Thyroglossal duct is an endodermal diverticulum whose origin, foramen caecum, is located between the bases for the development of tongue, that is, in front of the copula, to be more specific. The duct grows into the mesenchyme and grows distally. As early as the 7th week of embryonic development, thyroglossal duct is separated from the pharynx and it atrophies, except for its anterior, distal part which develops into a thyroid gland [1]. Thyroglossal duct atrophy occurs prior to the formation of the hyoid bone. In cases when there are remaining parts of the duct, the development of the hyoid bone can affect the final position of the duct or its residuals. Residual segments of thyroglossal duct are the basis for the development of cysts that can occur at any height from the base of the tongue to the sternum [1, 2].
They are located in the median or close midline of the neck and hence they are commonly referred to as medial cysts of the neck. They are noticeable at all ages and can reach the size of 1-5 cm. Furthermore, they are asymptomatic, except in the case of inflammation when they become painful and hard. In the adult population, thyroglossal duct cysts (TDC) have a prevalence of 7%. Malignant TDC incidence rate ranges from 0.7 to 1.5% [3, 4]. In the case of malignancies, it is most frequently the case of papillary epithelium carcinoma, and very rarely the case of the squamous epithelium carcinoma [5].

Although the cancer rarely occurs according to statistical data, the literature data warn us to be very careful when diagnosing each case [6]. TDC is presented as an oval tumor mass which should not be confused for an ectopic thyroid gland. Before making the final decision on treatment, ultrasound or scintigraphy should verify the size and morphology of the thyroid gland. TDC should be distinguished from branchiogenic cysts developed close to the midline, solitary cysts in the thyroid gland, hemangioma, cystic lymphangioma and cystic degenerated lymph nodes [7].

Comparative analysis of diagnostic procedures and treatments of TDC in children and adults did not show significant differences [8]. However, studies proving the existence of papillary carcinoma refer to adult patients, between the ages of 21 and 68 years [5]. The treatment of TDC is surgical, according to the Sistrunk Protocol [9]. Unfortunately, there are cases of cysts regrowth after the surgical treatment [10, 11].

Although the initial diagnosis of TDC can be set by clinical examination, most authors agree that its confirmation implies the usage of ultrasonography and cytological analysis, and in special cases, the magnetic resonance (MR) imaging [12]. So far, a relatively small number of studies have been published regarding the TDC cytology [13]. In most cases, cytological analysis of the cystic content enables us to differentiate benign from malignant lesions [5, 14]. In doing so, the positive, subjective effects of evacuation of cyst content should not be ignored.

This study was aimed at determining the cytological characteristics of cyst content, cell specificity, cellularity and the content of the extracellular matrix as well as the significance of cytological analysis in the detection of papillary carcinoma of a thyroglossal duct cysts.

**Material and Methods**

Cytological findings of 28 adult patients with TDC were analyzed. All patients were treated at the Center for Thyroid Gland in Banja Luka from 2004-2014. A soft tissue oval tumefaction of the anterior side of the neck was present on clinical examination. The patients underwent ultrasonography and fine-needle aspiration cytology (FNAC). Ultrasonography included the examination of the thyroid gland as well. The patients were between the ages of 16 and 64 years, the average age being 29.2 years. There were 17 women and 10 men (the ratio being 1.7: 1.0). In addition to the size of the cyst, ultrasonography established the localization and echo-structure of the content. The localization refers to the position of cysts in relation to the median line of the anterior side of the neck, and in relation to the hyoid bone according to the height. In accordance with usual regulations, the content of the cyst was classified into three categories: anechoic, homogeneous hypoechoic and heterogeneous content. Aspiration puncture was performed with a fine needle (23G), with the adjusted dose of 10 ml. The prepared smear was air-dried and then stained according to the May Grunwald – Giemsa procedure. The analysis was done with a wide field-of-view microscope, Axioscope, while the pictures were taken with a digital camera Canon EOS-450D.

