unhealthy alcohol drinking behaviors, was violent or unfaithful) had a significant impact on PD occurrence.[14] A higher incidence of PD in women whose partner did not want the pregnancy was also reported by Mukherjee et al.[15] Lack of support from family members was related to PD in a systematic review by Fisher et al.[14] Interestingly the lack of support in the relationship and seems to play a role in DS occurrence rather than the presence of relationship itself. Relationship status was found statistically insignificant.[14] Therefore, we suggested estimating the relations of pregnant women with their family members and attitude to their family situation during every medical examination.

Moreover the present study showed the highest incidence of DS to occur in the first trimester and its decline with the progression of pregnancy, reaching with the lowest numbers reached in the third trimester. Precise percentages of PD occurrence differ between populations. However, most authors report the general numbers to be high. According to the majority of authors the percentage of women suffering from PD during the first trimester of pregnancy oscillated around 25-30%.[16,17] However, occurrences as low as 7.4% or as high as 40.5% were also reported.[18-21] Large discrepancies in reported

**Table 1.** Characteristics of the study group

| | Study group N=346 | Women scoring <13 points N=216 | % | Women scoring ≥13 points N=130 | % | OR (95% CI) | p-value |
|---|---|---|---|---|---|---|---|
| Age (years) | 26.8±4.3* | 26.8±3.8* | | 26.8±5.1* | | 1.0 (0.95-1.05) | 0.99 |
| 17-24 years old | 115 | 62 | 28.7 | 53 | 40.8 | 1.9 (1.2-3.0) | 0.007 |
| Education | | | | | | | |
| junior high school | 11 | 5 | 23.1 | 6 | 4.6 | 2.40 (0.71-8.15) | 0.11 |
| secondary/vocational school | 134 | 77 | 35.6 | 57 | 43.9 | 1.48 (0.94-2.32) | |
| university | 201 | 134 | 62.0 | 67 | 51.5 | 1.0 | |
| Place of residence | | | | | | | |
| village | 87 | 57 | 26.4 | 30 | 23.1 | 0.92 (0.48-1.75) | 0.93 |
| town <20 000 | 43 | 26 | 12.0 | 17 | 13.1 | 1.14 (0.53-2.47) | |
| town 20000-100000 | 73 | 43 | 19.9 | 30 | 23.1 | 1.21 (0.62-2.36) | |
| city 100000-500000 | 69 | 43 | 19.9 | 26 | 20.0 | 1.05 (0.53-2.08) | |
| city >500 000 | 74 | 47 | 21.8 | 27 | 20.8 | 1.0 | |
| Occupation | | | | | | | |
| unemployed | 71 | 41 | 19.0 | 30 | 23.1 | 1.0 | 0.39 |
| mental | 152 | 92 | 42.6 | 60 | 46.1 | 0.89 (0.50-1.58) | |
| physical | 28 | 17 | 7.9 | 11 | 8.5 | 0.88 (0.36-2.16) | |
| partly mental and partly physical | 95 | 66 | 30.6 | 29 | 22.3 | 0.60 (0.32-1.14) | |
| Marital status | | | | | | | |
| single | 22 | 10 | 4.6 | 12 | 9.2 | 1.75 (0.64-4.77) | 0.89 |
| in a relationship | 324 | 206 | 95.4 | 118 | 90.8 | 1.0 | |
| Household income | | | | | | | |
| poor | 15 | 2 | 0.9 | 13 | 10.0 | 5.55 (1.18-26.05) | <0.001 |
| average | 89 | 41 | 19.0 | 48 | 36.9 | 1.0 | |
| good | 202 | 147 | 68.0 | 55 | 42.3 | 0.32 (0.19-0.54) | |
| very good | 40 | 26 | 12.0 | 14 | 10.8 | 0.46 (0.21-1.00) | |
| Primiparity | 247 | 160 | 74.1 | 87 | 66.9 | 1.41 (0.88-2.27) | 0.15 |
| Previous vaginal delivery | 84 | 45 | 20.8 | 39 | 30.0 | 0.61 (0.37-1.01) | 0.06 |
| Assisted reproductive techniques | 43 | 29 | 13.4 | 14 | 10.7 | 0.78 (0.39-1.53) | 0.46 |
| Smoking during pregnancy | 80 | 39 | 18.1 | 41 | 31.5 | 2.09 (1.26-3.47) | <0.01 |
| Alcohol consumption during pregnancy | 37 | 20 | 9.3 | 17 | 13.1 | 1.47 (0.74-2.93) | 0.27 |
| Trimester of pregnancy | | | | | | | |
| 1st | 182 | 129 | 59.7 | 53 | 40.8 | 1.0 | <0.01 |
| 2nd | 82 | 41 | 19.0 | 41 | 31.5 | 0.18 (0.09-0.35) | |
| 3rd | 82 | 46 | 21.3 | 36 | 27.7 | 0.08 (0.04-0.16) | |
| Unplanned pregnancy | 55 | 27 | 12.5 | 28 | 21.5 | 2.10 (1.16-3.82) | 0.03 |
| Infertility | 73 | 45 | 20.8 | 28 | 21.5 | 1.44 (1.08-1.92) | 0.04 |
| Mood disorders before the current pregnancy | 116 | 52 | 25 | 64 | 49.2 | 3.06 (1.92-4.87) | <0.001 |
| Mental disorders in family members | 86 | 38 | 44.1 | 48 | 55.8 | 2.74 (1.66-4.52) | <0.001 |
| Lack of partner support | 102 | 38 | 17.6 | 64 | 49.2 | 4.54 (2.78-7.42) | <0.001 |
| Lack of other family member support | 93 | 35 | 16.2 | 58 | 44.6 | 4.16 (2.53-6.87) | <0.001 |
| Unhappiness in relationship | 48 | 11 | 5.1 | 37 | 28.5 | 7.94 (3.87-16.29) | <0.001 |
| **Pregnancy complications:** | | | | | | | |
| Gestational diabetes | 22 | 13 | 6.0 | 9 | 6.9 | 0.86 (0.36-2.07) | 0.74 |
| Gestational hypertension | 14 | 7 | 3.2 | 7 | 5.4 | 0.59 (0.20-1.72) | 0.33 |
| Cholestasis of pregnancy | 4 | 2 | 0.9 | 2 | 1.4 | 0.60 (0.08-4.30) | 0.61 |
| Iron-treated anemia | 48 | 27 | 12.5 | 21 | 16.2 | 0.74 (0.40-1.37) | 0.34 |
| Vaginal bleeding during pregnancy | 60 | 34 | 15.7 | 26 | 20.0 | 0.75 (0.42-1.31) | 0.31 |
| Cervical insufficiency | 6 | 1 | 0.5 | 5 | 3.9 | 0.12 (0.01-1.01) | 0.02 |

* – average ± standard deviation

OR – odds ratio

95% CI – 95% coefficient interval

**Table 2.** Independent risk factors of depressive symptoms during pregnancy

| | Study group N=346 | Women scoring <13 points N=216 | % | Women scoring ≥13 points N=130 | % | aOR (95% CI) | p-value |
|---|---|---|---|---|---|---|---|
| Mood disorders before the current pregnancy | 116 | 52 | 25 | 64 | 49.2 | 2.68 (1.37-5.22) | 0.004 |
| Mental disorders in family members | 86 | 38 | 44.1 | 48 | 55.8 | 2.72 (1.24-5.98) | 0.013 |
| Lack of family member support | 93 | 35 | 16.2 | 58 | 44.6 | 2.73 (1.51-4.96) | <0.001 |
| Unhappiness in a relationship | 48 | 11 | 5.1 | 37 | 28.5 | 4.0 (1.77-9.01) | <0.001 |
| Good financial situation | 202 | 147 | 68.0 | 55 | 42.3 | 0.45 (0.25-0.80) | <0.01 |

aOR – adjusted OR (logistic regression)

95% CI – 95% coefficient interval

PD occurrence during the second and third trimester were also observed. Gaynes et al. found the incidence of PD being 8.5% in the second and third trimesters in the populations of England, Scotland, Norway, Portugal, the Netherlands, Australia, the United States, Canada, Hong Kong, and Japan.[20]her children, and other family members. Objectives. We systematically review the evidence on (1 Alqahtani et al. reported the incidence reaching 12.8% and Mikšić et al. - 23% in the third trimester, while Park et al. suggested it may even approximate 61.4% in the third trimester of pregnancy.[21-23] According to Koss et al. every third woman will suffer from depression during at least one trimester of her pregnancy, while for 25% of women the experience of depression will be limited only to one trimester.[24]

Our research, as well as previous studies, confirmed the presence of multiple issues which increase the incidence of DS and, therefore, increase the chance of PD development if present.[3,5,21,23] According to Dimidjian et al. a history of mood disorders constitutes one of the most important factors, which stays in line with our results.[25] In our study pre-pregnancy mood disorders were one of the most significant risk factors of DS. A similar odds ratio was reported by Gebremichael et al. who correlated previous history of depression with a significant impact on PD occurrence.[26]monthly income AOR (95% C.I Mood disorders in family members are an independent risk factor of PD. According to Gebremichael et al. a mental disorder in a close relative is associated with over a 3-fold higher risk of PD.[26]monthly income AOR (95% C.I Our study also showed that confirmed mood disorders in family members were an independent risk factor of DS.

According to our results socioeconomic status played a significant role in DS occurrence. Self-assessment of the financial status as "good" reduced the incidence of DS. Additionally, questionnaires returned via internet may promote honesty of the answers. A small study group and no verification possibility of PD occurrence in the studied cohort constitute a limitation of this study.

DS are not sufficiently reported by pregnant women, with up to 80% of the cases of PD remaining under-recognised by healthcare providers.[27] Implementing screening methods in modern technologies like smartphone applications or websites, which are commonly used by pregnant women, seems feasible and could be considered a way of enforcing a more private or confidential and less impersonal screening method.[27-29] Creating a universal strategy of educating medical professionals regarding the risk factors of PD and developing guidelines for ways of screening for it is also crucial.[29]

## Conclusions

Pregnant women commonly report depressive symptoms. The evaluation of relations with the family members, socio-economic status, former depressive symptoms and possible prenatal depression is essential for the proper screening of depression in pregnant women.

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**ORIGINAL PAPER**

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# Cytisine parameters measured in the 1.5 Tesla magnetic field

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**ABSTRACT**

**Introduction.** Cytisine, Cytisinum ($C_{11}H_{14}N_2O$), is an organic chemical compound. Cytisine is heterotricyclic compound that is the toxic principle in Laburnum seeds and is found in many members of the Fabaceae (legume, pea or bean) family.

**Aim.** The aim of the study is to measure the influence of water on the form of drug in the magnetic field 1.5 Tesla.

**Material and methods.** For this purpose, magnetic resonance imaging tests were performed to check the solubility of pure cytisine, Desmoxan tablets and Tabex capsules.

**Results.** From a pharmacological point of view, both Desmoxan tablets and Tabex capsules should exert the same effect on the human body, this is due to the identical content of the active substance, in this case cytisine (1.5 mg).

**Conclusion.** The differences in the results obtained may be related to additional excipients that contain medications, but it is believed that they should not have a negative impact on the action of the active substance.

**Keywords.** cytisine, desmoxan, magnetic resonance imaging, tabex

## Introduction

Commonly used smoking cessation preparations are based on cytisine. Cytisinum ($C_{11}H_{14}N_2O$) is an organic chemical compound obtained on an industrial scale from the seeds of the Laburnum anagyroides shrub, which have toxic properties.[1-4] Because of its similarity to nicotine, cytisine is used as a substitute for nicotine to satisfy craving associated with an attempt to quit smoking.[5] Tabex and Desmoxan have the same active ingredient, but they differ in excipients. Tabex and Des-

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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moxan are equivalent, that is, their action in the body is identical. Both contain the same active substance - cytisine, in an identical amount – 1.5 mg.[6-7] The differences only start at the stage of excipients and the form of the drug (capsule/tablet). In this case, the additive substances should not affect the drug's effect. In this work, we examined differences in parameters such as relaxation times for pure cytisine, Tabex tablets and Desmoxane capsules.[8-10]

## Aim

The aim of the study is to measure the influence of water on the form of drug in the magnetic field 1.5 Tesla.

## Material and methods

Measurements were made using Optima MR360 magnetic resonance imaging by General Electric Healthcare (Milwaukee, Wisconsin, USA) with a 1.5 Tesla field and a dedicated small flex transceiver. Basic sequences used for clinical trials were used. To compare popular pharmaceuticals, Desmoxan and Tabex tablets and pure cytisine were prepared (Figure 1). All MR scans were performed with Optima MR360 magnetic resonance from General Electric Healthcare (Milwaukee, Wisconsin, USA). The camera was supported in the SV23 software version. The prepared ependorf samples were placed in the MR tunnel and then a series of measurements was made to determine the relaxation times T1 and T2. The lung cancer cells in the vials were placed on the FLEX Small transceiver coil. To perform the measurements, the Fast Spin Echo (FSE) sequence was used with the following parameters: FOV field of view=10x10 [cm]; Matrix=320 x 224; NEX=2.0; Slice Thickness=1.0 [mm]; Spacing=0.5). TR time varied in the range of 48÷15000 [ms] (48, 50, 100, 150, 200, 300, 500, 1000, 1500, 2000, 3000, 5000, 10000, 15000 ms). TE time was 3 ms.

**Fig. 1.** Photo showing cytisine powder, Desmoxan capsule and Tabex tablet used for the study

## Results

The Desmoxan caapsule was ground in 5 ml of water. Directly after placing the powder in water, the relaxation time T1 was measured. With the following snapping parameters, the repetition time was changed in the range TR=48 to 15,000, TE=3 ms (Figure 2).

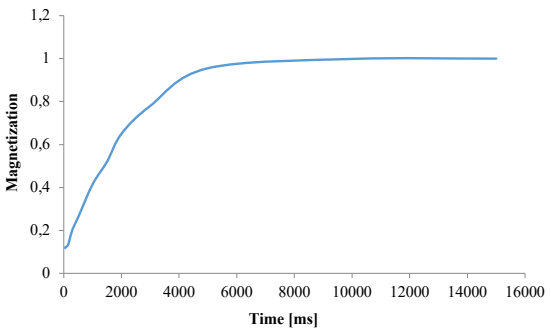


**Fig. 2.** T1 relaxation time curve for Desmoxan capsule induced in 5 ml $H_2O$

In the next step, after 24 hours, a series of measurements were again made to determine the relaxation time T1 (the tablet was lying in water all the time), the value of T1=1330 ms was obtained (Figure 3).

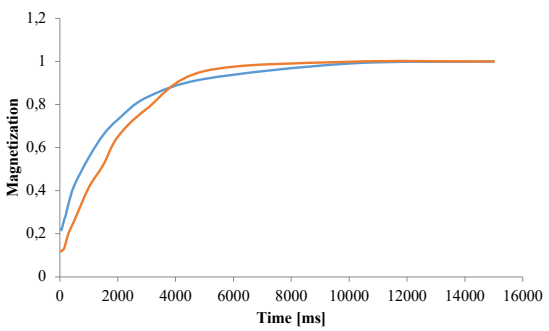


**Fig. 3.** T1 relaxation curves for the Desmoxan capsule induced in 5 ml $H_2O$ and the same capsule after 24h. The curve is marked in orange on the chart after 24 hours

In the next stage, after 72 hours, the measurements were repeated, and the relaxation time T1 was again determined, where its value strongly approached the water value and injected T1=3070 ms, which may indicate the dissolution of the tablet (Figure 4).

In the graph, the curve is marked orange after 24 hours, while the curve is marked green after 72 hours. On the graph, the curve is marked orange after 24 hours, while the curve is marked green after 72 hours. The results of relaxation times are shown in the table below (Table 1).

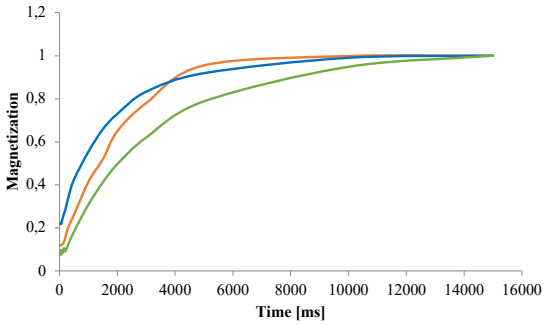


**Fig. 4.** T1 relaxation time curves for Desmoxan capsule induced in 5 ml $H_2O$ and the same capsule after 24h and 72h

**Table 1.** T1 relaxation times

| Sample | Desmoxan capsule | Desmoxan capsule after 24h | Desmoxan capsule tablet after 72h |
|---|---|---|---|
| $T_1$ [ms] | 1946 | 1330 | 3070 |

In the next step of the test, the oil solubility test of Desmoxan (5ml) was performed. The relaxation time T1 was determined which was T1=205 ms, then the series of measurements was repeated after 16h and the relaxation time was again determined which was T1=158 ms (Figure 5).

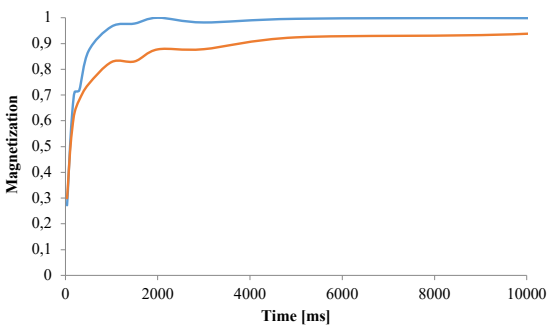


**Fig. 5.** T1 relaxation time curves for Desmoxan capsule induced in 5 ml oil and the same capsule after 16h

On the graph, the relaxation time curve marked immediately after placing the tablet into the oil is marked in blue, while the curve is marked orange after 16h. The results of relaxation times are shown in the table below (Table 2).

**Table 2.** T1 relaxation time

| Sample | Desmoxan capsule in oil | Desmoxan capsule after 24h in oil |
|---|---|---|
| $T_1$ [ms] | 205 | 158 |

To compare the results, scans of oil, water and vinegar were performed without the addition of drugs. The following relaxation time results were obtained: T1 oil=177 ms, T1 vinegar=1894 ms, T1 water=3200 ms.

The table 3 shows the results of T1 relaxation times for tablets with the addition of various solvents.

**Table 3.** T1 relaxation times for tablets with the addition of various solvents

| Sample | Cytisine | Desmoxan | Tabex |
|---|---|---|---|
| +5 ml vinegar | 648 ms | 573 ms | 1107 ms |
| +5 ml oil | 171ms | 169 ms | 170 ms |
| +5 ml water | 984 ms | 1066 ms | 1498 ms |

The table 4 shows the results of T2 relaxation times for tablets with the addition of various solvents.

**Table 4.** T2 relaxation times for tablets with the addition of various solvents

| Sample | Cytisine | Desmoxan | Tabex |
|---|---|---|---|
| +5 ml vinegar | 86 | 34 | 37 |
| +5 ml oil | 64 | 63 | 65 |
| +5 ml water | 90 | 38 | 47 |

## Discussion

Discovery of cytisine properties provides an opportunity for further interrogation of the physiological roles of nicotinic receptor.[11-13] Our observation during this experiment was that during 72 h in water, all form of discussed here drugs (Cytisine powder, Desmoxan capsules and Tabex tablets) were dissolved in water and both relaxation time of solutions decresed due to higher drug concentration in soluble form.

The addition of various solvent to cytisine shows decrese in T1 and T2 values when compared to cytisine dissolved in pure water. Desmoxan capsules and Tabex tablets showed similar behaviour in solubility. The amount and solubility in different media may induce changes in drug efficacy.[14-15] In our study we observed that only pure water was good media to analyze solubility of discussed drugs. The density of media in variable solvents is an important factor to discuss during a new drug preparations.[16-21]

## Conclusion

The diffrences between pure Cytisine, Tabex and Desmoxan are base on chemical composition of tablets. Desmoxan contein T lactose monohydrate, microcrystalline cellulose, talk and magnesium stearate. Tablex contein microcrystalline cellulose, corn starch, colloidal anhydrous silica and magnesium stearate. The results of Tabex and Desmoxan solubility are visible where compared to pure cytisine powder.

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**REVIEW PAPER**

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# The potential of intracellular $^{13}C$ MR spectroscopy to study the absolute configuration of endogenous and polarized alanine

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**ABSTRACT**

**Introduction.** Quantitative and accurate monitoring of tumor makes hyperpolarized carbon ($^{13}C$) Magnetic Resonance Imaging and Spectroscopy (MRI/S) a powerful tool for in vivo metabolic and structural study. Moreover, the studies of the properties and functions in tumor tissue of the compounds of carbon (C) that are organic, are fundamental to tumor biochemistry.
**Aim.** To review $^{13}C$ MR spectroscopy to study the absolute configuration of endogenous and polarized alanine
**Material and methods.** An analysis of literature regarding $^{13}C$ MR spectroscopy of polarized alanine.
**Results.** Current evidence suggests that the determination of absolute configurations of amino acids play significant role in physiological mechanisms during tumor growth and treatment.
**Conclusions.** Nearly 50% nuclear polarization for $^{13}C$ can be achieved in various organic molecules when Dynamic Nuclear Polarization DNP is performed in a strong magnetic field and at cryogenic temperatures.
**Keywords.** $^{13}C$ NMR, dynamic nuclear polarization, hyperpolarized carbon

## Introduction

From all types of spectroscopy, Magnetic Resonance Spectroscopy (MRS) can provide a useful analytical tool to determine the absolute configuration (L,D) of some metabolites in biomedical assays.[1-5] All enzyme catalyzed reactions in living matter are stereospecific and provide product in enantiomeric forms. By definition, the enantiomeric forms are mirror images of each other and their high-resolution spectra recorded in achiral media are identical. However, two enantiomers will

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lose the same chemical potential and consequently will have differ MRS spectrum, while interacting with a further chiral entity.[6-9] Moreover, observation of enantiomeric composition of samples, based on MRS in the presence of the chiral shift reagent can induce precise determination of the ratios of both enantiomers and induce its separation. The chiral entity cannot only separate two forms but it also has influence on the sensitivity of recorded spectra by establishing labile diastereomeric interactions with enantiomers.[10-15] The split of the resonances of the two forms can be exploited to assess quantitatively the enantiomeric composition of a living matter. It is obvious that stereochemical considerations of the enantiomeric forms are of high importance for understanding biochemical and physiological mechanisms in cancer tissue. Determination of absolute structure of metabolites, such as amino acids, might be a sufficient way to early detection of metabolic disorders that has implications for diagnosis and treatment. In particular, in order to target a biological receptor, the specificity and chirality of the recognition site must be correct. When such peptides are composed of L-amino acids, they are rapidly degraded *in vivo* by naturally occurring proteases but when composed of D-amino acids, the peptide is resistant to degradation, giving the possibility for therapeutic use.[16-20]

## Molecular asymmetry

Molecular asymmetry is fundamental for intracellular processes of chiral components in both healthy and cancer tissue. A molecule is chiral when it cannot be superimposable on its mirror image with the chiral object and its mirror image cannot be referred to as enantiomers. Almost all intracellular reactions are catalyzed by enzyme and usually provide two enanciomeric products with the absolute configuration of right-handed (D-) configuration and left-handed (L-) configuration. Recent studies have shown that both, D- and L- amino acids (AA) are present in animal and human body in high concentrations as building blocks of proteins, intermediates in metabolism and fulfill specific physiological functions. However, the structure and symmetry of organic molecules also play crucial role in many biochemical processes. One of the reasons for the increasing interest in the separation and quantification of both AA configurations lies in clinical diagnostics. The pros and cons of existing methods for enantioselective analysis of AA and their applications to biological samples are recent trends in the field but there are several research questions.[21-28]

## $^{13}C$ MRS

In particular, breast cancer cells show varied concentrations of AA using mostly chromatographic techniques; however their chirality was not studied yet. Therefore, the detection of D- and L- AA is of particular interest for our study since the determination of absolute configurations play significant role in physiological mechanisms during tumor growth and treatment. It is generally held that breast cancer cells express strong glycolytic activity and release large amounts of lactate, which can be produced or consumed with regard to the oxygenation of cells. D- and L-alanine was already found in gland and epithelial cells of mammals, but is typically quantified as the sum of their D- and L-enantiomers.[29-33] Moreover, alanine is known to be the predominant amino acid for the growth of breast cancer cells. Because the intracellular functions of alanine such as cell regeneration by L- form and damage by D- form are associated with absolute configurations, their ratio can be useful in diagnostic and therapeutic processes. We focused on the detection of absolute configuration of intracellular alanine *ex vivo* using carbon-13 magnetic resonance spectroscopy ($^{13}C$ MRS). The chemical shift range for 13C (~250 ppm) is much larger than that for proton (~15 ppm) and the $T_1$ relaxation time of $^{13}C$ in small molecules is much longer than that of $^{1}H$. However, in nature, about 1.11% of naturally occurring C is $^{13}C$ isotope, which is magnetically active and can be applied to probe molecular structures that correspond to physiological changes in tumor tissues. More than 98.89% of naturally occurring C is $^{12}C$ with no MR signal.[35-41] Several techniques have been used to overcome the $^{13}C$ MRS limitation by means of enhancing the polarization of nuclear spins. One of the techniques is proton decoupling, which eliminates the coupling of $^{1}H$ with $^{13}C$ by irradiating the entire $^{1}H$ resonance absorption range and consequently collapsing $^{13}C$ resonances to singlets. Although the MR signal of $^{13}C$ measured intracellularly corresponds to metabolic changes, signal intensity (SI) is too low to be relevant for quantitative and longitudinal study. However, the visualization of $^{13}C$ nuclei concentration may result in images and spectra with high signal to noise ratio (SNR) due to the hyperpolarization process. Nearly 100% nuclear polarization for $^{1}H$ and 50% for $^{13}C$ can be achieved in various organic molecules when Dynamic Nuclear Polarization (DNP) is performed in a strong magnetic field and at cryogenic temperatures. Replacing the $^{12}C$ (98.9% natural abundance) isotope with the $^{13}C$ isotope at a specific carbon or carbons in a metabolic substrate does not affect the substrate's biochemistry. With $^{13}C$ MRS, the body tissues are virtually invisible, and only regions where the hyperpolarized $^{13}C$-labeled substance is present will appear in the generated images.[36-39] In nature, C is abundant in all forms of life and all dead organic materials. Although the MR signal of $^{13}C$ *in vivo* corresponds to metabolic changes, signal intensity (SI) of naturally occurring $^{13}C$ is too low to be relevant for quantitative and longitudinal study. However, the visualization of $^{13}C$ nuclei concentration may result in images and spectra with high signal to noise ratio (SNR) due to the hyperpolar-

ization process. Hyperpolarization does not change any chemical or physical properties of the substances, however it allows acquisition of $^{13}C$ images and spectra in a relevant time frame. Hence, hyperpolarized $^{13}C$ MRI/S can directly inform about the biochemical tissue composition and chemical structure by generating frequency and spatial distribution of hyperpolarized atoms. In general, most suitable $^{13}C$ compounds for MR are small molecules (molecular weight ~120 $gmol^{-1}$) with a possibility to obtain information about molecular behavior *in vivo* due to rapid uptake in tissue. We review the applications of $^{13}C$ hyperpolarized techniques such as dynamic-nuclear-polarization (DNP) to monitor tumor targeting giving an overview of rapid $^{13}C$ MRI/S sequences used which is followed by the hardware enhancement such as coil design.[29-33] Hyperpolarization does not change any chemical or physical properties of the substances; however it allows acquisition of $^{13}C$ images and spectra within a relevant time frame. Hence, hyperpolarized $^{13}C$ MRI/S can directly inform about the biochemical tissue composition and chemical structure by generating frequency and spatial distribution of hyperpolarized atoms. In general, most suitable $^{13}C$ compounds for MR are small molecules (molecular weight ~120 $gmol^{-1}$) with a possibility to obtain information about molecular behavior *in vivo* due to rapid uptake in tissue. Prior to $^{13}C$ MRS *ex vivo* experiments, can be use presently available $^{13}C$ D-, L- alanine and $^{13}C$ L- Alanine to confirm $^{13}C$ resonance assignment in water solution phantoms. In these studies, we propose the utilization of at least three chiral lanthanide complexes such as (1) lanthanum(III)-tris (6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate, $C_{30}H_{30}F_{21}LaO_6$, (2) europium tris[3-(heptafluoropropylhydroxymethylene)-(−)-camphorate, $C_{42}H_{42}EuF_{21}O_6$ and (3) ytterbium(III)-tris[3-(heptafluoropropylhydroxymethylene)-l-camphorate, $C_{42}H_{42}F_{21}O_6Yb$, as a probe for differentiation of the enantiomeric chirality on the racemic D- and L- spectra and enantiomeric (D or L) spectra of amino acids solution.[33-36] Proposed chemical shift agents are highly water-soluble and suitable for study in the living matter. The chiral entity (1-3) cannot only separate two absolute forms of D- and L- alanine but it also has influence on the sensitivity of the recorded spectra by establishing labile diastereomeric interactions with enantiomers. The split of resonances of the two forms can be exploited to assess quantitatively the enantiomeric composition of the living matter.[37-41]

## Conclusion

Due to the beneficial impact of intracellular $^{13}C$ MR spectroscopy to study the absolute configuration of endogenous and polarized alanine in medicine there is a growing interest in analytical identification and quantification in vitro and in vivo.

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**REVIEW PAPER**

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# Radio Frequency MRI coils

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**ABSTRACT**

**Introduction.** Magnetic Resonance Imaging (MRI) coils technology is a powerful improvement for clinical diagnostics. This includes opportunities for mathematical and physical research into coil design.

**Aim.** Here we present the method applied to MRI coil array designs.

**Material and methods.** Analysis of literature and self-research**.**

**Results.** The coils that emit the radiofrequency pulses are designed similarly. As much as possible, they deliver the same strength of radiofrequency to all voxels within their imaging volume. Surface coils on the other hand are usually not embedded in cylindrical surfaces relatively close to the surface of the body.

**Conclusion.** The presented here results relates to the art of magnetic resonance imaging (MRI) and RF coils design. It finds particular application of RF coils in conjunction with bore type MRI scanners.

**Keywords.** field strength 1.5 Tesla, magnetic resonance imaging, radio frequency coil

## Introduction

Radio Frequency (RF) coil is commonly used in configurations for MR imaging.[1-9] The RF signal emitted by tissue is detected by monitoring the alternating voltage induced in antenna wires near the patients.[10] These coils may be used also as to transmit the radio frequency pulses that are applied to the patient, or that separate coils may transmit the radio frequency emitted by the tissue may be detected using receive only coils.[12] The theoretical basis for the electromagnetic analysis is the solution of Maxwell equation for the electric field expressed in the terms of the vector and scalar potentials.[10-15]

$$\nabla \times \vec{E} = -\frac{\partial \vec{B}}{\partial t}$$

$$\nabla \times \vec{B} = \mu \vec{j} + \mu\varepsilon \frac{\partial \vec{E}}{\partial t} \qquad \text{(Equ. 1).}$$

$$\varepsilon \nabla \cdot \vec{E} = \rho$$

$$\nabla \cdot \vec{B} = 0$$

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Maxwell's equations form the basis for any electromagnetic field analysis. They describe them in an exhaustive way and allow for full analysis. The Lorenzt gauge condition is used to eliminate the scalar potential and with harmonic time dependence.[16-18] $B_x$ component is symmetric along the XZ, YZ, and XY planes therefore static $B_1$ field characteristic, static coil geometry and vector current density is defined.

$$B_0 = B_0\hat{z}, i.e. B_{1x}, B_{1y} \quad \text{(Equ. 2).}$$

The goal is to find the appropriate current density distribution which will generate a static field component along the X, Y and Z directions with the specified uniformity. In order to generate $B_x$ component of the RF field, the current density distribution is viewed as a vector superposition of two components, along the z-direction and the other along the azimuthal direction.[19-27] Thus, the general expression of the current density distribution is:

$$\vec{J}^a(\vec{r}) = \left[j_{\emptyset}^a(\emptyset, z)\hat{\alpha}_{\emptyset} + j_z^a(\emptyset, z)\hat{\alpha}_z\right]\delta(\rho - a) \text{ (Equ. 3).}$$

The expression for both components of the current densities can be written as:

$$j_{\varphi}^a(\varphi, z) = \cos\varphi \sum_{n=1}^{\infty} c_n \sin k_n z \quad \text{(Equ. 2.4),}$$

$$\text{where } |z| \leq \frac{L}{2} \quad \text{(Equ. 2.5);}$$

Where $C_n$ is the Fourier coefficient and kn is factor given as

$$k_n = \frac{(2n-1)\pi}{L} \quad \text{(Equ. 2.6).}$$

The RF field can be expressed in terms of radial $B_\rho$ and azimutal $B_\varphi$ components of the magnetic field:

$$B_x = B_\rho \cos\phi - B_\varphi \sin\varphi \quad \text{(Equ. 2.7).}$$

Receiving coils needed to be designed and positioned so that they are maximally sensitive to emitted radio frequency signals, that is so that they have the largest possible voltage induced in them by the radio frequency signals emitted by the tissue.[28]

The approximation is made as accurate as necessary by increasing the number of such monopoles. Any two consecutive monopoles are defined as a V-shaped dipole over which a testing function is defined. Satisfying the boundary conduction that the current vanishes at both ends of the dipole. The n-th testing function is non zero only when the V-shaped dipole corresponds to the V-shaped dipole and zero otherwise. The analysis of electromagnetic fields in today's era is carried out by specialized software packages (Fig. 1).[29-32] The enormous development of numerical methods, which has become possible as a result of the continuous increase in computing power of computers caused that very complex 3D objects are analyzed. The project starts with making assumptions and developing a drawing of a 3D object. Next, each component is assigned material properties and an analysis area is assumed after which the results are most often presented in the form of colored graphs or maps of the distribution of the analyzed parameters.[33-38]

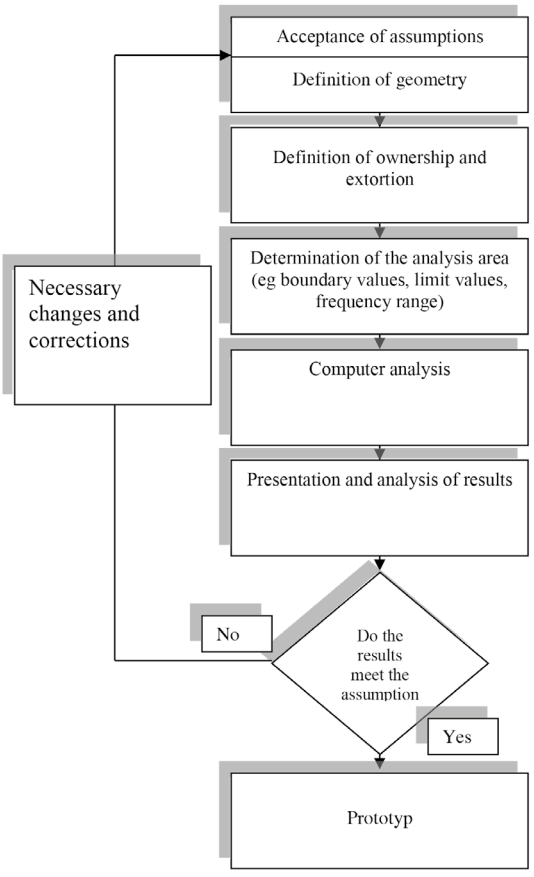


**Fig. 1.** Simplified computer design scheme

Figure 2 presents two types of birdcage coils. Both types of coils can be used in magnetic resonance systems. The proper selection of geometric dimensions as well as the values of capacities included in the circuit define the characteristics of the coils. From the point of view of the principles of operation of magnetic resonance systems, the receiving coils are resonant circuits with strictly defined properties. The fundamental and most important is the resonant frequency f0 which

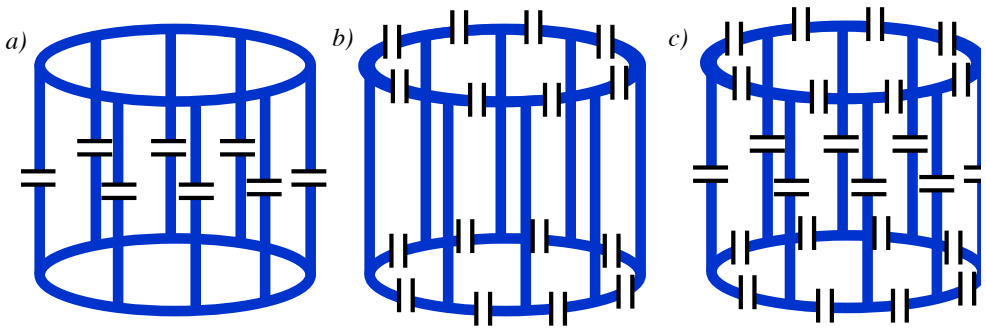


**Fig. 2.** Birdcage coils, a) high-pass coil, b) low-pass coil, c) quadrature transmit coil

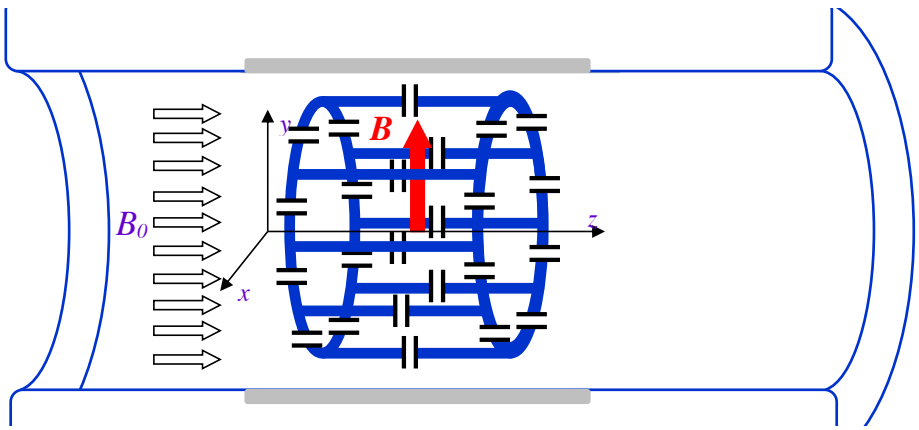


**Fig. 3.** Schematic diagram of magnetic resonance magnet

strongly depends on the geometry of the circuit. Another important parameter is the Q quality of the resonant system as well as the wave impedance. Birdcage type coils are practically the most used transceiver devices currently in MR systems. Their structure is based on two rings connected with conductive rods. The number of these parallel element's ranges from 8 to 32. Condensers are placed between the conductive elements.

Figure 3 presents an illustrative schematic of the MR system. The main element is a magnet that generates a constant magnetic field and induction B0 = 1.5 [T]. Inside it there are additional coils to generate magnetic gradients and coils to level the field. Noteworthy is the BODY coil, which is the most often transceiver-receiving device and is built into the MR system permanently. The birdcage coil presented can be both a transmitting and receiving element. This type of coil allows the reception of a signal generated by a variable magnetic component in the X-Y plane. In the drawing, this vector is marked in red.

## Conclusions

We discussed the major orations of MRI coils and discussed the major physical phenomenon involved in MRI together with RF coil design.

## Acknowledgements

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## The list of abbreviations:

**a** - radious of RF coil, **L** - total length of the coil, **r** (Φ, z) - current vector

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**REVIEW PAPER**

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# The role of MRI in the central nervous system

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**ABSTRACT**

**Introduction.** Magnetic Resonance Imaging (MRI) has modified the practice of radiology. MRI is base on safe interaction between radiowaves at a particular frequency and hydrogen nuclei in the body. Metabolic encephalophaties are by definition those disorder of the central nervous system that are not due primarily to structural abnormalities.

**Aim.** Here we present the $^{1}$H MRI and functional MRI (fMRI) method applied to diagnosis of disorders of the central nervous system.

**Material and methods.** Analysis of literature and self-research.

**Results.**We have discussed the major MRI applications in the characteristic of the central nervous system. The relationship beteen the motion of flowing blood and the representation of the blood on images is complex. This work is an introduction to the basic ideas and techniques of fMRI. Therefore, both, $^{1}$H MRI and functional MRI, methods are ued in neuroscience.

**Conclusion.** Nonivasive MRI and functional MRI are daily diagnostics methods in neurology.

**Keywords.** $^{1}$H MRI, functional MRI, metabolic brain disfunctions

## Introduction

Magnetic Resonance Imaging (MRI) has modified the practice of radiology. MRI is base on safe interaction between radiowaves at a particular frequency and hydrogen nuclei in the body. Metabolic encephalophaties are by definition those disorder of the central nervous system that are not due primarily to structural abnormalities.

## Fundamental applications of $^{1}$H Magnetic Resonance Imaging in metabolic brain disfunctions are:

- in herpes simplex encephalitis
- in neurosacarcoidosis
- in Sjögren's syndrome
- in toxoplasmosis
- in isolated angiitis of the nervous system

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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## In herpes simplex encephalitis

Cerebro spinal fluid (CSF) characteristically shows a lymphotic pleocytosis with mild or moderate elevation of protein and local normal glucose.[1] CSF analysis is a set of laboratory tests. CSF test includes visual observation of color and clarity, tests for glucose, protein, lactate, lactate dehydrogenase, red blood cell count and white blood cell count. Documentation of temporal lobe involvement may be obtained by ECG, radionuclide brain scan, computed tomography (CT) scan, or Magnetic Resonance Imaging (MRI). The role of each of these metabolities in the diagnosis of herpes simplex virus encephalitis is changing.[1] The ECG has been felt by the most consistently useful diagnostic modality early in the curse of infection. Radionuclide and CT scans should be used together since either test may be initially normal. The diagnostic accuracy of MRI as compared to radionuclide and CT has much greater clarity of morphologic details has been shown to provide elegant localizing informations in herps simplex encephalitis and in particular with gadolinium enhancement should provide earlier more precise localization of temporal lobe injury than can be achieved using CT.[1]

## In neurosacarcoidosis

A brain CT scan can detect discreate brain mass lesions, multiple nodles hydrocephalus, diffuse inflammation and areas of periventricular white metter hypodensity.[2] CT scaning is helpful in monitoring disease activity as juged by the size of a mass lesion or the degree of enhancement of diffuse inflammation.[2] MRI is very sensitive in detecting of sarcoidosis and is good for evaluating the paraseller area, posterior fossa and spinal cord. MRI has demonstrated hypothalamic lesions not seen on CT scan and has revealed clinically unsuspected disease involving white matter in the periventricular white matter in the periventricular regions. Althought both CT scan and MRI scan easily demonstrated hydrocephalus, MRI can better define an obstructing lesion at the levelof cerebral aqueduct or fourth ventricle. Furthermore, MRI can access CSF flow at sites of possible obstructing lesion at the level of the cerebral aqueduct. MRI with contrast agent is the preferred technique for the evaluating sarcoidosis. Enhanced images frequently reval diffuse or focal leptomeningeal disease and can demonstrate iflamation. A normal enhanced MRI scan does not exclude the diagnosis of sarcoidosis. MRI angiography has little to contribute in sarcoidosis.[2]

## In Sjögren's syndrome

Sjögren's syndrome is an autoimunne disease affecting lacrimal and salivary glands causing symptoms of xerophthalmia and xerostomia (sicca syndrome). Diagnosis is based on electencephalography and cerebrospinal fluid analysis are each abnormal in about two-thirds of patients with active central nervous system manifestation. MRI of the head has proved positive in 75 percent of cases with manifestations of Sjögren's syndrome.[3]

## In toxoplasmosis

The lumbar puncture is not helpful in making the diagnosis of toxoplasmosis. The useful finding is a nonspecific lymphocytic pleocytosis. Imaging of the brain with CT is the most useful test for toxoplasmosis.[4] MRI appears to be more sensitive than for demonstrating the lesions o f cerebral toxoplasmosis.