The cell type, cellularity and content of the extracellular matrix were analyzed. According to the type, the following were described: follicular cells, squamous epithelial, macrophages, erythrocytes, lymphocytes, granulocytes, and erythrocytes. Cellularity was expressed with semi-quantitative method as A (low), B (moderate) and C (high), where cellularity A represented the total number of cells less than 10 in 10 consecutive visual fields; cellularity B represented 10-100 cells in 10 consecutive visual fields and cellularity C represented more than 100 cells in 10 consecutive fields. Granulocytes and erythrocytes were not included in the semi-quantitative analysis.

The extracellular matrix or substrate was descriptively shown as dense-opaque, translucent and scarce. The presence of cholesterol crystals or cellular debris in the matrix was noted. There was no evidence of malignancy in the study period. Three patients underwent surgical treatment according to the Sistrunk protocol. Other patients refused surgical treatment because they did not feel any functional disorders or esthetic deficiency.

**Results**

All cysts were ultrasonographically identified in the medial or close midline. Most of them, i.e. 25 cysts were placed distally from the hyoid bone. The cyst was adjacent to the hyoid bone in only three cases: one directly above it, and in two cases closely below it. The size of cysts varied from 1.5 to 6.0 cm in diameter. The cysts were of anechoic, homogenous hypoechoic and heterogeneous structure in 14, 8 and 6 cases, respectively. The thyroid gland was properly developed, without pathological changes in 23 patients. Smaller nodules of benign characteristics were observed in the thyroid gland in 4 patients. One patient had agenesis of the left lobe. The amount of aspirated content ranged from 0.5 - 7 ml. According to the layout, the content was mostly densely-brown or clear-yellowish.
Cytological analysis indicated significant differences in relation to the type of cells presented in the analyzed content. In one case, the content of the cyst was acellular! Follicular cells were detected in 5 cysts, squamous epithelial cells in 4, macrophages in 27, erytrophages in 1, granulocytes in 4, and older red cells in 1 cyst. In terms of cellularity, semi-quantitative method indicated that low cellularity (A) was present in 2 smears, moderate cellularity (B) in 21 smears, and the remaining 5 smears were hypercellular (C) (Table 1).

In group A, one smear consisted of only a few macrophages and transparent, pinkish colored matrix. In another smear, there were no cells, and the matrix was extremely dense, dark purple with numerous cholesterol crystals (Figure 1).

Group B consisted of 21 smears of moderate cellularity. Macrophages, few squamous epithelial cells, cholesterol crystals and amorphous extracellular mass were observed in 17 smears (Figure 2). In 4 cases, macrophages, numerous granulocytes and detritus were observed.

In the third, C group, there were 5 cystic nodes. Four cysts contained a higher number of macrophages, single or in small clusters of follicular cells, dense colloid and a slight amount of cholesterol crystal (Figure 3).

Apart from the classic macrophages, the remaining, fifth cyst consisted of erythrophages, individual follicular cells and many old and partially destroyed red blood cells. In the extracellular matrix, there were cholesterol crystals in as many as 19 smears. The proportion between the smear density and the amount of cholesterol crystals was not observed.

Cytological analysis did not show any changes that would unequivocally imply the presence of carcinoma. Histological diagnosis was established only in three cases. Two out of five patients with hypercellular content and a patient from the first group underwent the surgical treatment primarily due to esthetic reasons. In all three cases, TDC of benign characteristics was determined pathohistologically. All other patients are controlled periodically. The monitoring period varies from 1 - 10 years. During this period, “recharging” of the cyst did not occur in 8 patients after the initial evacuation of the content. In the same period, punctures had to be repeated in four patients because of a slight enlargement of the cyst. It should be mentioned that the time interval before repeated punctures was 1-2 years.
No important difference in relation to earlier cytological picture was observed in the smears of repeated punctures.

Discussion

Clinically, TDC can often be confused with the low-lying branchiogenic cyst, anterior cyst of thyroid gland or lymphoepithelial cyst [13]. Thyroglossal duct cyst is typically located in the median line of the anterior side of the neck, below the hyoid bone. Although results vary, studies show that 40% of TDC lie outside the midline i.e. laterally to the supposed medial line in adult population. When it comes to the height, over 82% of TDC are located at the infrahyoid muscles, and only 5% at the suprahyoid ones [12].