## In isolated angiitis of the nervous system

CT and MRI show multiple lesions in both gray and white matter. Normal results of any of the above tests can be suggestive of angiitis and exclude more common explanations for central nervous system. Angigraphic findings in cerebral artretis are classically described as intermittet narrowing and dilatation of blood vessels.[5] Angiitis confined to small vessels has been found at postmortem in case with normal angiography.

## Functional Magnetic Resonance Imaging

The field of functional magnetic resonance (fMRI) has extended enormously since mid-1990 and is dominated by basic neuroscience.[6-11] FMRI is very interdyscipinary and is providing the area to study a detailed map of the brain activation.[11-15] The possibility of measuring changes in brain blood flow associated with neural activity in the brain was known from 1998. FMRI results depend from blood volume. ach dot of light on the fMRI, the voxel (the pixel of fMRI screens), captures blood flow in the region of approximately 80,000 neurons and more than 4 million synapses.[15-24] This dot of light on the screen is the average measurement of activity over one second in this region. However, neuronal signaling occurs a thousand times faster, in the milliseconds. [24-34]

## Conclusion

MRI is used in practically the entire body. However, technological advances enable this technique to be applied in neuroscience.

## Acknowledgements

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**REVIEW PAPER**

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# Glycosylation of immune system proteins and its role in autoimmune diseases and cancer

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**ABSTRACT**

**Introduction.** Structural glycans have great biological significance and are involved in signaling and cell communication of the immune system. They are attached to proteins and lipids in an enzymatic process called glycosylation where glycosyltransferase and glycosidases bind sugar residues and lead to the formation of bioconjugates.

**Aim.** In this paper we describe the importance of glycosylation in the immune system and its changes in diseases.

**Material and methods.** This review was performed according to systematic literature search of major bibliographic databases.

**Results.** Proper glycosylation ensures the functioning of the organism, however, defects in structural glycans of immune system changes their properties and can lead to disorders and further to autoimmune diseases. It has been also proven that glycosylation of autoimmune system is changed during cancer. In this paper we described types of structural glycans, significance of glycosylation of selected components of the immune system and its modifications in disorders.

**Conclusions.** Knowledge about changes in the glycosylation in diseases is the key to understanding the processes of autoimmune diseases and may allow the development of new treatments in the future.

**Keywords.** Glycosylation, Immunity, Cellular, Humoral, Autoimmune Diseases

## Introduction

Glycosides are elementary biomolecules that are involved in many biological processes, both in prokaryotic and eukaryotic cells.[1] Proteins are one of the most commonly glycosylated structures. It is estimated that the structures of the human proteome are at least 40% glycosylated and that glycans can constitute up to 90% of the molecular weight of certain glycoproteins. Changes in glycomes lead to new properties of cells and are often caused by changes in environmental and genetic conditions.[2] However, these modifications can be both positive and negative and can manifest themselves in various diseases.[3]

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## Aim

In this paper we describe the importance of glycosylation in the immune system and its changes in diseases. Understanding the mechanisms, modifications and changes occurring in glycan of specific diseases is very important and can help in the development of new diagnostic methods, therapies, and treatments.

## Analysis of the literature

Glycosylation is an enzymatic process involving the attachment of carbohydrate groups (sugars, oligosacharids) to proteins, lipids or other oligosacharides by glycosidic bond. In this reaction, important bioconjugates such as glycosphingolipids, glycoproteins, glycosaminoglycans, and proteoglycans are formed. Glycosylated proteins (glycoproteins) of great biological significance are created in the post-translational processing of proteins in endoplasmic reticulum (ER) and Golgi apparatus.[4] Fucose (Fuc) and mannose (Man) residues are attached to many proteins which guarantees them successful folding of the in the ER and provides them with protection against enzymatic degradation - proteolysis in and outside of the cell. Oligosaccharides attached to proteins also have functions in inter-cellular interaction and cell signaling which ensures – among other – that the structure is properly transported to the target organelle.[1,5]

Glycosyltransferases and glycosidases, mostly found in the ER, are crucial for glycosylation process. On the other hand, several enzymes of this type which attach single sugar residues can be found in the cytosol. Glycosyltransferases catalyze the selective transfer of a glycosidic bond using sugar donors. One of the most common are: nuclesides diphosphate (e.g. GDP-Man) but also derivatives of: monophosphate nucleosides (e.g. CMP-NeuAc); lipids phosphates (e.g., dolichol phosphate oligosaccharides); or unsubstituted phosphates. In contrast, glycosidases hydrolyze glycosidic bonds, thereby releasing saccharide molecules.[5,6]

There are two basic types of glycosylation depending on the atom connecting carbohydrate group with protein, forming N- and O-glycans.[7] N-glycans are linked through a glycosidic bond between the amide group of an asparagine residue in the Asn-X-Ser/Thr combination (where: Asn – asparagine; Ser – serine; Thr – threonine; X is any amino acid residue except proline) and Nacetylglucosamine (GlcNAc). Through the function of membrane enzymes – glycosyltransferases and glycosidases – located in the ER and Golgi apparatus, three basic N-glycans are created: oligomannose, hybrid, and complex type. Each of them contains the basic $Man_3GlcNAc_2Asn$ core (Fig. 1A). Oligomannose structures consist of many mannose molecules (Man) but a maximum of nine. Each of them, form α(1,6) and α(1,3) bonds, leading to a branching of the core structure but the terminal Man are connected by α(1,2) bonds. Hybrid N-glycans are formed from the oligomannose type, due to the action of transferases in the Golgi apparatus in which some of Man residues are removed and replaced with GlcNAc, sialic acid (SA), fructose, and galactose (Gal) residues. Subsequently, the structure can be subjected to further reactions which leads to complex structure formation.[2,7,8] O-glycans are linked by a glycosidic bond with the oxygen atom of the hydroxyl group

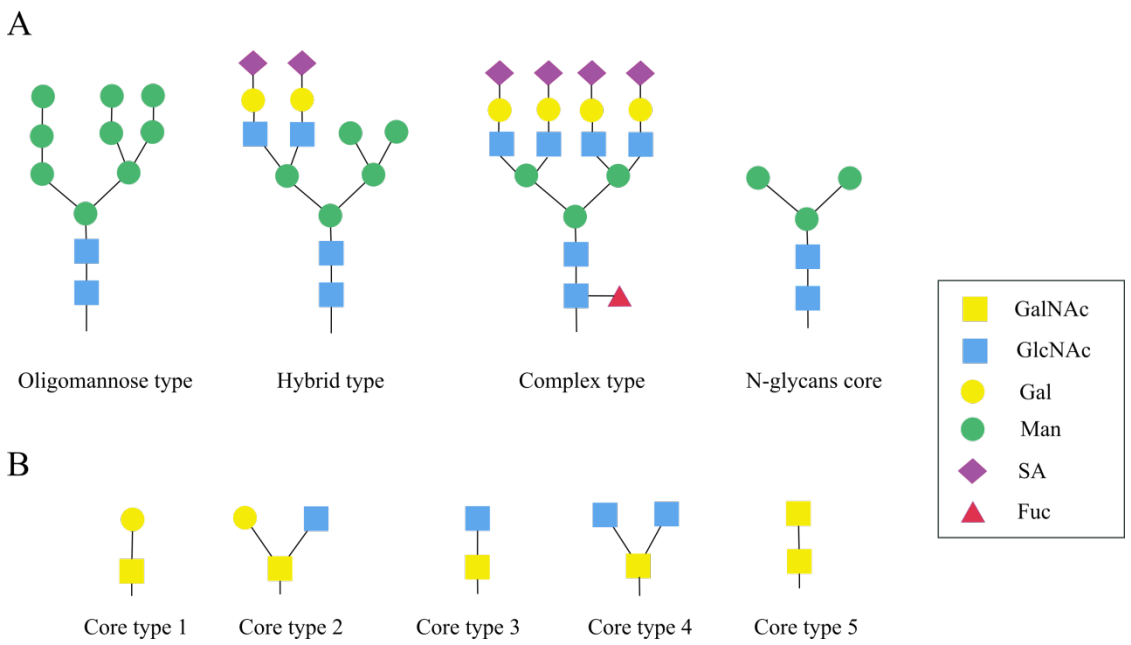


**Fig. 1.** The basic structures A) N-glycans B) O-glycans. Abbreviations: GalNAc – Nacetylgalactosamine; GlcNAc – N-acetylglucosamine; Gal – galactose; Man – mannose; Fuc – fucose; SA – sialic acid

of a serine or a threonine residue with GalNAc (Nacetylgalactosamine) or GlcNAc. In contrast to N-glycans with one core structure, O-glycans present five core combinations which may additionally differ in configuration and manner of binding of sugar residues. Each of the cores can be further modified and extended by further mono- or oligosaccharides (Fig. 1B).[9] In addition, they can be created even in the cytosol where N-glycosylation does not occur.[4] Furthermore, a common terminal structural element is the LacNAc (N-acetyllactosamine), which can be attached to various protein sites in both O- and N-glycans, leading to the formation of a structure called polylactosamine[2].

As already mentioned, glycosylated biochemical structures are involved in signaling and intercellular communication.[1,5,10] It applies to the immune response which is based on recognizing and responding to the presence of antigens. It is due to the ability to distinguish host cells from others which in turn is possible due to the various glycans present on the surface of both host's immune system and other, foreign cells.[11] The entire range of glycosylated proteins allows for appropriate immune responses that are associated with: leukocyte migration; cell guidance and apoptosis; activation of B and T lymphocyte receptors; antibody functions; antigen presentation by MHC (major histocompatibility complex) as well as differentiation of lymphocyte subpopulation.[12] Below, selected specific types of glycosylation of molecules and cells, responsible for the body's immune responses are presented.

Lymphocytes are an important group of cells involved in cellular and humoral responses. During the formation of T lymphocytes in the thymus, already in the early fetal development, there is an intense production of the T cell receptor protein (TCR), basic for recognizing antigens presented by MHC.[13] It is one of many immune-system proteins that are glycosylated. Heterodimer unit in the form of αβTCR or γδTCR is a membrane protein that contains at least 7 potential Nglycosylation sites.[14,15] β(1,6) glycosylation of GlcNAc and further branching with residues LacNAc, allows binding of galectin 3 (Gal-3) – an endogenous protein capable of binding oligosaccharides – to the TCR receptor which regulates the activity of T lymphocytes.[16] Galectin 3 defines the binding surface of the TCR receptor which reduces the affinity of MHC and blocks its activity by preventing disorders leading to autoimmune diseases.[14,17,18] Another glycosylated protein of immune system is the CD45 membrane protein. Change in the structure of N- and O-glycans of this protein for both, B and T lymphocytes is a determinant of the stage of cell differentiation. For example, naive and memory B cells are characterized by linear structures of N-glycans (poly) LacNAc.[19,20]

Immunoglobulins are proteins secreted at various concentrations by B lymphocytes in the immune response. Five classes of human immunoglobulins are distinguished: IgA, IgD, IgG, IgE, IgM, but the most common is IgG.[21] They all undergo glycosylation which regulates their properties such as conformation and stability, half-life as well as the ability to bind specific antigens and other immune system proteins such as receptors and lectins.[22] These proteins are mainly N-glycosylated, but O-glycosylation of IgA1, IgD, and IgG3 also occurs, however IgA1 is characterized by 3 to 5 glycosylation sites with mucin-type structures which contain terminal GalNAc residues.[23-25]

IgG participates in humoral and innate immune responses. It consists of four polypeptide chains – two heavy and two light chains, forming the characteristic shape of the protein in the form of the letter Y. The antibody has two domains – Fab (antigen binding fragment), responsible for antigen binding and Fc (crystallizable fragment) that binds to receptors.[26,27] Proteins of this subclass have only one Nglycosylation site at $Asn^{297}$ in Fc domain where glycosylation prevents a change in protein conformation, guaranteeing characteristic chain arrangement and receptor binding ability.[28] It has been shown that the crystallizable Fc region undergoes glycosylation changes during disease states, affecting anti-inflammatory properties. In non-pathological conditions, 15-25% of the Fab surface are glycans and it contains more GlcNAc structures, multi-mannose branches, and much more α2,6-sialic acid residues, compared to Fc, which in turn is characterized by increased fucosylation.[29] High fructose content inhibits the cytotoxic cellular response while high content of terminal SA, especially in the α(2,6) configuration, inhibits the inflammatory response.[28,30]

Glycosylation disorders lead to the development of various diseases, including autoimmune diseases and cancers (Table 1).[2,4,11,14,17,31-43] Lack of appropriate sugar residues in receptor proteins interferes with their function, thereby blocking signaling pathways. Dysregulation of the N-glycosylation pattern can lead to the pathological behavior of T cells.[10] For example, defects in the synthesis of Nglycan complexes on T-cell surfaces, results in the enhancement of the TCR receptor signal, resulting in disorders manifested by lower specificity of T lymphocytes of detection to antigens. This phenomenon is observed on autoimmune encephalomyelitis or glomerulonephritis whereas kidney disease occurs in humans as a consequence of type 2 diabetes.[33,44] Glycosylation disorders are very often the outcome of mutations in the genes responsible for enzymes attaching sugar residues. Ohtsubo et al. have shown that glycotransferase disorders transferring GlcNAc on mannose residues in N-glycosylation, lead to glucose transporter receptor (Glut-2) disorders and thereby interfere with insulin secretion, leading to type 2 diabetes.[32] Another example is the lack of the *Mgat5* gene, coding for

**Table 1.** Summary of glycosylation disorders and their effect on autoimmune and cancer diseases

| | Glycosylation disorder | Glycosylation type (N/O) | Outcome of disorder | Diseases | References |
|---|---|---|---|---|---|
| 1 | Disabling GlcNAcT-IVa glycosyltransferase | N | Receptor defect for glucose transporter (Glut-2) | Type 2 diabetes | 32 |
| 2 | Disabling β(13)-N-acetylglucosaminyltranserase action V | N | Reduction of TCR receptor glycosylation | Lower specificity for antigens; increased susceptibility to autoimmune diseases | 14 |
| 3 | Disabling β (1-3) -N-acetylglucosaminyl transferase II | N | Reduction of TCR receptor glycosylation | Lower specificity for antigens; increased susceptibility to autoimmune diseases | 33 |
| 4 | Limiting glycosylation of T lymphocytes | N | Increased T cell response; less specificity for antigens | Autoimmune encephalomyelitis; glomerulonephritis | 11,17 |
| 5 | Molecular chaperone Cosmc glycosyltransferase defect | O | Lack of sugar residues galactose and sialic acid on the Tn antigen | Haemolysis; thrombocytopenia | 34,35 |
| 6 | No sialic acid residues or abnormal binding to CD45 receptor | O | Intercellular signaling disorder by lectin binding disorder | Rheumatoid arthritis; systemic lupus erythematosus | 36 |
| 7 | Lack of sialic acid residues in the Fc domain of IgG antibodies | N | Chronic inflammation | Multiple sclerosis; rheumatoid arthritis; cancer of the prostate, stomach, large intestine and bladder | 37-40 |
| 8 | Tn antigen sialylation | O | Cancer hosting; neoplasia | Cancer | 35,41 |
| 9 | Additional mannose residues in IgG | N | Stimulation of cancer cell growth and survival | Lymphomas | 42 |
| 10 | Sialylation of cancer cell surfaces | - | Disorder of cancer cell recognition by the immune system cells as abnormal | Cancer | 31,43 |

the Nacetylglucosaminyltranserase V (GnT-V) branching enzyme responsible for attaching the GlcNAc β (1-6) residue to the TCR receptor which results in hyperactivity of T lymphocytes.[14] A similar dependence was observed in the mutation of the gene encoding the enzyme attaching the N-acetyllactosamine residues – GnT-II (β(1-3)-Nacetylglucosaminyltransferase).[17] The occurrence of autoimmune diseases is not only associated with changes in the structure of N-glycans, but also in O-glycans disorders of mucin-type proteins. An example is the Tn syndrome characterized by the lack of a developed Tn antigen (galactose and sialic acid defects) on the surface of blood cells which leads to hemolysis and thrombocytopenia (thrombocytopenia). Glycosyltransferase malfunction is responsible for this glycosylation changes and it is caused by the Cosmc molecular chaperone defect of this enzyme, crucial for O-glycosylation of proteins.[34,35]

A compound that plays a large role in the development of human immunity, starting from fetal development, is sialic acid, which affects the activity, half-life and transport of significant proteins such as immunoglobulins.[45] An example of a disorder caused by the loss of free sialic acids in oligosaccharide branches is the undesirable formation of CD45 receptor dimers. Reduced glycosylation of the protein leads to inhibition of signal transduction which results in the arrest of lymphocyte activity, thereby causing autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus.[36,47] In addition, sialylated IgG in Fc domain produced by B lymphocytes, have a role in the process of rheumatoid arthritis because they exhibit anti-inflammatory properties. It has already been proven that among people suffering from rheumatoid arthritis, the presence of sialylated IgG is undetectable, but its concentration increases significantly during remission[39]. Similarly, changes in the glycosylation of IgG effected by the decrease in the level of sialylation have been demonstrated in patients with multiple sclerosis in which the presence of pro-inflammatory factors leading to a chronic inflammation in the body is also characteristic.[37,38] It is interesting that the appearance of Tn antigens with sialic acid in the O-glycan structure (Neu5Acα2,6GalNAcα1-Ser/Thr) may be a marker for detecting the early phase of tumor formation.[35,41]

As mentioned above, the manifestation of glycosylation aberrations in cells of the immune system also applies to cancer processes. Glycans are important for the communication of cancer cells during their invasion and metastasis, as well as for fooling and escaping from the immune system, and even for their drug resistance by influencing the mechanism and absorption of the drug.[47,48] Changes in glycosylation have been proven in many cases. Glycosylation aberrations in IgG have been described for prostate, stomach, colorectal and urothelial type bladder cancer. Tanaka et al. showed that in prostate (P) and bladder (U) cancers, the sIgG number significantly decreased in both study groups compared to the healthy controls (C). In addition, Tanaka et al. showed that the

U group had a lower IgG concentration than the others.[40] In different types of lymphomas, Nglycosylation sites in host antibodies are added. They are modified by oligomannose structures bound by Mannose Binding Lectin (MBL) with oligomannose structures, triggering stimulus signals, thereby promoting tumor growth and survival.[42] Compounds on the membranes of cancer cells may also be glycosylated. The high sialylation surface on the cell surface disrupts the recognition pathways of non-host structures and as a result, cells that should be destroyed – are not. In this way, inter alia, are interrupted: signals for CD8+ T cells or ligand for the Fas receptor, favoring the development of the disease.[31,43]

## Conclusions

Glycosylation is a very important process that determines the survival of the organism. It is the basis of the immune system, by giving high variability to molecules ensuring its functioning. Its disorders can lead to the development of diseases characterized by autoaggression towards the host organism. The progress of science towards understanding the mechanisms of glycosylation in the immune system is key to understanding the processes of autoimmune diseases. Comprehension the mechanisms that determine the course of immune responses in healthy and sick people will allow us to develop new treatments in the future.

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**REVIEW PAPER**

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# Treating kidney cancer – a review

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**ABSTRACT**

**Introduction.** Kidney cancer in the structure of registered cases was in 6th place in men and 8th in women.

**Aim.** Understanding the molecular biology of renal cell carcinoma has made it possible to produce new diagnostic methods.

**Material and methods.** This review was performed according to a systematic literature search.

**Results.** Minimally invasive techniques seem to have a bright future in kidney cancer. However, they still require many clinical trials before they enter the general clinical use.

**Conclusion.** Photodynamic therapy, thanks to research conducted in kidney cancer, will find application in cancer of other organs.

**Keywords.** kidney cancer, malignant tumors, MRI

## Introduction

The number of malignant tumors in Poland over the last three decades has more than doubled, reaching more than 140.5 thousand cases in 2010, affecting about 70,000 men and 70.5 thousand women.[1] The incidence of malignant tumors in Poland in 2015, in absolute numbers, was 81,659 for men and 81,661 for women. These numbers, in Podkarpacie, were respectively 4,665 and 4,276.[2] The mortality rate for malignant neoplasms in Poland in 2015 totaled in absolute numbers 55,663 men and 44,938 women, and 28,586 in the Podkarpacie region alone for men and 1,904 for women. Kidney cancer in the structure of registered cases was in 6th place in men (3.9%) and 8th in women (2.4%). In Podkarpacie, kidney cancer took one place higher - 5th place in men (5.1%) overtaking stomach cancer, and 7th place in women (3.1%) overtaking cervical cancer.[2] Although the incidence of kidney cancer in men was lower than the average for EU countries, in 2010 the mortality rate was about 25% higher than the average for EU countries (data from 2009).[3] In 2013/14, the percentage of deaths from cancer was about 30 percent. lower than the average for Poland, however, malignant kidney tumors are still a serious epidemiological problem.[4] Almost 90%

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of malignant kidney tumors are renal cell carcinomas (RCC), while 80% of renal cell carcinomas are clear cell carcinomas (RCCC).[5-6] The most important prognostic factors in 5-year survival are the clinical stage, grade, local stage of the tumor, the status of local lymph nodes and the presence of distant metastases.[7-16]

## Material and methods

This article is based on an analysis of articles posted on the PubMed website (https://www.ncbi.nlm.nih.gov/pubmed), books and websites.

## Results

**Minimally invasive techniques**

Percutaneous ablation and cryotherapy were originally intended only for patients with one kidney, patients with multiple tumors, elderly patients, and for those patients who did not qualify for surgery. However, the percentage of these procedures has recently increased in patients with small tumors, most frequently detected incidentally.[17] Eight percent of patients in the US are treated with minimally invasive methods, compared to 1998, when only 4% were treated using these methods.[18] This is possible thanks to the positive opinions of preliminary reports on the control of the cancer process.[19-23]

## Operational techniques

**Surgical treatment should be divided into two categories:**

a) saving operations. The goal of sparing treatment should be optimal regional tumor control, combined with minimizing ischemia, ideally under 30 minutes.[24] Patients with T1a and T1b tumors (i.e. tumors that do not exceed 7 cm in the largest dimension) and the normal function of the other kidney have comparable results to those operated with the radical nephrectomy technique.[25-27] Nevertheless, some patients cannot be operated on with saving techniques due to the unfavorable location of the tumor or local advancement. In the hands of an experienced surgeon, the results obtained using the laparoscopic, laparotomy and robotic methods are comparable.

b) radical nephrectomy. During this operation the kidney, perirenal fat tissue, regional lymph nodes and adrenal glands are removed.[28] This is the preferred technique for tumors growing in the inferior vena cava. However, it leads to an increased risk of chronic kidney disease and increased mortality associated with the cardiovascular system.[29-31]

Sparing therapy is more likely to preserve kidney function, reduce the number of cardiovascular events and reduce mortality therefore, radical treatment should not be used when a saver treatment is possible.[32-33] Studies show that there are no significant differences in the group of lymphadenectomy patients and in the group of patients who did not remove lymph nodes.[34] Lymph nodes are pre-operatively assessed in computed tomography and/or in magnetic resonance imaging as well as intraoperative palpation. Some authors report that CT/MRI may not show the presence of small metastases to regional lymph nodes.[35] Adrenalectomy should only be performed if the adrenal glands appear suspicious in preoperative imaging or when the tumor is located in the upper pole of the kidney near the adrenal glands.[36-37] Metastasomy is performed in patients with diagnosed synchronous metastases or in patients with metachronic metastases in the general good state, which metastases responded to therapy and are removable. This treatment should be considered because complete removal of metastases improves the prognosis.[38]

**Systemic treatment**

**a) chemotherapy**

Most kidney cancers develop from proximal tubules. These coils have a high level of expression of the P-glycoproteins responsible for drug resistance. For this reason, chemotherapy is not routinely used. The only exception is the combination of 5-fluorouracil with immunotherapy, but these data need confirmation.[39]

**b) immunotherapy**

IFN-alpha in randomized studies has demonstrated survival compared to patients with metastatic renal cancer receiving hormone therapy.[40] The combination of biological drugs is of interest to several randomized trials. For now, there is no evidence that combining drugs gives better results than monotherapy.[41]

**c) angiogenesis inhibitors**

Understanding the molecular biology of renal cell carcinoma has made it possible to produce new drugs. These include two recently-registered drugs in the US and Europe - these drugs target the vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) genes.[42]

Sorafenib is an inhibitor of many kinases that have activity against, inter alia, Raf-1, VEGFR-2, PDGFR. Three months of taking the drug increases the percentage of patients with progression free from 43% to 75% compared to placebo.[43] Sunitinib is an oxindole tyrosine kinase inhibitor (oxindol tyrosine kinase (TK) inhibitor). The median of progression-free survival in patients using sunitinib (11 months) is longer than in those using TNF-alpha (5 months).[44]

**Photodynamic therapy**

Currently, photodynamic therapy in renal cancer is only used in preclinical studies.[45] The first study in mice 46 reported tumor necrosis to a depth of 3-5mm without any side effects. In 2008, the first studies with photo-

dynamic therapy in in vitro kidney cancer appeared. It turned out that the uptake of marker (hypericin) and apoptosis occurs in almost 100% of cancer cells.[46,47] An additional discovery was the increase in radiosensitivity. In clinical practice, photodynamic therapy is used to assess surgical margins during sparing procedures.[48]

## Conclusion

In the last century, the treatment of choice in kidney cancer was nephrectomy. The more the procedure was performed with a wider margin, the better. Currently, thanks to a better understanding of molecular biology and the conduction of numerous studies, we come to the conclusion that saving treatments should not be just an alternative. Radical nephrectomy should be another option, not the first. Particular mention should be made of photodynamic therapy. It seems to be a safe and at the same time extremely effective and easy to carry out therapy. It is to be hoped that patients with renal insufficiency and other complications of nephrectomy will soon be only a fraction of a percent of patients with kidney cancer. Photodynamic therapy, thanks to research conducted in kidney cancer, will find application in cancer of other organs.

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**CASUISTIC PAPER**

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# Rare Benign cystic teratoma in the parotid gland

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**ABSTRACT**

**Introduction.** A teratoma is a tumor developed of several different types of tissue, like hair, muscle, teeth or bone.

**Aim.** Mature benign cystic teratomas are very rare in the salivary glands and just few cases were reported.

**Description of the case.** A 13 years old female was presented to our dental clinic of Princes Basma Hospital in Irbid in north of Jordan, with a painless, insidious progressive swelling in left parotid region without any significant family and personal history. Parents were cancer phobic and nervous from the condition of their daughter, they were very confused. There was no pain or any history of trauma. It was present just inferior to left ear cartilage. The mass was of size 3x3cm, non-tender, fixed, soft to firm in consistency, having smooth surface.

**Conclusion.** Teratoma in parotid region is an extremely rare entity. Lack of any pathognomonic feature, it is hard to diagnose preoperatively. Lumpectomy is advisable to remove the mass because the risk of damaging facial nerve in young patients and recurrence is rare. A definitive diagnosis is achieved after the histopathological study. Teratoma should be kept in account while evaluating a case of a soft tissue mass of parotid gland as a differential diagnosis.

**Keywords.** benign cystic teratoma, parotid gland, benign parotid tumours

## Introduction

Parotid tumors are the most common type of salivary gland tumors over all, they are for 80 to 85 percent of all salivary gland tumors. While most parotid tumors are noncancerous (benign), the parotid glands are where about 25 percent of cancerous (malignant) salivary gland tumors develop.

The parotid glands, are seen just in front of the ears on each side of the face, are the largest of the three pairs of major salivary glands. They are responsible for producing serous saliva to aid in chewing and digesting food.

Parotid tumors present a variety of characteristics. In the case of a parotid tumor, we may notice a swol-

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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len mass in your jaw area that may or may not be painful. If the tumor suspected to be malignant, it may also affect facial nerves, causing pain, numbness, a burning or prickling sensation, and finally loss of movement in the face.

A teratoma is a tumor developed of several different types of tissue, like hair, muscle, teeth or bone. Typically they form in the ovaries, testicles, or tailbone and rarely in other areas.[1] Teratomas found in babies, children, and the adults. Teratomas of embryonic origin are most often found in new borns, in young children, and, since the advent of ultrasound imaging technique, in fetuses. Mature benign cystic teratomas are very rare in the salivary glands and just few cases were reported.[2]

Fine needle aspiration (FNA) cytology is an important procedure in the primary diagnosis and management of cystic parotid gland lesions even it is not reliable. The diagnostic accuracy of this procedure can be improved by establishing a detailed clinical history, obtaining an adequate cellular specimen, and having comprehensive knowledge of the variety and frequencies of possible diagnostic entities that may present as cystic parotid gland lesions.

## Aim

Mature benign cystic teratomas are very rare in the salivary glands and just few cases were reported.

## Description of the case

A 13 years old female was presented to our dental clinic of Princes Basma teaching Hospital in Irbid in north of Jordan, with a painless, insidious progressive swelling in left parotid region without any significant family and personal history. Parents were cancer-phobic and nervous from the condition of their daughter, they were very confused. There was no pain or any history of trauma (exist several pathological diseases with same symptoms). It was present just inferior to left ear cartilage. The mass was of size 3x3cm, non-tender, fixed, soft to firm in consistency, having smooth surface.

Fine needle aspiration cytology came out to be of cystic lesion. CT (computed tomography) of that area revealed a hypodense space occupying lesion of size 3x3cm, after hematological investigations, ECG, chest x ray, patient was prepared for surgery.

Post tragus Incision done to perform lumpectomy. The mass was well encapsulated without interfering the facial nerve. It was smoothly dissected except the upper part was attached to ear cartilage, so we needed to detach by scalpel and it was punctured, then yellow discharge was released. We removed small part of the surface of the ear cartilage (fig.1). The mass was sent to histopathology department in 10% formalin solution (fig.2). After one we week stiches were removed and she came after two weeks with the histopathology report,

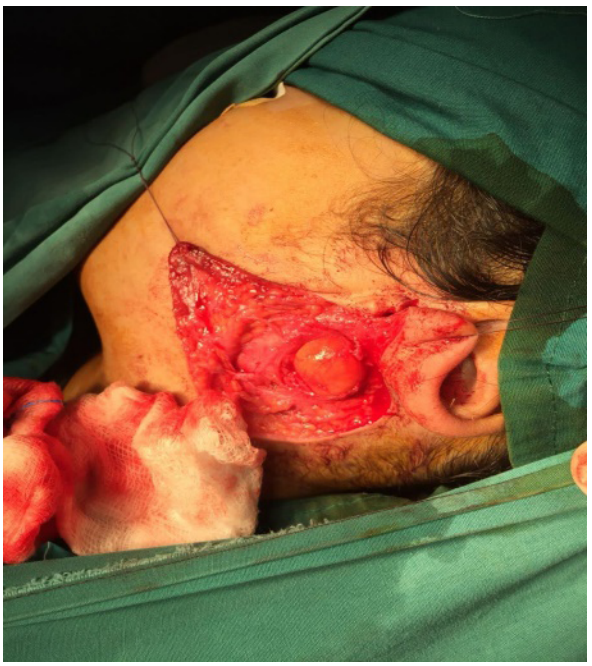

**Fig. 1.** Operation view

**Fig. 2.** Biopsy in formalin

which final diagnosis was benign cystic teratoma (fig.3). Patient was doing well.

Histopathologic examination showed a cystic cavity lined by keratinized stratified squamous epithelium resembling epidermis. The fibrous connective tissue wall contains sebaceous glands, hair follicles, skeletal muscle, and foci of hyaline cartilage (fig.4.).

After one year patient came to our clinic without any problems or signs of recurrence and the wound is healed fine.

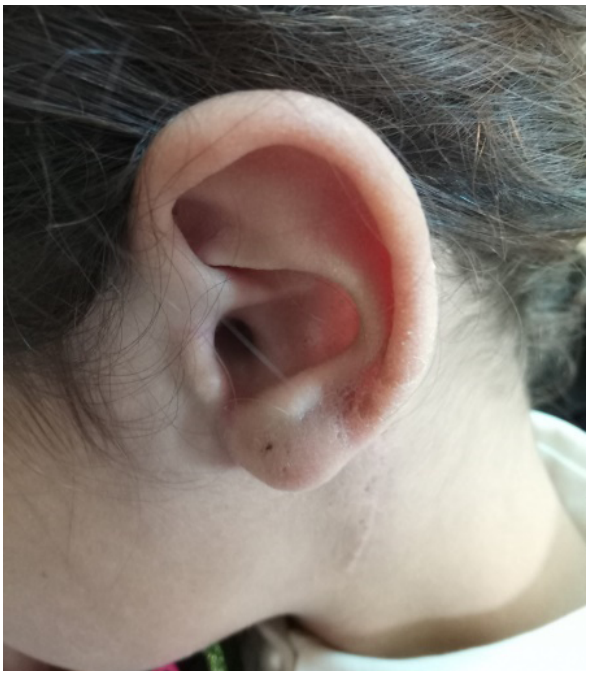

**Fig. 3.** Postoperative scar

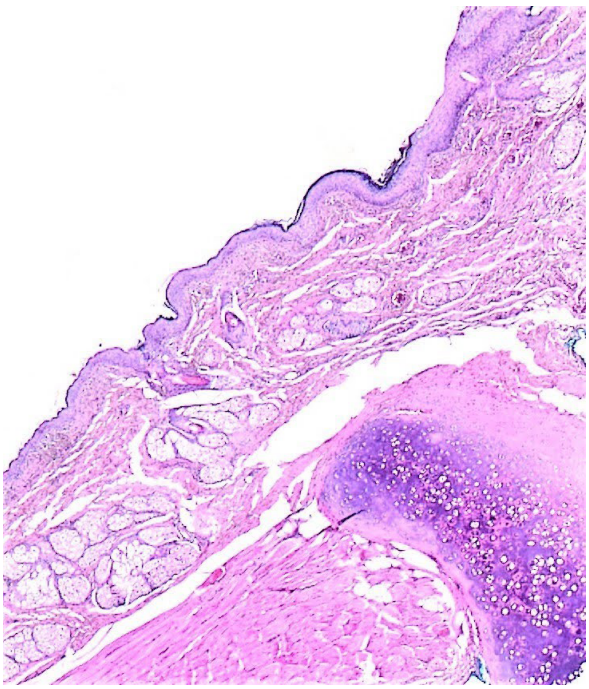

**Fig. 4.** H&E stain, original magnification X40

## Discussion

Teratomas are part of a category of tumors known as nonseminomatous germ cell tumour. All tumors of this category are the sequel of abnormal development of pluripotent cells: germ cells and embryonal cells. Teratomas of embryonic origin are always congenital; teratomas of germ cell origin may or may not be congenital developed. The kind of pluripotent cell appears to be unimportant, apart from constraining the location of the teratoma in the entire body.

Teratomas derived from germ cells are found in the testes in men and ovaries in women. Teratomas derived from embryonic cells often occur in the midline of the brain, elsewhere in the skull, in the nose, in the tongue, under the tongue, and in the neck called (cervical teratoma), mediastinum, retroperitoneum, and attached to the coccyx. Teratomas may also occur elsewhere: very rarely in solid organs (most notably the liver) and hollow organs (such as the stomach, heart and bladder), and more commonly in the skull sutures. More rarely such as in brain matter, teeth or eyes.[3,4]

A mature cystic teratoma in parotid salivary gland was first described in 1975 by Shadid et al.[3]

Parotid is a common site for cysts which may be congenital or acquired. Congenital cysts can be branchial cleft cyst, branchial pouch cyst, congenital duct cyst or dermoid cyst.[5,6] Acquired cysts may be of traumatic, neoplastic, calculi or parasitic i.e. hydatid cyst. Cysts can occur at any part of parotid. CT is better than Ultrasound to define the teratoma extent, relation to the surrounding organs and in evaluate cystic wall. The recommended treatment for parotid teratomas is surgical excision and recurrence is rare.[6]

It is very difficult to diagnose teratoma before final excision and rare recurrence make planning for total parotidectomy is problematic and here the risk of facial nerve injury is higher. Lumpectomy is satisfied procedure to preserve facial nerve, but follow up is very important because of risk of malignant transformation.

## Conclusion

Benign cystic teratoma is very rare in salivary glands. Surgical excision is for treatment and final diagnosis. In the parotid gland we must take into account high risk of facial nerve damage, so we prefer to make lumpectomy with periodic follow up.

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**CASUISTIC PAPER**

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# Splenic hydatidosis with abdominal pain – a rare presentation in a developing nation

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**ABSTRACT**

**Introduction.** Primary extrahepatic hydatid cysts are rare, and primary splenic hydatid cysts even rarer. Splenic hydatidosis constitutes 2% to 3.5% of all hydatid cysts.

**Aim.** To present a case report of splenic hydatidosis with abdominal pain.

**Description of the case.** We report here a case of isolated splenic hydatid cysts in a 23 year old female, who presented with dull dragging pain in the left hypochondrium. Diagnosis was made on computed tomography imaging of the abdomen and microscopic examination of the laminated hydatid cyst wall and supplemented with positive enzyme linked immunosorbent assay for hydatid antibodies.

**Conclusion.** The incidence of splenic involvement by hydatid cysts is very low. Man is an accidental intermediate host, as entry of the larval forms into humans represents an end stage in its life cycle. Until recently the gold standard treatment for splenic hydatidosis was splenectomy, as medical therapy seems to be ineffective. However, the last two decades have shown a tendency towards splenic conservative surgery in suitable cases, to reduce opportunistic post splenectomy infection.

**Keywords.** abdominal pain, histopathology, hydatidosis, spleen

## Introduction

The first description of splenic hydatid cyst was given by Berlot in 1790.[1] Hydatidosis also known as echinococcosis, is a parasitic infection of liver and other organs by flatworm, echinococcus. Echinococcus granulosus is the causative organism of hydatid cysts. Most hydatid cysts are acquired in childhood with a latent period of five to twenty years before the diagnosis.[2] The growth of hydatid cyst is very slow, as a very crude estimate of an increase in the diameter by about two to three centimeters each year.[3] The rate of growth of hydatid cysts depends not only on immunologic relationship between the parasites and humans but also on the resistance offered by the enveloping structure. The incidence of hydatid cysts has decreased in the endemic areas due to enforcement of public health measures and livestock handling procedures. Various measures like, public education about the disease and its transmission, instructions for vigorous hand washing after contact with canine species, elimination of vegetables grown at

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ground level from the diet, and discontinuation of the practice of feeding the entrails of slaughtered animal to dogs have led to the decrease in the occurrence of hydatidosis.[4] A mean incidence of splenic hydatid cyst in India is 4.3%, with the disease affecting all age groups and both sexes with equal frequency.[4]

## Aim

To present a case report of splenic hydatidosis with abdominal pain.

## Description of the case

A 23-year-old woman presented to the general surgery clinic with dull aching pain in the left upper quadrant of the abdomen for the past 3 months. Physical examination revealed tenderness and an ill-defined firm mass on the left side. Hemogram showed mild eosinophilia. Abdominopelvic computed tomography (CT) scan showed a 10×9×5 cm loculated cyst presenting as an unenhanced hypodense mass with well-defined borders in the spleen (Figure 1).

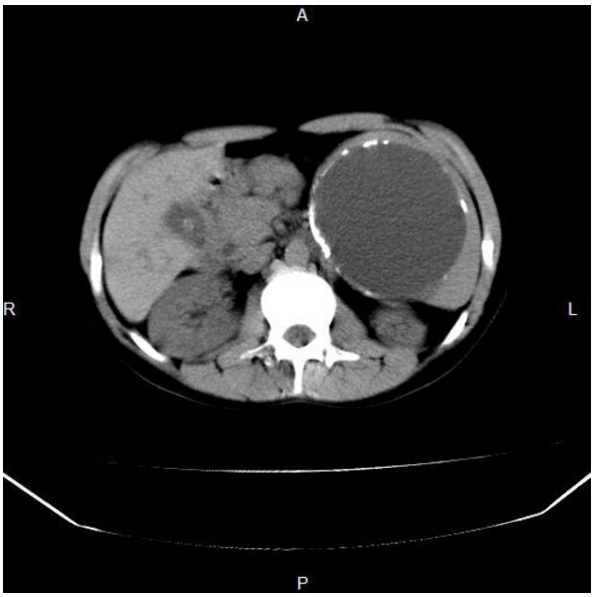


**Fig. 1.** Abdominopelvic computed tomography (CT) scan showed a single 10 × 9 × 5 cm loculated cyst presenting as an unenhanced hypodense mass with well-defined borders in the spleen

A CT scan of his chest did not show any cysts. On exploration, a huge hydatid cyst in the spleen of size 10.5×8.7×6.1 cm, filling the left side of the abdomen was seen. The contents of the splenic hydatid cyst was aspirated and all the endocysts were removed, followed by splenectomy. On microscopic examination, the hydatid cyst wall showed the characteristic laminated adventitial layer adjacent the red and white pulp of the spleen (Figures 2, 3, 4 and 5). The postoperative period was uneventful, and she was discharged with albendazole treatment with a dose of 10 mg/kg/day for 6 months, on the postoperative day.