In everyday work, ultrasonography has the priority in determining the position and size of TDC over the more expensive techniques such as computed tomography CT/MR. In this analysis, ultrasonography determined the size, position and echogenicity of the cysts. There were no significant difference in comparison with the data in the above cited studies [2, 12].

Cytological examination of stained smears showed different characteristics in terms of types of the cells present as well as in terms of their number. Distribution and density of the extracellular matrix and the presence of cholesterol crystals showed significant differences. Although they are not numerous, previous studies have shown that in most TDC there are macrophages, and that all other cell types are sporadic [13]. In this analysis, as many as 27 out of 28 smears contained macrophages; whereas mature, stratified squamous cells and follicular cells were found in 9 and 4 smears, respectively. In some cases, TDC clinically look like an anterior cyst of the thyroid gland. The difference in cytological imaging of the TDC smear and a cyst in the thyroid gland is not always clear. Cysts of the thyroid gland usually contain neither stratified nor squamous epithelial cells nor cholesterol crystals [7]. The presence of any of these parameters with the clear absence of follicular cells refers to TDC. Otherwise, TDC smear in which only phagocytes and follicular cells are seen cannot be cytologically distinguished from colloid cystic nodules of the thyroid gland.

A greater number of granulocytes which indicated the inflammatory process were observed in 4 smears, although there was neither pain nor redness in the clinical picture. After additionally conducted analysis of erythrocyte sedimentation rate (ESR), white blood cells (WBCs) and C-reactive protein (CRP), an adequate antibiotic therapy was administered. According to the literature, up to 30% of patients come to the first check-up with signs of intracystic infection [15]. A big difference was noticeable in terms of cellularity, i.e. the number of cells in the smear. The smear of one cyst was completely acellular! Purple colloid and cholesterol crystals were visible. In contrast, five smears were described as hypercellular. Although it was expected, the turn in terms of atypia was not noticed. Two out of five were treated surgically, and the histological diagnosis was: TDC.

No malignant cells were detected in any smear. According to previous studies, cytological analysis of TDC cancer showed about 60-66% accuracy [5, 16]. Diagnostic criteria for papillary carcinoma in TDC did not differ from the criteria for the same kind of cancer in the cystic nodule of the thyroid gland. This in itself justifies the request of the majority of authors to make cytological analysis of the TDC aspirates an obligatory diagnostic procedure. None of 28 patients belonged to pediatric population. Although some authors claim they have not noticed significant differences in behavior of children and adult patients with TDC, they are united in opinion that the cytological analysis is a useful diagnostic method, regardless of age [8, 17]. In contrast, Lee et al. stated that in children under 10 years of age, cytological analysis showed a diagnostic sensitivity of 70% and a positive predictive value of 41% so it is not recommended as a routine procedure in the stated age [18].

Comparing cytological findings of this analysis with the similar ones reported in literature, containing a larger number of histological diagnoses, we can assume that none of our patients suffered from the cancer in TDC. This is supported by the fact that there were no elements of tumor growth related to TDC in the process of clinical monitoring, which was 10 years long for some patients. Nevertheless, we believe that surgical treatment of TDC is justified and definite.

Conclusion

Cytological analysis shows that thyroglossal duct cysts do not have a uniformed cytological picture. Most thyroglossal duct cysts contain phagocytes and cholesterol crystals. Although cellularity of the smear was different, no difference in clinical status of thyroglossal duct cysts was observed during the specified period.
Thyroglossal duct cysts that do not contain cholesterol crystals and squamous cells in the smear are not cytologically different from colloidal-cystic nodules of the thyroid gland. The presence of granulocytes in the thyroglossal duct cyst smears requires additional processing and antibiotics, in spite of the absence of any elements of inflammation in the clinical picture.

Despite the fact that typical characteristics of papillary carcinoma were not detected in the analyzed smears, we believe that cytological analysis is of great importance in its detection.

References