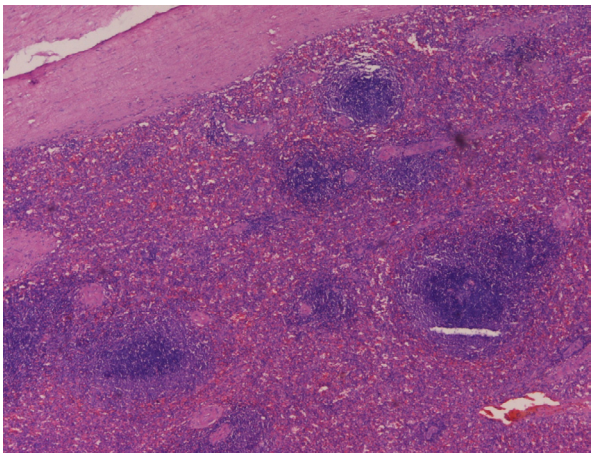

**Fig. 2.** Microscopic examination shows different sized lymphoid follicles in the splenic pulp with foci of the hydatid cyst wall with the characteristic laminated adventitial layer (10X) (H&E stain)

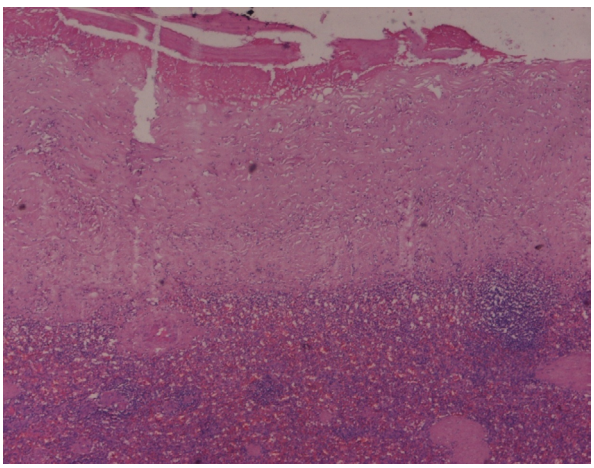

**Fig. 3.** Tissue section shows the hydatid cyst wall showed the characteristic laminated adventitial layer adjacent the red and white pulp of the spleen (10X) (H&E stain)

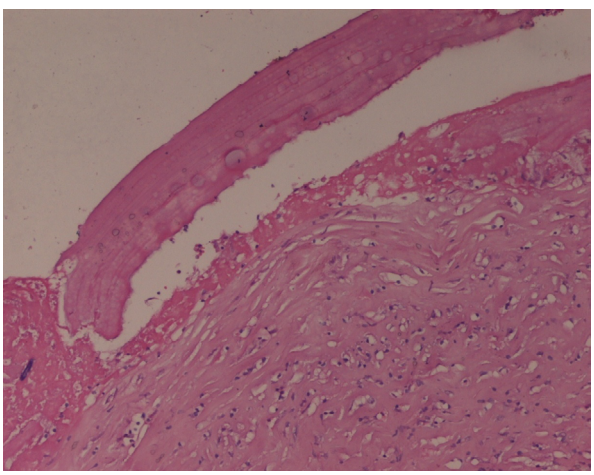

**Fig. 4.** Tissue section shows the hydatid cyst wall with the characteristic laminated adventitial layer (10X) (H&E stain)

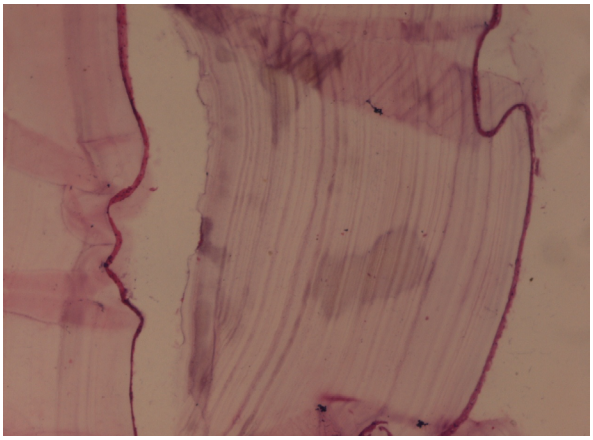

**Fig. 5.** High power of Figure 4 (40x) (H&E stain)

## Discussion

Hydatid cyst is the only parasitic cyst of the spleen.[5] In India, a mean incidence of splenic hydatidosis is 4.3%.[5] Liver is the most common site where hydatid cysts develop (70%), which acts as a first filter, followed by the lungs (15%), which acts as the second filter. No organ is immune to infestation by hydatid disease.[6] The rare sites of hydatid cysts include thyroid, omentum, pancreas, gall bladder, central nervous system and the kidney.[7-9]

Echinococcal cysts of the spleen usually grow very slowly and patients may be asymptomatic for 5-20 years before diagnosis. The symptomatology is usually mild with usual complaints of mild discomfort or pain in the left hypochondrium. The symptom complex produced by splenic hydatid cyst are mainly due to mechanical displacement and pressure effect on the adjacent organs.[10] Approximately 30% of splenic cysts are asymptomatic and detected incidentally. In addition 40% have no detectable physical signs.[10,11] Physical examination may reveal either a large palpable spleen or a hard, round and smooth mass, which follows the respiratory movements of the diaphragm.

Traub reported a splenic cyst causing hypertension by renal artery compression.[10] The splenic cyst can rupture and disseminate all over the peritoneal cavity. Other reported complications of splenic hydatid cysts are acute abdomen produced by splenic hydatid cysts coexistent with bilateral ovarian tumor, rupture of long standing splenic hydatid cyst into bronchial tree, traumatic rupture of splenic echinococcal cyst with anaphylactic shock.[11,12] Laboratory evaluation of patients with hydatid disease often yields non-specific data. Eosinophilia is not significant in endemic areas.[4]

A large battery of serological tests are available but their importance have been diminished by increased reliance upon modern imaging modalities like ultrasound (USG), computed tomography (CT) and Magnetic resonance imaging (MRI). Several serological tests are specific to hydatidosis and are used to confirm the diagnosis. Serum immunoelectrophoresis is currently the most reliable, with a sensitivity of approximately 90%.[4]

Complement fixation, enzyme linked immuno-sorbent assay (ELISA) and western blot analysis have also been used. The Casoni skin test is sensitive but not specific, and also remains positive for years after eradication of the organism. Plain radiology of abdomen can reveal a soft tissue shadow with or without calcification, displacement of left diaphragm upwards, stomach to the right and transverse colon with splenic flexure downwards. Celik et al have suggested displacement of neighbouring organs is characteristic of cyst of spleen rather than splenomegaly from other causes.[13] Ultrasound and Computed tomography alone or in combination established the definite diagnosis of splenic hydatid cysts in almost all the cases. These procedures can identify daughter cysts and hydatid sand, both of which are specific to echinococcal infestation.[4] Ultrasound is cost effective and particularly valuable for follow up screening. CT is more accurate than ultrasound in localizing and delineating extent of the cyst.[14] Postoperative histopathology will confirm the parasitic nature of this splenic cyst.

The development of echinococcal cysts in the spleen is uncommon because hexacanth embryos are usually trapped in the liver (first Lemman's filter) and/or lung (second Lemman's filter) but will be trapped in the splenic capillaries once in the systemic circulation.[8] Splenic echinococcosis may also arise by retrograde spread from the liver to the spleen via the hepatic portal and splenic veins in portal hypertension. The spleen may also be affected by rupture of a hepatic echinococcal cyst into the peritoneal cavity.[1,5]

Splenic echinococcosis needs to be differentiated from non-parasitic cysts, epidermoid cysts, haemangiomas, sarcomas, pseudocysts and tumours of the diaphragm, stomach, colon, left kidney or pancreas. The diagnosis is made by the history, physical examination, the presence of peripheral calcification or daughter cysts within a large cystic lesion or coexistent cystic lesions in the liver or other organs.[13,15]

The surgical procedures employed are total splenectomy and cyst enucleation and tube drainage of the cavity. The diagnosis of echinococcal cyst is confirmed by histopathology in all of the resected specimens.Medical treatment comprises of Mebendazole (60 mg/kg/day for 6-24 months) or Albendazole (10 mg/kg/day for 6 months). The treatment of hydatid cysts is principally surgical. However, pre- and post-operative 1-month courses of Albendazole and 2 weeks of Praziquantel should be considered in order to sterilize the cyst, decrease the chance of anaphylaxis, decrease the tension in the cyst wall (thus reducing the risk of spillage during surgery) and to reduce the recurrence rate post-opera-

tively.[4] Intra-operatively, the use of hypertonic saline or 0.5% silver nitrate solutions before opening the cavities tends to kill the daughter cysts and therefore prevent further spread or anaphylactic reaction. [4]

## Conclusion

The incidence of splenic involvement by hydatid cysts is very low. Man is an accidental intermediate host, as entry of the larval forms into humans represents an end stage in its life cycle. Until recently the gold standard treatment for splenic hydatidosis was splenectomy, as medical therapy seems to be ineffective. However, the last two decades have shown a tendency towards splenic conservative surgery in suitable cases, to reduce opportunistic post splenectomy infection.

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**CASUISTIC PAPER**

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# Atypical extra nasopharyngeal angiofibroma in an unusual location: tonsil posterior pillar (oropharynx)

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**ABSTRACT**

**Introduction.** Angiofibroma is a benign, locally aggressive highly vascular tumor that typically affects young adolescent males and has a pathognomonic epicenter of origin in the nasopharynx. The atypical angiofibromas share the same histological features as that of Juvenile nasopharyngeal angiofibroma, however they differ significantly in their clinical features.

**Aim.** Here we are reporting a very rare case of atypical angiofibroma in an adult male presenting as a non-vascular mass in the oropharynx with posterior pillar as the subsite.

**Description of the case.** A 26-years old male patient presented with chief complaints of foreign body sensation and mass in throat for one and a half months which was gradually progressive in size. Histopathological examination revealed a lesion with an intricate mixture of blood vessels, irregular fibrous stroma with loose edematous and fibrous area along with multinucleated stromal cells which was suggestive of angiofibroma.

**Conclusion.** Oropharyngeal angiofibroma, being an atypical angiofibroma in terms of site and presenting complaints, presents a diagnostic challenge. Though rare, it should always be kept as a differential diagnosis in any oropharyngeal mass irrespective of its vascularity, typical age or sex of the patient.

**Keywords.** atypical angiofibroma, extra nasopharyngeal (ENA), oropharynx

## Introduction

Angiofibroma is a benign, locally aggressive highly vascular tumor that usually arises from the lateral wall of the sphenopalatine foramen and pterygoid base.[1] But it has an early submucosal spread to nasopharynx and it mainly affects young adolescent males thus also known as Juvenile Nasopharyngeal Angiofibroma (JNA). However, it has also been reported at very young ages, elderly, and female patients and at the sites other than the nasopharynx. These rare variants have been termed as atypical angiofibromas.[2] The atypical angiofibromas share the same histological features as that of JNA, however they differ significantly in their clinical features.

Angiofibromas rarely originate outside the nasopharynx. Reports of primary extra nasopharyngeal angiofibromas (ENA) have appeared sporadically in the

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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literature. The most common site for atypical angiofibromas is the nasal septum.[3] Other less frequently involved sites are the maxilla, inferior turbinate, middle turbinate, ethmoid sinus, sphenoid sinus, oral cavity, oropharynx, larynx, ear, trachea, larynx, middle cranial fossa, infratemporal fossa, tonsil, retromolar region and conjunctiva.[4,5] To date, there have been only 10 cases reported of angiofibromas originating from the oropharynx in the literature.

## Aim

Here we report another rare case of atypical angiofibroma in an adult male patient originating from the oropharynx with posterior pillar as its subsite and this being the second case report in PubMed and google scholar literature.

## Description of the case

A 26 years old male patient presented in the O.P.D. of ENT department with the chief complaints of foreign body sensation and mass in the throat for one and a half months which was gradually progressive in size. He also complained of difficulty in breathing on lying down for one month. He had no history of any oral bleed or pain in the throat.

On examination, a single, pale, multilobulated mass with smooth surface was seen in the oropharynx arising from the right side posterior tonsillar pillar inferiorly reaching till right laryngopharynx (Figure 1). The mass was non pulsatile, soft on palpation, and did not bleed on touch. The rest of the ENT examination was normal. A contrast enhanced MRI was done which showed a polypoidal heterogeneously enhancing mass of size 8×17×20mm

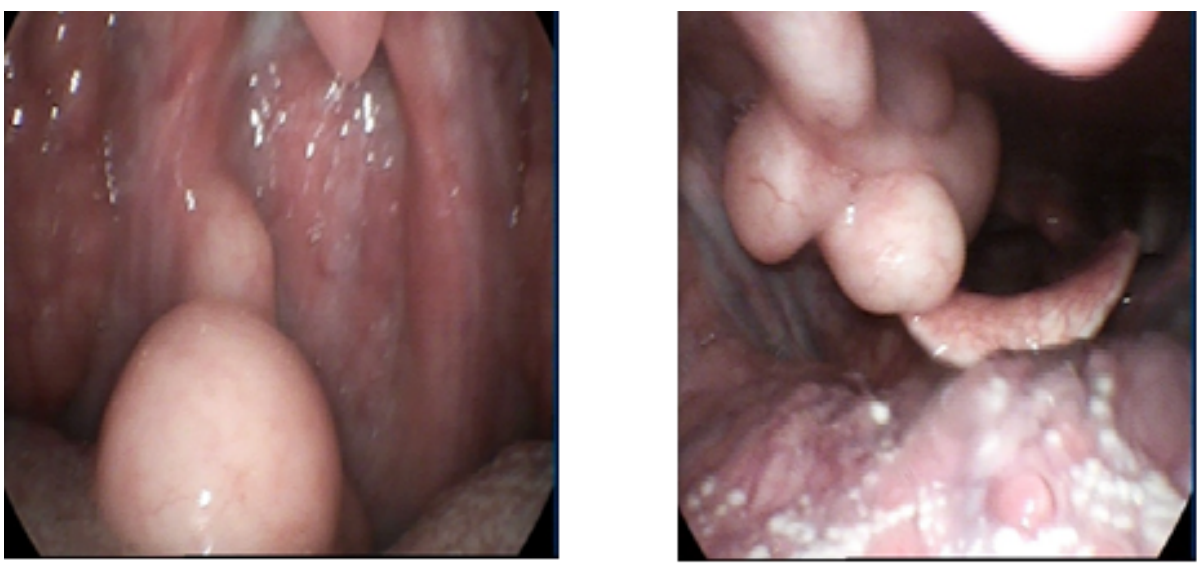

**Fig. 1.** Intraoral mass from the right side posterior tonsillar pillar inferiorly reaching till right laryngopharynx

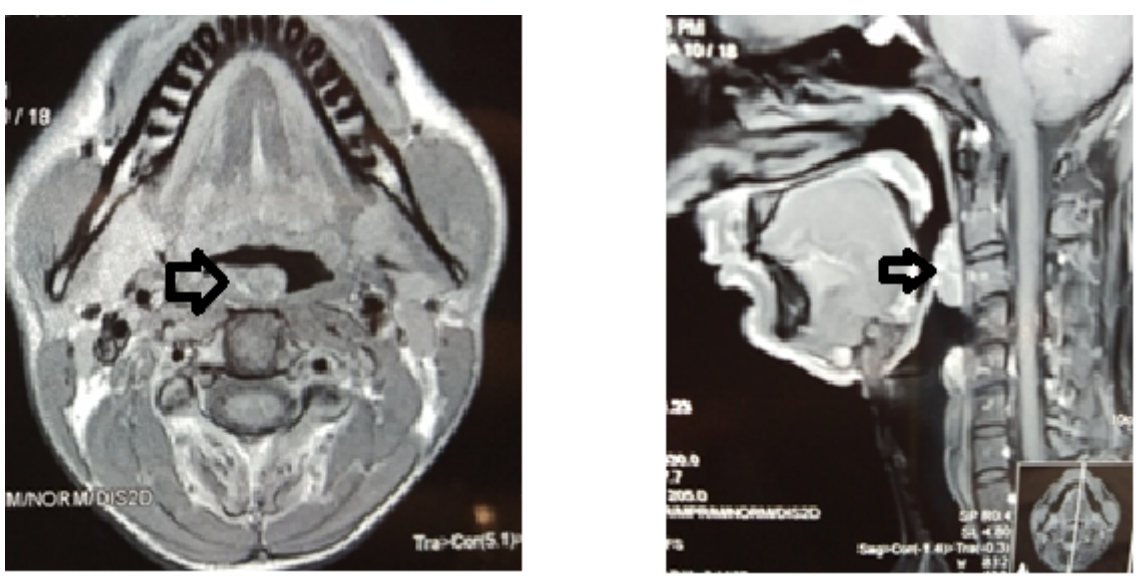

**Fig. 2.** Contrast enhanced MRI

on the right side of oropharynx (Figure 2). Excision of the mass was planned under general anesthesia.

Intraoperative findings revealed a 1.5 × 1 cm multilobulated mass attached to posterior tonsillar pillar on right side. The mass was completely excised using a coblator and the site of origin was also coblated (Figure 3). There was minimal intraoperative bleeding. The excised mass was sent for histopathological examination. Histopathological examination revealed a lesion with an intricate mixture of blood vessels, irregular fibrous stroma with loose edematous and fibrous area along with multinucleated stromal cells (Figure 4a, 4b). The images highlight the characteristic histopathological features of angiofibroma at 4X (fig 4a), 10X (fig 4b) magnifications. Immunohistochemistry panel applied:

- S-100: Non specific positive. Negative in tumor cells.
- Myo D1: Negative
- Desmin: Negative
- Ki-67: 1-2% Positive (Low proliferative index)

Above features found suggestive of angiofibroma.

**Fig. 3.** Excised mass intended for histopathological examination

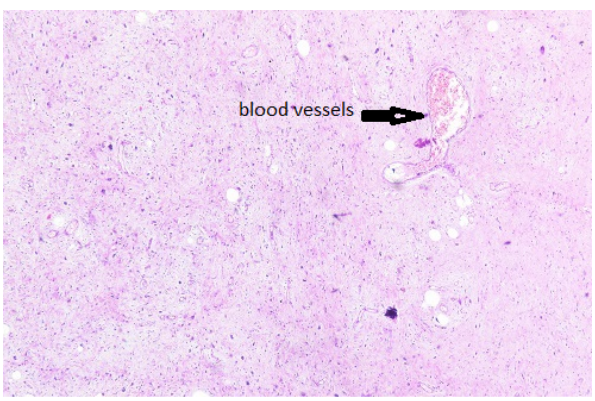


**Fig. 4a.** Histopathological examination of excised mass (4×)(H&E stain)

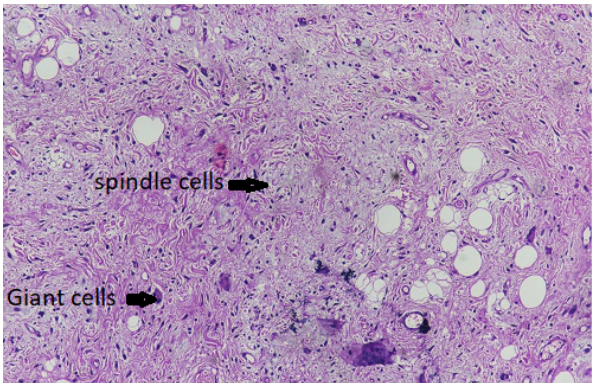


**Fig. 4b.** Histopathological examination of excised mass (10×)(H&E stain)

The patient was discharged on the third post-operative day and has remained disease free on his regular follow up for more than one year (Figure 5).

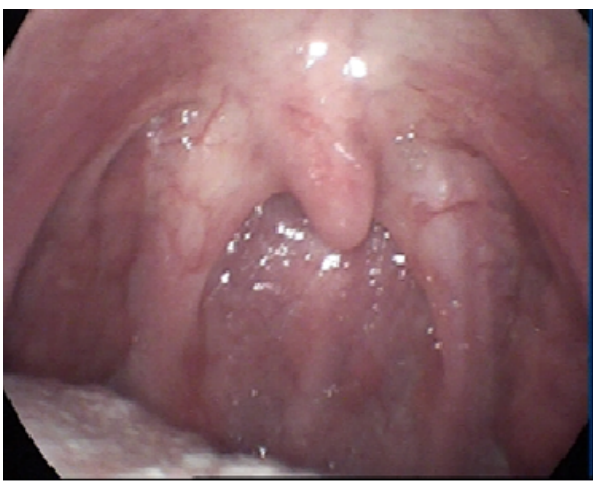

**Fig. 5.** Postoperative oropharynx

## Discussion

Angiofibroma is the most common benign vascular tumor in the nasopharynx, making up 0.5% of all head and neck tumors.[4] They are typically seen in adolescent males and are thus known as 'juvenile nasopharyngeal angiofibroma'. Celik et al. in 2005 proposed that angiofibromas presenting with at least one of the following criteria such as origin or location other than nasopharynx, presenting complaints other than nasal obstruction or epistaxis, age younger than seven or older than 25, female sex, atypical histopathology and multifocality were considered as atypical. Our case meets criteria via three parameters: age, location and presenting complaints, thus labeled as 'atypical angiofibroma'.[2,5-8]

Extra nasopharyngeal Angiofibroma (ENA) is a separate and rare entity. As compared to JNA, ENA occurs at older age with mean age of 22 years and the male to female ratio was 2.75:1. That is, there is a higher incidence of extra-nasopharyngeal angiofibroma in females compared to JNA. Windfur and Vent published a litera-

**Table 1.** Atypical extranasopharyngeal angiofibroma arising in oropharynx: literature review

| S. No. | Author/ Year | Age/ Sex | Subsite | Presenting Complaints | Symptoms duration | Management | IHC | Reference |
|---|---|---|---|---|---|---|---|---|
| 1. | Beeden (1971) | 1/M | Posterior pharyngeal wall | Oral and nasal bleed | 5 months | Radiotherapy further f/b transoral excision | Not Mentioned | [9] |
| 2. | Kim (1972) | 22/M | Not Defined | Dyspnea, Dysphagia, Altered Speech | 4 Months | Transoral Resection | Not Mentioned | [12] |
| 3. | Ali (1982) | 28/F | Left Tonsil | Foreign Body Sensation,Occasion Oral Bleed | 24 Months | Transoral Resection | Not Mentioned | [13] |
| 4. | Chung (1995) | 21/M | Soft Palate on Rt side | Accidental Finding | - | Transoral Resection | Not Mentioned | [14] |
| 5. | Cejas Mendez (2000) | 35/M | Right Tonsil | Dysphagia, Foreign Body Sensation | Few months | Right Tonsillectomy | Not Mentioned | [15] |
| 6. | Celik (2005) | 15/M | Right Tonsil | Dysphagia | 1 Year | Transoral Rt Tonsillectomy | Not Mentioned | [2] |
| 7. | Eftekharian (2008) | 19/M | Right Posterior Tonsillar Pillar | Foreign Body Sensation in Throat | 6-7 Years | Transoral Resection | Vimentin (Strong) Actin (Occasional) | [11] |
| 8. | Mendoza Ramirez (2012) | 60/M | Right Tonsil | Dysphagia | 8 Months | Right Tonsillectomy | CD 34 CD 31 D2-40 | [16] |
| 9. | Szymanska (2013) | 49/M | Right Tonsil | Dysphagia | 1 Month | Right Tonsillectomy | Not Mentioned | [17] |
| 10. | Nitin (2018) | 3/M | Posterior Part of Soft Palate and Uvula | Dysphagia, Mouth Breathing, Intermittent Noisy Breathing | 15 Days | Transoral Resection | Not Mentioned | [10] |

ture review of 174 cases of extra nasopharyngeal angiofibroma from 170 publications published till 2015.[3] The most common site being nasal septum followed by maxillary sinus.[3] Out of the 174 cases, 39 (22.4%) cases had their origin outside nose and paranasal sinuses.

Many theories for pathogenesis have been put forward for origin of JNA but all have been debated. Origin from conjoined pharyngobasilar and buccopharyngeal fascia was suggested by Burner (1942). This theory might hold true for etiopathogenesis of oropharyngeal angiofibroma.

Till now only 10 cases of oropharyngeal angiofibromas have been described in the literature. The first case was reported by Beeden AD in 1971.[9] Out of the 174 cases reviewed by Windfur and Vent till 2015, only 9 cases had their origin in oropharynx. Later on in January 2018 another case of oropharyngeal angiofibroma originating from soft palate was reported in a 3 year old child by Nitin et al .[10] The ENAs can originate from any mucosal structure within the head and neck region, including the oral and nasal cavities. According to the literature, ENAs are most frequently localized in the maxillary sinus.[3,7-9]

Out of the 10 reported cases, five cases had their origin from tonsil, two from the soft palate, one from posterior pharyngeal wall, one from the posterior tonsillar pillar while in one case subsite of origin was not defined. Our case had its origin from posterior tonsillar pillar on right side, very similar to the case reported by Eftekharian A.[11] Literature review of atypical extranasopharyngeal angiofibroma arising in oropharynx is presented in Table 1.

The most common presenting symptom for JNA is nasal obstruction and epistaxis. However, for oropharyngeal angiofibroma it is dysphagia. Other symptoms are foreign body sensation in throat, dyspnea, and change in voice if it extends in the laryngopharynx. On examination these generally appear as a vascular mass, however in our case it was a pale polypoidal mass not bleeding on touch.

Contrast enhanced CT/MRI is the investigation of choice for angiofibroma which reveals the extension and vascularity of mass along with its relation to surrounding structures.[18] Contrast enhanced CT Scan helps us to differentiate between JNA and ENA as JNA produces strong and homogenous enhancement as compared to heterogenous or even no enhancement by ENA due to its poor vascularity. Complete surgical excision of the mass is the treatment modality of choice which prevents further recurrence. The surgical specimen should be sent for histopathological analysis.

The definitive diagnosis of angiofibroma is made by histopathologic analysis of the surgical specimen. Ma-

lignant transformation has been reported in literature in JNA cases with radiotherapy treatment being the main cause.[19] However, in ENA cases no malignant transformation has been reported till date as the literature available is very sparse. Close follow up is warranted.

## Conclusion

Oropharyngeal angiofibroma being an atypical angiofibroma in terms of site and presenting complaints presents a diagnostic challenge. Though rare but should always be kept as a differential diagnosis in any oropharyngeal mass irrespective of its vascularity, typical age or sex of the patient.

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**CASUISTIC PAPER**

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# Parotid pilomatrixoma: Diagnostic trap and management dilemma

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**Abstract**

**Introduction.** Pilomatrixoma is a benign cutaneous adnexal neoplasm originating from the matrix cells of the hair follicles. Usually a slow growing and painless lesion, it must be considered in differential diagnosis of a preauricular swelling. Rapidly progressive lesion with skin fixity and missed subtle cytological features may lead to a misdiagnosis of parotid neoplasm resulting in management dilemma.

**Aim.** This report emphasizes consideration of pilomatrixoma as a differential diagnosis in a similar clinical scenario, the role of frozen section during surgery and fascia lata interposition to prevent Frey's syndrome. A brief review of literature is presented.

**Description of the case.** We present a similar dilemmatic case of a 19 years old male with preauricular swelling. Based on cytology and image findings, a diagnosis of parotid neoplasm with possible malignancy was made. Surgical exploration revealed primarily a subcutaneous lesion with partial attachment to superficial surface of parotid. Lesion was excised with a cuff of normal parotid tissue. Frozen section confirmed it to be a nonmalignant lesion with possibility of pilomatrixoma. Fascia lata was interposed between parotid and thin skin flap to avoid gustatory sweating. Patient is on follow up for 6 months without recurrence or any complication.

**Conclusion.** Pilomatrixomas can be misdiagnosed in case of lesions in subcutaneous plane in parotid region. In such cases, the differential diagnosis should include tumor and non-tumor lesions of skin and parotid gland. Importance of frozen section should also be kept in mind and the pathologist should be engaged at the time of surgical excision of the tumor. Interposition of soft tissue between parotid and thin skin flap helps prevent gustatory sweating in such cases. A high index of suspicion is needed for proper diagnosis and management of these lesions.

**Keywords.** parotid gland, parotid neoplasms, pilomatrixoma

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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## Introduction

Pilomatrixomas are the benign skin tumors which arise from the matrix cells of hair follicles. Synonyms: pilomatricoma, calcifying epitheloma of Malherbe.[1] Pilomatrixoma is a rare adnexal neoplasm of children and young adults first described in 1880. It presents clinically as a solitary lesion especially in head & neck region and upper extremities.[2] Pilomatrixoma are slow growing tumors with size ranging between 0.5 to 3 cm, the larger versions known as giant tumor (max diameter = 5 cm). These tumors have good prognosis however some become locally aggressive and also have tendency to reoccur, while others have potential to transform to pilomatrix carcinoma.[3,4] Surgical excision is the treatment of choice.[5]

## Aim

This report emphasizes consideration of pilomatrixoma as a differential diagnosis in a similar clinical scenario, role of frozen section during surgery and fascia lata interposition to prevent Frey's syndrome. A brief review of literature is presented.

## Description of the case

We present a dilemmatic case of 19-year-old male patient who presented to our hospital with slow growing and painless swelling over right preauricular region for 9 months. Clinical evaluation revealed a 1.5cm × 1cm firm, non-fluctuating, non-tender, non-pulsatile swelling over right side preauricular region 1.5cm below the right zygoma. Skin overlying the swelling was non pinchable with no discharge or sinus. Swelling was mobile in all directions.

USG of the swelling showed a well-defined heterogeneously hypoechoic lesion measuring 1.2cm × 0.9 cm in the subcutaneous planes of right preauricular region (fig 1). Contrast MRI showed well defined lesion measuring 1.4 cm × 1 cm in subcutaneous plane in superficial lobe of right parotid gland with indistinct fat planes. Post contrast scan showed mild peripheral and central variegated enhancement (fig 2). FNAC showed features suggestive of possible malignancy. General examination as well as systemic examination of the patient did not reveal any co-existent anomaly. Since the diagnosis was uncertain in this particular scenario, right superficial parotidectomy with intraoperative frozen section analysis to assess the malignant status of the tumor was planned. Modified Blair's incision was given. Superficial musculoaponeurotic system (SMAS) flap was elevated to expose parotid. Over the lesion, only skin flap could be elevated. Lesion was attached to lateral aspect of superficial lobe but did not seem to arise from it. Hence, it was excised with a surrounding cuff of normal parotid tissue. Frozen section was suggestive of a nonmalignant lesion with possibility of pilomatrixoma (fig 3). Initial decision of parotidectomy was withheld. Tensor fascia lata was interpositioned between parotid and thin skin flap to avoid gustatory sweating. Peri and post-operative period were uneventful.

Histopathology report showed irregular islands of epithelial cells in a characteristic organization with a bi-

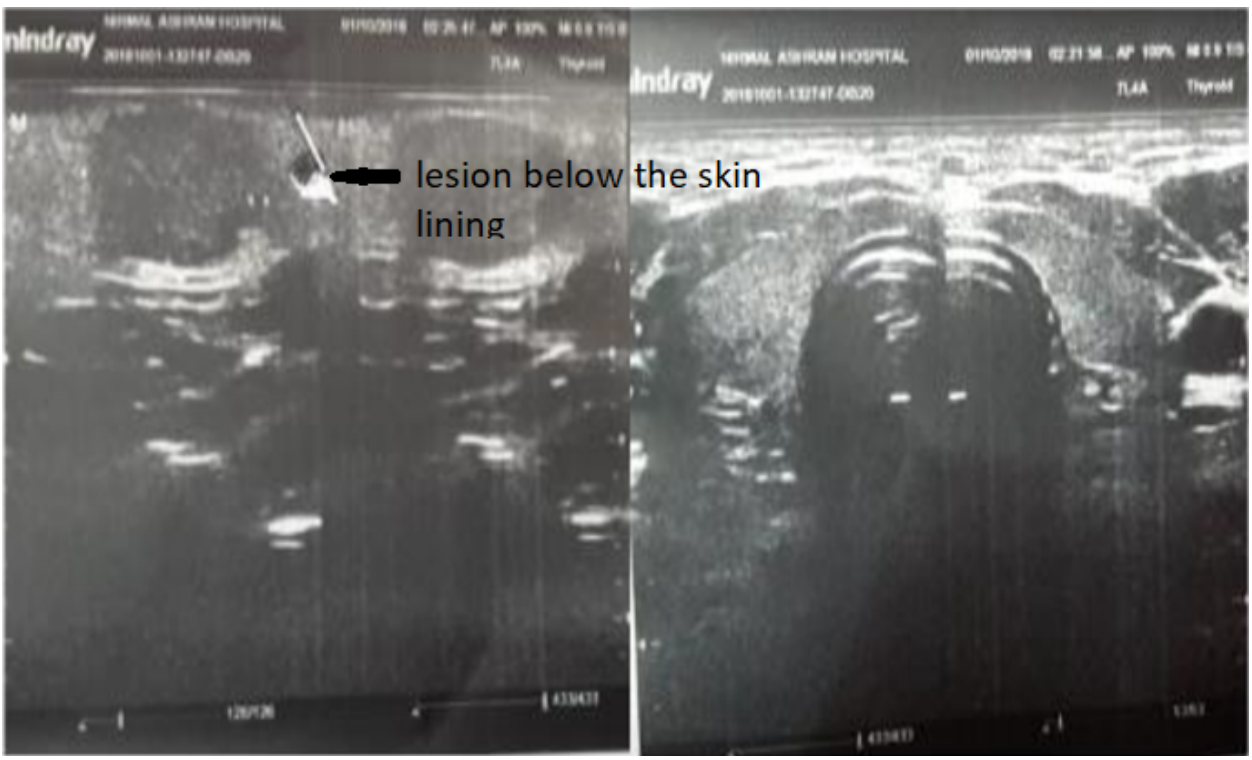


**Fig. 1.** USG of the swelling

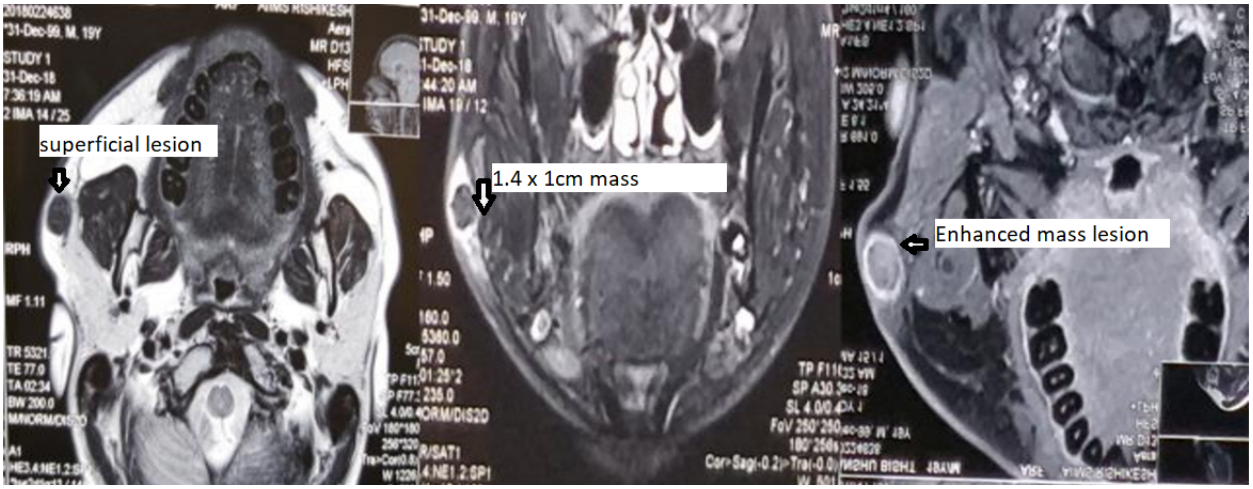


**Fig. 2.** Post contrast scan

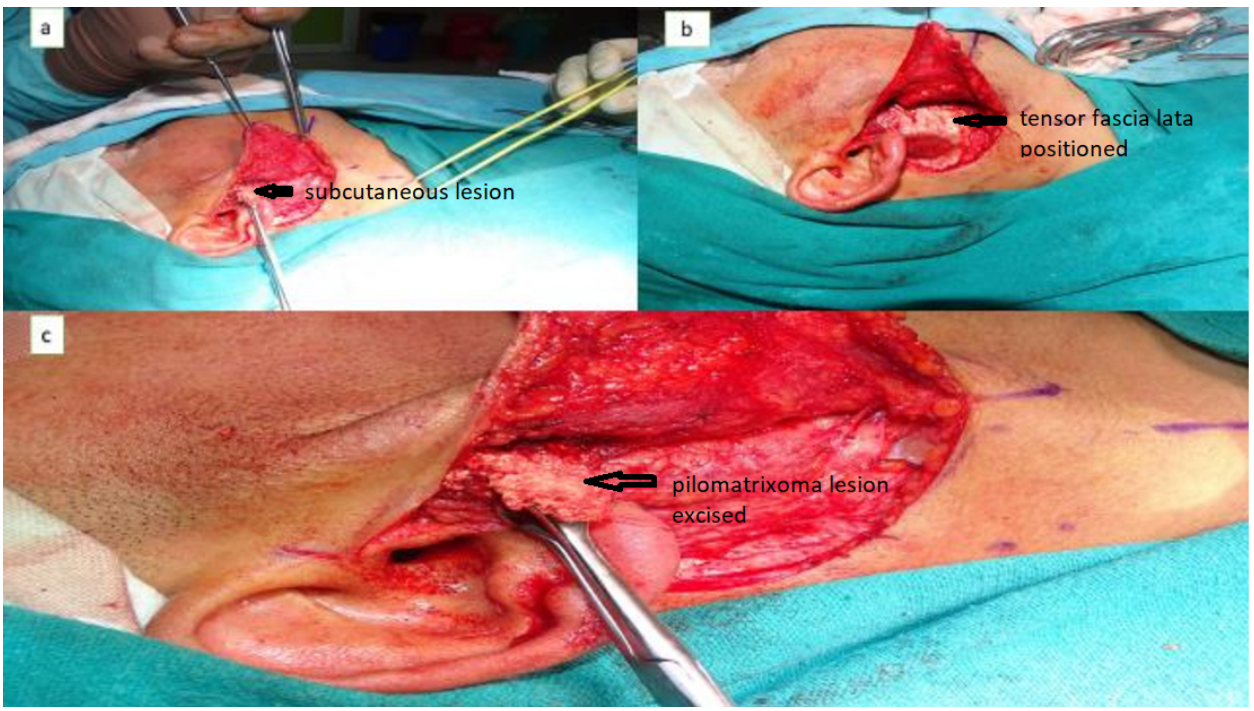


**Fig. 3.** Frozen section was suggestive of a nonmalignant lesion

phasic architecture composed of central ghost cells and varying amounts of basaloid cells in the periphery suggestive of pilomatrixoma (fig 4). Ghost cells are basically an enlarged epithelial eosinophilic cell which had shed their nucleus and contain only cytoplasm.

A final diagnosis of right parotid region pilomatrixoma based on HPE report was made. The patient is on regular follow up for past 8 months and there is no feature suggestive of loco regional recurrence.

## Discussion

Pilomatrixoma originates from epidermal basal layer from stem cells that can differentiate as hair matrix cells.[6] The published data indicates the incidence range between 0.001% to 0.0031% from all dermatological histology specimens.[7] Pilomatrixoma is the most common benign skin tumor of pediatric age group accounting for 20% of skin appendages tumor in most casuistries.[8] Various studies show a bimodal age distribution for this tumor with highest incidence during the 1st and 2nd decade of life and a second lower peak between the age group of 50-65 years of age.[9-11] These tumors had a female preponderance with a female to male ratio ranging from 0.43:1 to 2.45:1.[10,11] Our patient is a male who developed the lesion at an age of about 18 years.

Pilomatrixoms has a propensity to develop in head and neck regions with head as the more frequently involved region. In the head most common subsite being

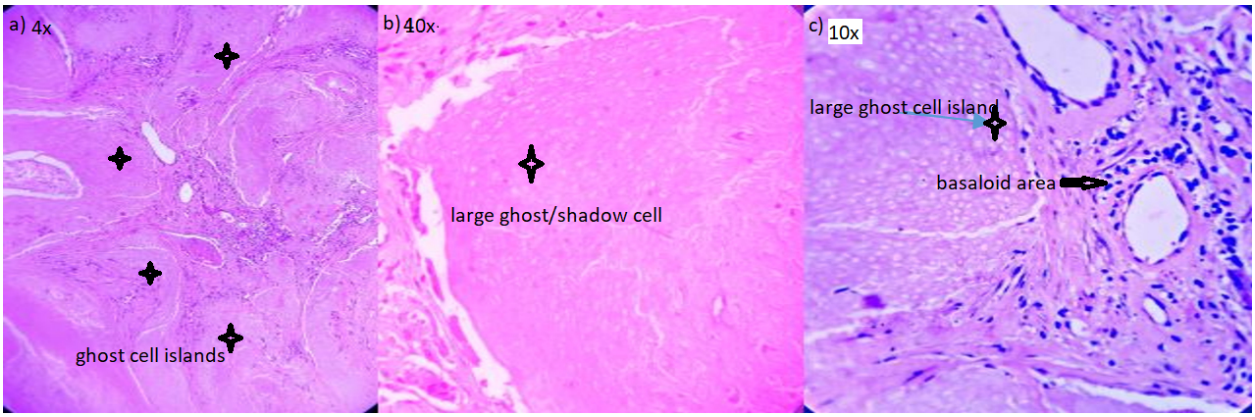


**Fig. 4.** Histopathology report – fig 4a highlights the islands of ghost cells at 4× magnification, fig 4b shows an enlarged ghost cell at 40× magnification and fig 4c image represents the basaloid cell area along with large ghost cell island at 10× magnification (H&E stain)

the cheeks followed by periorbital and preauricular regions.[4,8,11] About 90 -98% lesions reported in literature are found to be solitary lesions similar to our case with isolated lesion in right parotid region whereas 2-10% have been reported as multiple tumor lesions in the same individuals.[4,9,11]

The various causative factors include history of trauma, insect bites, surgery, vaccination.[9,12] Mutations in APC gene (adenomatous polyposis coli), CT NNB1 gene (catenin beta -1) are also been reported. An over expression of bcl-2 proto-oncogene has also been documented in literature to be associated with such tumors.[13] Pilomatrixomas are also seen associated with various genetic disorders like turner's syndrome, Rubinstein–Taybi syndrome, myotonic dystrophy, trisomy 9, Gardner syndrome, celiac disease, 21-hydroxylase deficiency, Soto syndrome, trisomy 18, myosin heavy chains (MYH)-associated polyposis, and xeroderma pigmentosum. Also associated are some chronic diseases like sarcoidosis, hypercalcemia, increased levels of parathyroid hormones related protein and angiomyxoma.[10] This case neither had any syndromic manifestation, nor had any history of earlier described causative factors. Hence, genetic mutation or over-expression could be a possible cause of the lesion here.

Clinically the lesion is slow growing tumoral mass, firm in consistency, non-tender and mobile. The presence of calcification gives firm characteristic to the lesion this is known as tent sign.[14] Seesaw shaped skin "teeter-totter" sign, increases the chance of a right diagnosis.[11]

As the clinical diagnosis is a dilemma, the other differential diagnosis should be kept in mind which include sebaceous cyst, dermoid cyst, epidermoid cysts, adenopathies, brachial remnants, degenerative fibroxanthomas, neurofibroma, basal cell carcinoma, preauricular sinuses and sialadenitis of the parotid gland.[4,11] As in this case, a short duration with overlying non pinchable skin raised a possibility of malignancy. But at the same time, a non-tender, mobile lesion with no history of facial weakness and pain pointed to a possibility of benign lesion.

As per literature review, the accuracy of clinical diagnosis for pilomatrixomas varies between 28.9% and 46%.[1] In a review conducted by et al., preoperative diagnosis was consistent with the pathological diagnosis of pilomatrixoma in only 100 cases (28.9%) out of 346 cases.[8] In Kumaran et al. study, the diagnosis was achieved preoperatively in 46% of patients. Other diagnoses included sebaceous and dermoid cysts, foreign body reaction, calcification in lymph gland, and fat necrosis. Factors contributing to misdiagnosis include cystic lesions with varying consistency, punctum-like appearance, atypical location, and absence of clinically recognizable calcification.[18]

Imaging modalities are useful tools in differentiating pilomatrixomas by identifying calcifications, excluding parotid tumors in case of preauricular lesions, ruling out vascular or lymphatic tumors.[15]

However, the key role in diagnosing this tumor is held by histopathology examination. Typical features seen on histopath examination include enucleated ghost cells in the center and lobulated pattern with basaloid cells at the periphery.[4] Cytology in our case was not confirmatory and revealed features with possibility of malignant lesion. As per literature as well, on FNAC the basaloid cells can be mistaken for intermediate cells of mucoepidermoid carcinoma, illustrating the risk of misdiagnosing pilomatrixoma for malignant tumor of parotid gland.[19] So, these tumors can be misdiagnosed both clinically as well as pathologically.

Rarely complications are seen in PM cases, occasionally they grow to giant size i.e. > 5cm in diameter that cause compression symptom like facial nerve palsy.[10,13,16] Malignant transformation is seen very rarely with PMs especially, in older patients and in patients with repeated tumor excision.[9,16,17]

Recurrence rate ranges between 0% and 3% and most likely, this is due to inadequate surgical excision, and rarely is due to malignancy.[4,8,9] Our patient is in follow-up for past 8 months with no symptoms or signs of recurrence or complication.

An exhaustive search of literature/PubMed was done using MeSH terms as pilomatrixoma, parotid/preauricular region. A total of 113 cases were recorded in preauricular/parotid region which included 69 males and 44 females with a male to female ratio of 1.56:1. The mean age was found to be 30.6 years. All cases presented with swelling as the main chief complaint. All the cases were confirmed based on postoperative histopathological report. The size of tumor varied from as small as 0.2 cm to even more than 5 cm. All the lesions were managed by surgical excision. A maximum follow-up of 3.5 years was done and recurrence was reported in only 3 case post-surgical excision (1 case had recurrence after an interval of 3 months). These cases were managed with second surgical excision.

## Conclusion

Pilomatrixomas can be misdiagnosed in case of lesions in subcutaneous plane in parotid region. In such cases, the differential diagnosis should include tumor and non-tumor lesions of skin and parotid gland. Importance of frozen section should also be kept in mind and the pathologist should be engaged at the time of surgical excision of the tumor. Interposition of soft tissue between parotid and thin skin flap helps prevent gustatory sweating in such cases. A high index of suspicion is needed for proper diagnosis and management of these lesions.

## Compliance with ethical standards

The study has not received funding from any organization or institution and does not involve any potential conflict of interest (financial and non-financial). Procedure performed in the study was in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments.

## Informed consent

Informed consent was obtained from patient in the study.

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**CASUISTIC PAPER**

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# Primary pure squamous cell carcinoma of the gall bladder – a case report of rare and aggressive entity with adverse prognosis

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**ABSTRACT**

**Introduction.** The most common malignancy of the biliary tract is gall bladder carcinoma and the main subtype according to the histological classification is Adenocarcinoma. Pure squamous cell carcinoma of the gall bladder is very rare entity accounting for only 1.1-3.7% of the gall bladder carcinomas. It is highly malignant with poor prognosis due to high proliferative rate and local invasiveness to the adjacent organs. The patients are usually diagnosed at an advanced stage with a bulky tumor owing to its aggressive behavior.

**Aim.** In this paper, we describe a female patient with primary pure squamous cell carcinoma of the gall bladder.

**Description of the case.** A 42-year old female patient presented with chief complaints of pain in abdomen associated with nausea and vomiting and gradually progressive jaundice since 02 months. Contrast Enhancing Computed Tomography (CECT) abdomen showed an enhancing mass lesion in gall bladder involving adjacent organs for which she underwent extended cholecystectomy with pancreaticoduodenectomy.

**Conclusion**. Diagnosis as well as the management of this exceptionally rare type of tumour is undoubtedly challenging because of non-specific clinical as well as imaging findings. This case report is an attempt to add to the literary evidence for better pathological as well as clinical understanding of this rare and aggressive entity thereby providing additional material for the early diagnosis as well as the development of effective targeted therapies which will certainly help in increasing the lifespan of these patients.

**Keywords**. adenocarcinoma, cholecystectomy, gall bladder, pure squamous cell carcinoma

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**Participation of co-authors:** A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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## Introduction

The most common malignancy of the biliary tract is gall bladder carcinoma and the main subtype according to the histological classification is Adenocarcinoma (AC) followed by adenosquamous carcinoma (ASC) and squamous cell carcinoma(SCC).[1] There is considerable geographic as well as gender variation demonstrated by gall bladder carcinomas. The incidence is greater in Asian countries particularly India and Japan with a female preponderance of 3:1 partly because of higher incidence of gallstones in females and presents between 4th to 6th decade of life.[2] Delhi, the capital of India has the highest gallbladder cancer incidence rates for females (21.5 per 100,000).[3] Squamous differentiation can be encountered in about 7% of all the gall bladder adenocarcinomas. Those with squamous differentiation in less than 25% of the tumour are reported as focal squamous change; those with 25%-99% qualify as adenosquamous carcinoma.[4] Finding areas of squamous cell carcinoma in cases of otherwise usual adenocarcinoma of gall bladder is not a rare finding.[5] Pure squamous cell carcinoma without any glandular component is extremely uncommon. Pure squamous cell carcinoma of the gall bladder is very rare entity accounting for only 1.1-3.7% of the gall bladder carcinomas.[6] Squamous cell carcinoma of the gall bladder usually presents with an ill- defined clinical course. It is often diagnosed at an advanced stage and is characterized by extensive local spread and rarely distant metastasis. Though the exact mode of spread of these tumors is still not clear it has been observed that they spread widely by local infiltration and rarely metastasize to the regional lymph nodes or distant organs.[7] According to the report of Charbit et al. the growth rate of the squamous component is twice as fast as the adenocarcinomatous component. Hence the SCC is more aggressive, usually diagnosed when the tumor is large and locally advanced thereby having a poor prognosis in comparison to stage matched advanced gall bladder adenocarcinoma cases.[8]

## Aim

In this paper, we describe a female patient with primary pure squamous cell carcinoma of the gall bladder.

## Description of the case

A 42-year old female patient presented with chief complaints of pain in abdomen associated with nausea and vomiting and gradually progressive jaundice since 02 months. There was no history suggestive of gastric outlet obstruction. Her past medical history was non- contributory. Physical examination revealed deep icterus (bilirubin 14.8), a right hypochondrial hard lump and inferior border of liver is not separately palpable from mass. Laboratory tests revealed neutrophilic leukocytosis with total bilirubin 14.8 mg/dl. However, the values of liver enzyme test results were within normal limits. Contrast Enhancing Computed Tomography (CECT) abdomen was done showing an enhancing mass lesion in gall bladder involving duodenum and head of pancreas, multiple hepatoduodenal, periportal and retro pancreatic lymph nodes, involving right hepatic artery (fig.1.). She underwent extended cholecystectomy with pancreaticoduodenectomy with right hepatic artery ligation and the specimen sent to the Department of Pathology.

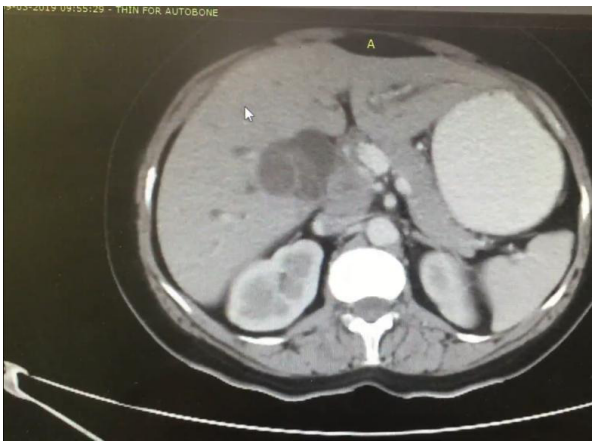
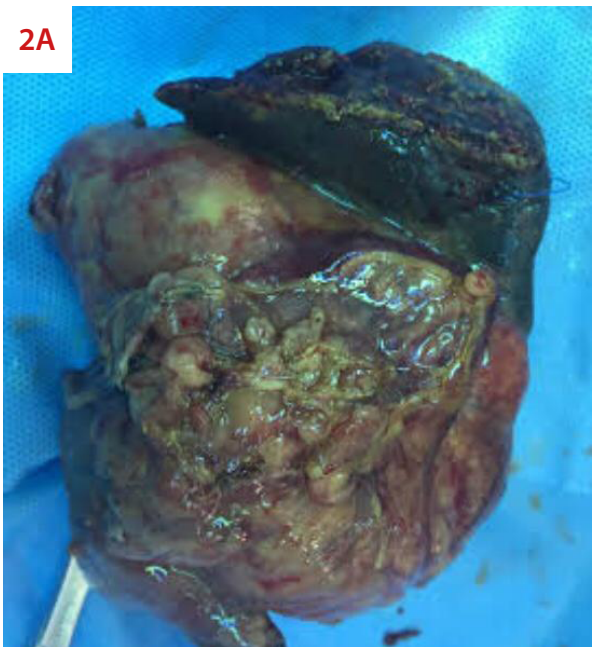



**Fig. 1.** CECT showing an enhancing large mass lesion in gall bladder

Grossly the specimen was submitted in two segments one labelled as radical cholecystectomy with liver wedge, distal stomach and proximal duodenum (fig.2A.) and second labelled pancreaticoduodenectomy specimen (fig.3A.). On cut opening the specimen it was observed that the entire gall bladder was tumorous with a large highly friable grey white infiltrative tumour creamy in consistency showing large areas of necrosis and haemorrhage. Grossly the tumor was seen invading the liver, pancreas and the adjacent duodenum, however the stomach attached appeared free of tumour (fig.2B,3B.). Multiple lymph nodes were dissected out from the specimen.

Microscopic examination showed mostly ulcerated gall bladder mucosa lined by columnar epithelium with an abrupt transition to an invasive tumor composed of masses and sheets of dysplastic squamous epithelium embedded in large pools of keratin (fig. 4.).

The tumour cells show moderate to marked nuclear pleomorphism with enlarged hyperchromatic nuclei, coarse chromatin, prominent nucleoli and moderate to abundant amount of eosinophilic cytoplasm. Extensive keratinization with presence of numerous prominent keratin pearls, dyskeratotic cells as well as intercellular bridges which are characteristic of squamous differentiation were quite evident in the sections. Dense fibrocollagenous stroma surrounding the tumour showed



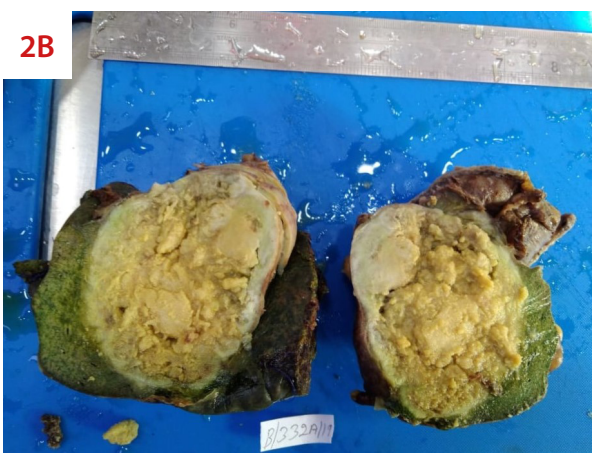
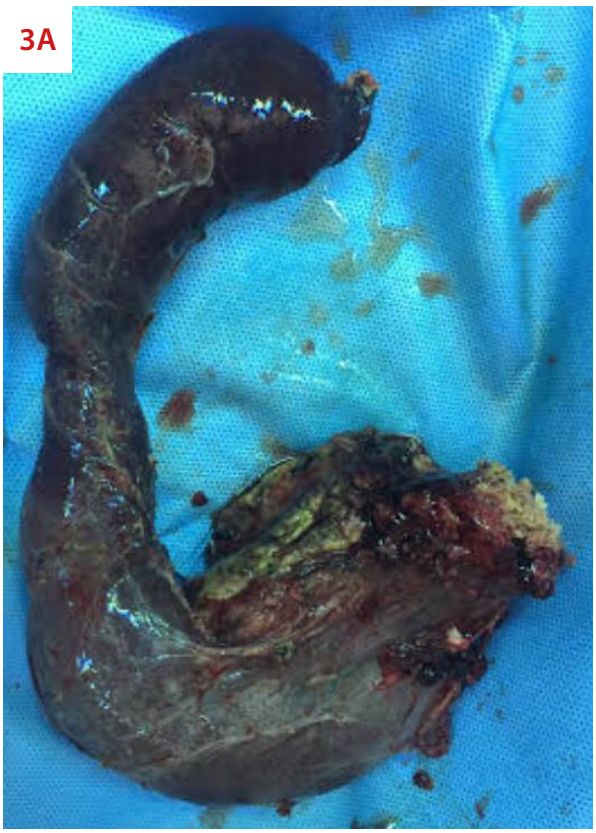




**Fig. 2A.** Gross specimen of gall bladder with liver wedge, **2B.** Cut surface of the specimen showing large grey white tumour infiltrating into the adjacent liver

marked desmoplastic reaction with lymphoplasmacytic infiltration, few neutrophils, eosinophils, many lymphoid aggregates and foreign body giant cell reaction. Atypical Mitotic figures seen with areas of necrosis. Foci of lymphovascular and perineural invasion identified. No invasive glandular element was identified in spite of multiple sectioning. The tumour was seen invading the liver, pancreas and duodenum, however the stomach was free of tumour. The liver vessels were free of tumour infiltration in the sections examined. The sections from all the surgical resection margins including the cystic duct were free of tumour. Out of total 11 lymph nodes identified 05 showed metastatic tumour deposits (fig. 5A, 5B, 5C, 5D, 5E, 5F.).

Final report was dispatched as Squamous cell carcinoma, G2: Moderately differentiated (TNM stage: PT-4N2M0). Postoperatively the patient developed sepsis and succumbed to death.



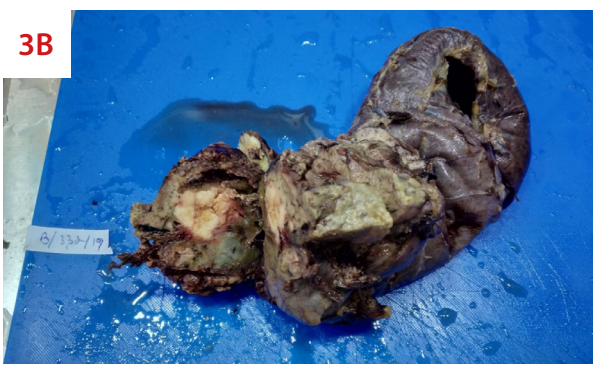




**Fig. 3A.** Pancreaticoduodenectomy specimen, **3B.** Cut surface of the specimen showing a grey white tumour infiltrating pancreas and duodenum

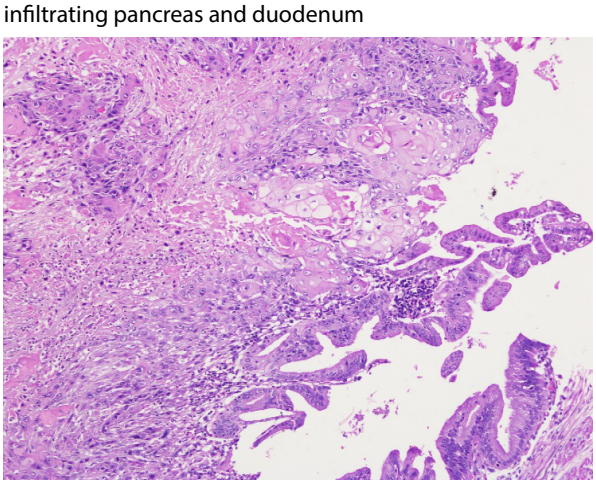


**Fig. 4.** Gall bladder columnar lining epithelium with an abrupt transition to invasive squamous cell carcinoma (H&E stain, 10x)

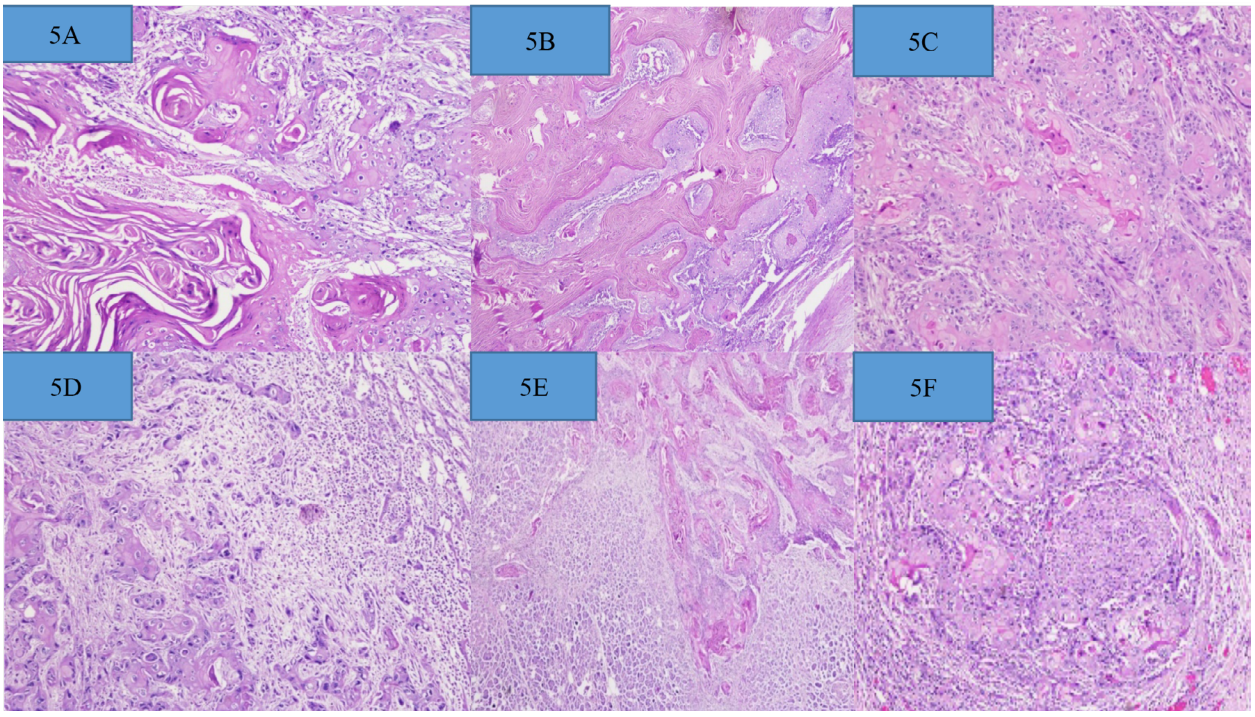



**Fig. 5.** Histopathological findings: **5A-5B.** Sheets of dysplastic squamous epithelium with large pools of extra cellular keratin. Many prominent keratin pearls noted (40x), **5C.** The tumour cells showing moderate to marked nuclear pleomorphism with enlarged hyperchromatic nuclei, coarse chromatin, prominent nucleoli and moderate to abundant amount of eosinophilic cytoplasm (40x), **5D.** The tumour invades into the liver parenchyma (10x), **5E.** The tumour invades into the adjacent pancreatic parenchyma (10×), **5F.** Foci of perineural invasion identified (40×) (H&E stain).

## Discussion

Primary pure squamous cell carcinomas of the gall bladder are rarely reported thus leading to sparse meaningful and sometimes underestimated diverse literature. Rapid growth, wide infiltration, dissemination and metastasis are the characteristics of primary pure squamous cell carcinoma of the gall bladder.

Due to the paucity of studies in the literature the knowledge on etiology, pathogenesis, clinicopathological characteristics as well as prognosis of this rare entity remains scarce. The gall bladder originates from foregut and hence most of the gall bladder carcinomas are heterogeneous during neoplastic transformation making pure SCC of gall bladder a rare entity. The etiopathogenesis of SCC of gall bladder is still not very well defined or understood, though several theories have been proposed. The source of origin is the obvious question as there is no squamous epithelium in a normal gall bladder. The origin of squamous cell carcinoma of gall bladder is theorized either from a squamous metaplasia of an existing adenocarcinoma or from a metaplasia – dysplasia-carcinoma sequence. One of the theory states that the squamous cell carcinoma arises from the pre-existing squamous metaplasia of the gall bladder. While another and more plausible theory suggests that it originates from the squamous differentiation of the neoplastic cells of adenocarcinoma via expression of mixed phenotypes within a single tumour.[9,10] Apart from the metaplasia-dysplasia – carcinoma sequence other presumptive causative possibilities suggested are chronic cholecystitis, cholelithiasis and parasitic infestation.[6,11] There are genetic changes also that have been identified along with mutations that consist of decreased expression of 23nm and overexpression of c-erb B2 gene product.[12] Dong et al deduced that CD109 may promote the proliferation of Gall bladder squamous cell carcinoma) GBSCC by suppressing the TGF-β signal component.[13] Another recent study has reported the mutation of ERBB and PTEN in GBSCC.[14]

Squamous cell carcinoma often appears necrotic grossly. They also tend to have significant amount of inflammation which may lead to misdiagnosis of cholecystitis at the time of clinical presentation. The gall bladder tumors can be identified by radiological imaging and abdominal ultrasound followed by CT scan is the initial diagnostic procedure of choice for biliary diseases but since the tumor lacks specific diagnostic test the confirmed diagnosis is always made by histopathological as well as immunohistochemical tests. Gall bladder tumors can be considered in elderly patients with radiological evidence of diffuse gall bladder wall thickening and intraluminal masses.[9] The definite diagnosis of gall bladder carcinoma is confirmed only by histological examination of the resected mass.[15] The char-

acteristic feature of pure squamous cell carcinoma is prominent keratinization in the form of keratin pearls without any evidence of malignant invasive glandular differentiation. Squamous differentiation can be represented in various patterns ranging from prominent keratinization to poorly differentiated with a pavement pattern, abundant eosinophilic cytoplasm, individual cell keratinization or intercellular junctions as the only evidence.[6]

## Conclusion

For proper understanding of the behavior and thereby deciding the mode of treatment of both the common as well as unusual types of gall bladder carcinomas thorough knowledge of the various types of the gall bladder cancer and their precursors is needed. Most of the patients present with nonspecific findings like abdominal pain, discomfort and weight loss. The characteristics of GBSCC are rapid growth, metastasis, with wide infiltration and dissemination. Only few literary descriptions and studies majority of which are case reports regarding the clinical and biological behavior are available because of the rarity of this entity. The diagnosis and management of this rare entity is quite challenging and the management guidelines are also not very clearly well defined. Incriminated to be a worst malignancy advanced radical surgery is the treatment of choice of squamous cell carcinoma of the gall bladder in which the resection of the involved organs is done where the lesion tends to remain localized without any metastasis or peritoneal deposits. However, radical surgical resection with no remaining lesion can be potentially curative if identified early offering a better prognosis as well as survival as the extent of the tumor at the time of diagnosis is the most important parameter in determining the survival. Multimodal therapy with surgical resection, chemotherapy and radiotherapy has shown to increase survival in some studies. However, till date there are no consensus and randomized trial data to evaluate as well to assess the benefit of adjuvant therapy. Due to the low incidence of the disease and no universally accepted treatment protocol, further studies are needed to emphasize the treatment. The better understanding of the tumor biology of the various types of gall bladder cancers including both common as well as unusual type and their precursors can certainly aid in understanding the behavior will definitely help in treatment as well as the development of effective targeted therapies which will certainly help in increasing the lifespan of these patients.

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**CASUISTIC PAPER**

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# External ophthalmomyiasis by sheep botfly – a report from Sirmaur hills

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**ABSTRACT**

**Introduction.** Myiasis is caused by larvae of flies infesting animal or human tissues and organs.

**Aim.** In this report we present 2 cases of external ophthalmomyiasis by sheep botfly.

**Description of the cases.** We report a case of two patients who presented with a history of foreign body sensation in the left and the right eye respectively. Slit lamp examination revealed larvae of *Oestrus ovis* (sheep botfly). In both cases, there was no contact history with sheep or goats. Signs of conjunctival inflammation and corneal involvement were absent in both cases. In most of the previous reports, corneal and conjunctival inflammation was present.

**Conclusion.** Treatment for external ophthalmomyiasis is based on larvae removal and application of topical antibiotics and steroids.

**Keywords.** myiasis, ocular, signs

## Introduction

Myiasis is the invasion of dipterian fly larvae in humans or animals (living or dead).[1] It can occur on any exposed part of the body but has also been reported to involve the intestine, bones and the urogenital tract.[2] Animals such as sheep are the natural hosts of the fly *Oestrus ovis* and humans are an accidental host. Other species that cause human myiasis are *Dermatobia hominis* (human botfly) and *Cordylobia anthropophaga* (tumbu fly).[3]

## Aim

In this report we present 2 cases of external ophthalmomyiasis by sheep botfly.

## Description of the cases

*Case 1.*

A 25-years-old immuno-competent male presented to the ophthalmology department with a history of foreign body sensation in the left eye for the past two days. There was no other ocular complaint nor any other significant history. He was a teacher by profession. There

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was no significant medical, surgical, family, traumatic or other drug intake history. Routine ocular examination was carried out and his visual acuity was 6/6 in both the eyes; pupillary reactions, ocular movements, fundus and intraocular pressure were within normal limits. On gross torch light examination, the left eye looked normal (fig.1.) until slit lamp examination was performed which revealed a barely visible multiple, translucent larvae over the upper palpebral conjunctiva, around 1 mm in length.

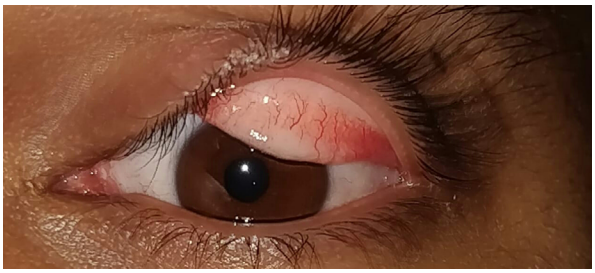

**Fig. 1.** Slit lamp examination of eye of first patient

The larvae were removed with sterile cotton buds under topical proparacaine eye drops, mounted on a glass slide and sent to microbiology department for identification. The specimen was identified as the larvae of *Oestrus ovis* (the sheep nasal botfly) (fig.2.). It had the characteristic spindle shape, segmented body along with a pair of darkly colored sharp, oral hooks at the anterior end. Surprisingly, our patient did not have any conjunctival congestion or corneal signs. We prescribed him topical moxifloxacin+ketorolac eye drops. His complete blood count and blood sugar was within normal limits. He was asymptomatic from the very next day.

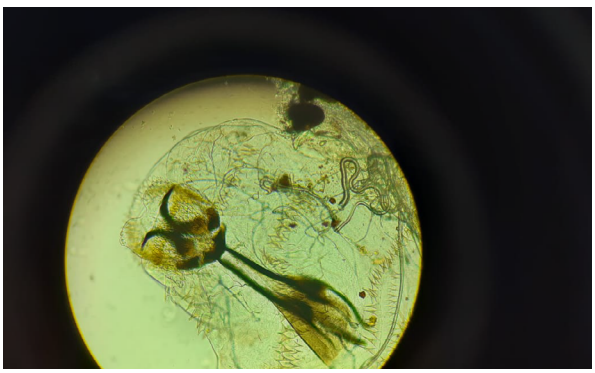

**Fig. 2.** Larvae of *Oestrus ovis*

*Case 2.*

A 22-year-old female was admitted to the emergency department with symptoms of foreign body sensation in the right eye for the past one week. She was a housewife. There was no significant medical, surgical, family, traumatic or other drug intake history. Routine ocular examination was carried out and her vision was 6/6 in both the eyes; pupillary reactions, ocular movements, fundus and intraocular pressure were within normal limits. Slit lamp examination revealed multiple translucent larvae over the right palpebral conjunctiva (fig.3.). The larvae were removed with sterile cotton buds under topical proparacaine eye drops, mounted on a glass slide and sent to microbiology department for identification. The specimen was identified as the larvae of *Oestrus ovis*. Surprisingly, the patient did not present any conjunctival congestion. We prescribed her topical moxifloxacin+ketorolac eye drops. Her complete blood count and blood sugar was within normal limits.

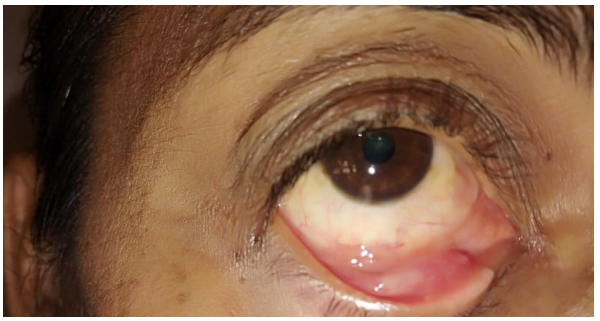

**Fig. 3.** Slit lamp examination of eye of second patient

## Discussion

*Oestrus ovis* is a common cause of myiasis in humans. Its larva cannot survive beyond the first larval stage in humans and are believed to die within ten days if not removed.[4] Ophthalmomyiasis is classified into two types. Ophthalmomyiasis externa has conjunctival involvement while ophthalmomyiasis interna is the term used if there is an intraocular penetration of the larvae. Cases are commonly reported in farmers and shepherds especially during spring and summer. Ocular findings can be in the form of acute catarrhal conjunctivitis, and if untreated, can lead to corneal ulcer, endophthalmitis, iridocyclitis and even blindness.[5]

Shepherds, horse groomers, and people living in poor hygienic conditions are susceptible to infestation by the organism. Further, compromised periorbital tissues as a result of surgery, malignancy, and infection predisposes the patient to myiasis.[6] Treatment for external ophthalmomyiasis is based on larvae removal and application of topical antibiotics and steroids.[7] Timely diagnosis and treatment prevents serious complications associated with this disease.

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**CASUISTIC PAPER**

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Justyna Podgórska-Bednarz [1,2(ABDFGH)], Agnieszka Guzik [1,2(ABDFGH)]

# Effects of sensory integration therapy in a 7-year old child with epilepsy following craniocerebral trauma – a case study

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**ABSTRACT**

**Introduction**. Post-traumatic epilepsy develops as a complication of a serious craniocerebral trauma, frequently an open head injury, resulting in neurological impairments.

**Aim.** The study was designed to discuss problems associated with sensory integration dysfunction observed in a seven-year old girl with epilepsy which occurred as a result of craniocerebral injury.

**Description of the case.** The case study is based on information gained from the girl's medical records, an interview with her guardians and a sensory function questionnaire. Additional examinations assessed the girl's balance control, and her functional performance according to Barthel scale. The girl received a three-week sensory integration therapy which included exercise involving tactile, vestibular and proprioceptive stimulation, as well as balance exercises and self-care training.

**Conclusions.** In this particular case, application of sensory integration therapy produced beneficial results. The findings show improvement in balance, coordination as well as self-care.

**Keywords.** epilepsy, rehabilitation, sensory integration

## Introduction

The timing of the first epileptic seizures following craniocerebral trauma is varied. The incidence of post-traumatic seizures in children is difficult to determine due to the scarcity of the related population studies. Generally, the prevalence of seizures in children, irrespective of the type (early or late seizures), the children's age, as well as severity of the trauma, is estimated to range from 5.5 to 21%.[1] Patients with post-traumatic epilepsy (PTE) account for about 5% of all the individuals referred for treatment to epilepsy centres. PTE is frequently drug resistant. In addition to their capacity to induce seizures, head injuries may also adversely affect the progress of the condition in patients with epilepsy.[2] Etiological factors of the condition occurring in children include maturation of the central nervous system (CNS), perinatal or intrauterine damage to the CNS and any genetically conditioned disorders of pubertal devel-

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opment.[3] PTE, which usually contributes to the affected person's severe disability, is the predominant long-term complication of craniocerebral injury. It typically develops during the first five years following the head injury.[4]

During the first years of life a child acquires the largest number of skills and the largest amount of information about the surrounding world. These experiences are collected via sensory perception. Deficits connected with the disease may hamper normal functioning and consequently disturb normal development.[5] Sensory integration involves processing of sensory information from our body and environment, for use in our daily life.[6] Sensory integration therapy focuses on the child's abilities in three areas: modulation of motor information processing, differentiation of the information, and its integration with action.[7]

Sensory integration method was developed in the 1960s, by Jean Ayres, an educational psychologist and occupational therapist. The author created the theory of sensory processing dysfunction. Ayres observed that sensory integration deficits affect postural response, muscle tone, movement planning, speech development, behaviour, emotions and cognitive functions. Initially the therapy was used in children with learning difficulties, and today it is also applied in children with delayed psychomotor development.[5]

In its hypothetical model of development, the method distinguished development of sensory, proprioceptive and vestibular perception, balanced responses, integration of primitive reflexes, development of body image, ability of motor planning, as well as visual and auditory perception. These at a later stage are followed by development of precise hand movements (lateralisation, ability to differentiate between the sides of the body), visuomotor coordination as well as spatial perception.[6] The therapy is mainly designed to normalise perception of stimuli by applying varied frequencies, to normalise muscle tone by means of relaxation, balance training, and spatial positioning of the body; it also aims at reconstruction of body image.[6] Impaired sensory processing is associated with inability to use information obtained via senses to enable smooth functioning in the daily life. Sensory modulation is described as regulation of sensory input by the brain. This is linked to a child's response to stimuli. Owing to sensory stimulation, a child can determine which sensory input they must pay attention to and which can be ignored. Impairments in sensory modulation are mainly associated with abnormal enhancement or inhibition of stimuli in the vestibular system, proprioception, as well as senses of touch, vision, hearing and smell.[7,8]

Sensory integration therapy may be administered to children with epilepsy. However, it is necessary to take into account the possible overloading of the nervous system with excess sensory stimuli, e.g. resulting from accumulation of the child's sensory experiences during the day. Movement is an indispensable factor organising brain activity, and for a child it is linked with pleasure; furthermore, research shows that good physical condition favourably affects the course of epilepsy. However, during sensory integration training it is necessary to keep in mind certain limitations and avoid intense simultaneous vestibular and visual stimulation, intense vibration stimulation particularly applied to auricular region, the skull bones, and neck muscles, intense stimulation of superficial sensibility (gentle, skin-stroking massage) which particularly affects the nervous system. During a therapeutic session, in the case of these children, rest intervals should be used between exercises much more often; these could be a good opportunity to introduce proprioceptive stimuli.[9,10]

## Aim

The purpose of the study was to present sensory integration dysfunction observed in a seven-year old girl with epilepsy resulting from a craniocerebral trauma, and to assess effects of the impairment in the girl's functioning.

## Description of the case

The present case study is related to a 7-year-old girl who at five months of age, under unclear circumstances, experienced craniocerebral injury, as a result of which she received a surgery due to epidural and subdural hematoma. Long-term consequences of these events included spastic quadriparesis, epilepsy and delayed speech development. The girl was delivered naturally, at 42 weeks of pregnancy; 10 points in Apgar scale at birth.

Neurological examination identified horizontal nystagmus, speech deficits (the girl could say isolated words), discrete left-side hemiparesis, gait abnormality, no meningeal signs. Laboratory tests, including examinations towards toxoplasmosis, Lyme disease and thyroid hormones showed normal results. Head MRI showed malacic lesions bilaterally in parieto-occipital region, accompanied with peripheral gliosis and dilatation of the occipital horn and trigone of the right lateral ventricle; lesions resembling glial scars in both cerebellar hemispheres; thin corpus callosum; dilatation of supratentorial system and Evans ratio.

Physical examination showed such abnormalities as wide-based gait, need for protection while walking up the stairs, impaired balance and motor coordination, fear of unknown surfaces, lack of targeted graphomotor skills and multi-point grip, difficulties in self-care (the gilr does not like combing, washing and touching; she cannot get dressed without help, she rarely communicates her physiological needs; no sphincter control). She depends on other people. The child received antiepileptic treatment (Depakine Chronosphere), and currently experiences no seizures. Visible absence seizures were

confirmed by EEG examination (weak sleep spindles; In the occiput on the right side continuous disconnections of non-typical IFs, and low IFs of 1.5 c./s.).

The girl has difficulty adopting to new environments, she is anxious and fearful and does not like to be left by herself. She does not initiate contact with others, and does not understand complex instructions. Her speech development is delayed, she can imitate sounds and selectively follow simple instructions. Psychological examination did not identify autistic behaviours.

The child currently has very limited contact with her parents and remains in the custody of her grandparents. They live in a detached house with a large backyard. The girl attends an outpatient neurorehabilitation unit five days a week. As reported by her guardians, the main problems are connected with emotional adaptation (attacks of squealing, screaming, crying), difficulty falling asleep, physiological needs, and fear of medical personnel. In the past there were problems during meals, because she was unable to eat by herself. The situation has improved, and now the girl can use a spoon and fork. The interdisciplinary team taking care of the girl includes therapists and specialists in ophthalmology, neurology, rehabilitation, neurological speech therapy and psychology. Her disability has been certified by specialists.

The program of sensory integration therapy included exercise stimulating tactile system, deep sensation, visual perception, and fine motor skills, and exercise in movement planning. Exercise affecting vestibular system focused on balance control, visuo-motor coordination, muscle tone, and spatial orientation. Exercise stimulating proprioception focused on sense of the body, spatial orientation, and muscle tone. Self-care training included exercise focusing on fine motor skills and learning body image.

The therapy was conducted every day for three weeks. The exercises were carried out in a playful form.

The examination was performed before the start of the therapy and after 15 days of the training, in the same conditions and using the same measurement tools. Effects of the therapy were assessed with the following tools:

1. Questionnaire, designed for assessing school-age children with symptoms of sensory integration deficits, proposed by Grzywniak, and containing statements related to specific symptoms of impaired sensory integration and their intensity

(does not like to travel by a fast driving car, especially while it is taking a turn, suffers from motion sickness, supports his/her head with hands while sitting in class, reads slowly with disturbed rhythm pattern, is hyperactive, faces difficulties trying to focus, faces difficulties while getting dressed, is shy and withdrawn, gets angry frequently, hates some scents, does not tolerate some flavours or textures of food, likes to swing or turn on a merry-go-round, and never has enough, falls asleep faster if he/she can hear specific sounds).[10]

Assessment is based on the following scores:
0 — no symptoms, 1 — symptom occurs rarely, 2 — symptom occurs occasionally, 3 — symptom occurs frequently with some intensity, 4 — symptom occurs always or almost always.[10]

2. Functional assessment according to Barthel scale – measurement of performance in 10 basic activities of daily living (feeding, transfers, grooming, toilet use, washing, bathing, walking, climbing stairs, dressing/undressing, faecal continence, urinary continence); it does not assess psychological and social functioning. The scale rating: I. 86-100 points – "mild" condition, II. 21-85 points – "moderate" condition, III. 0 - 20 points – "severe" condition.[11]

3. The Step Test – enables assessment of the ability to maintain balance in dynamic conditions, typical during walking in the presence of obstacles. The trial measures the number of steps performed with one foot onto and off a 7.5 centimetre high block, within 15 seconds. The subject is asked to perform the activity as quickly as possible, without any help, and the other foot must remain in full contact with the floor. Both healthy legs are tested.[12]

4. Get Up and Go Test – the subject is asked to perform a few simple activities, i.e. to stand up from chair (seat height of 46 cm) from a sitting position, walk along a flat surface at a distance of 3 metres, cross the line marking the distance, turn 180°, return to the chair and resume the sitting position. Time is measured from the moment the person, seated on a chair, is given the command to "start", to the moment he/she resumes a sitting position. The subject is asked to perform the task as quickly as possible, at a pace which is safe for him/her.[12]
5. One Leg Standing Test – it is an easy-to-conduct test which does not require any equipment except for a stopper. It assesses the subject's ability to maintain static balance, while standing on one foot without any support. The time is measured from the moment the subject's foot has been lifter to the moment it makes contact with the floor again.[12]

## Discussion

After the questionnaire was completed, significant deficits in sensory integration were identified. The score amounted to 95 points, and the most frequently selected responses, 3 and 4, may reflect severe dysfunctions in the girl.

Assessment of the girl's performance based on Barthel scale showed moderate disability, in accordance

with the score of 50 points (category II: 21-85). In the present case study, following the therapy administered, the child presented a slight improvement in coordination and balance. In the Step Test, after 15 days of the therapy, the girl achieved improvement of two steps and one step in the right and the left leg, respectively, compared to the baseline. The result in Up and Go Test was also improved after the therapy, the girl was able to perform the task 3.5 seconds faster than at the start of the program. In One Leg Standing Test there was also improvement; after the therapy the girl was able to stand on the right leg for 2 seconds, while at baseline she failed to complete the task of standing on both the right and left leg (Table 1).

**Table 1.** Comparison of results in the tests, before and after the exercise with elements of sensory integration training, as well as effects of rehabilitation

| Tests | Before | After | Effects of rehabilitation |
|---|---|---|---|
| The Step Test in 15 seconds | 6x(repetitions)- right foot 4x- left foot | 8x(repetitions)- rights foot 5x- left foot | + 2x<br>+1x |
| Up and Go Test in seconds | 15 seconds | 11.5 seconds | - 3.5 seconds |
| One Leg Standing Test in seconds | Failure to perform the task – both legs | 2 seconds – right leg Failure to perform the task – left leg | Improvement in the right leg |

x- number of repetitions

The present study showed that a rehabilitation program combined with elements of sensory integration therapy, even with short duration of such treatment, produced beneficial effects in the girl's ability to focus on the motor task performed. It also led to relaxation of the body and decrease in muscle tone. Supporting a child's development with the use of sensory integration therapy is very important. After a specified duration of the exercise it is possible to observe effects relative to therapeutic assumptions and goals, such as improvement in the child's psychomotor condition.[7]

Rehabilitation is a process which should be initiated at the same time as a medical treatment, e.g. in this case pharmacological therapy, at each stage of its advancement. This is because such disorder as epilepsy may significantly affect the results of treatment, the outcome of therapy will not depend exclusively on the sensory deficits of the child. The condition may lead to general weakness, and lack of willingness to perform motor exercise. Due to this, children tend to like sensory therapy, as it is carried in a playful form, and it does not require precision of movement. The small patients eagerly perform such activities because therapy is tailored to the functional condition of each child. Rehabilitation with elements of sensory integration enables adequate stimulation of the vestibular system in patients with balance deficits.[8]

According to Emmons et al., rehabilitation based on sensory integration techniques enables adequate stimulation of the vestibular system in patients with balance disorder. This method is particularly effective when applied to small children. Since they affect the vestibular system and proprioception, sensory integration techniques may be an alternative or, in the case of older children, a supplement to standard vestibular habituation training used in treatment of balance disorders.[12]

Before the specific therapeutic program is designed for children with deficits it is necessary to perform in-depth diagnosis of the system of balance and sensory integration. It is more difficult to perform diagnosis in children than in adults due to problems connected with assessing specific ailments. Therefore, support from parents in identifying any disorders is of critical importance for every aspect of rehabilitation and examinations. In order to assess integration abnormalities, and determine whether they are caused, e.g. by incomplete myelination occurring by the age of 4-5 years, it is helpful to perform examinations and comprehensive observations, because it is only after this stage that children can be examined with the use of tests for adults.[13-17] According to the scarce data reported in the literature children with vestibular disorders may benefit from rehabilitation focusing on reduction of sensory deficits. Children usually respond to this type of exercise more rapidly than adults, because of the greater plasticity of the central nervous system and its capacity to compensate for and to adapt to a vestibular dysfunction.[18]

The present case study shows that therapy plays a significant role in the process of learning new activities and in improving the child's quality of life. After the therapy program was introduced it was possible to observe decreasing reticence in the girl, she started making visual contact, was able to say more words, create short sentences, and express her willingness or unwillingness to perform some exercise. In order to assess effectiveness of sensory integration therapy applied in the present study, the findings could be compared to research by other authors focusing on a similar problem and applying this type of therapy. Any discrepancies may be linked with the different disease occurring in the child because there are no articles discussing this particular condition. Krzywińksa- Wiewiórowska et al. report that rehabilitation should be introduced in the case of children with chronic diseases.[19]

The findings reported by Fazlioglu et al., who conducted a study in a group of 30 children with autism, ranging in age from 7 to 11 years, confirm effectiveness of sensory integration therapy.[20] Effectiveness of sensory

integration therapy in children with Autism Spectrum Disorder was also shown in review studies.[21,22]

In summary, the above findings suggest that sensory integration training may beneficially affect functional capacities, balance control and coordination in a child with post-traumatic epilepsy due to head injury.

## Conclusions

Sensory integration therapy produced beneficial results in a 7-year-old child with PTE due to craniocerebral injury. In this specific case improvement was observed in self-care skills, balance control and in motor coordination. It is necessary, however, to perform further research involving a larger sample, and employing a greater number of measures, in order to draw far-reaching conclusions.

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