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ORIGINAL ARTICLE

Comparative Study of the Level of Inflammatory Factors of Erythrocyte Sedimentation Rate and Reactive Protein C in Benign and Malignant Thyroid Nodules

Mohsen Eshraghi[1], Seyed Jalal Eshagh Hosseini[1], Fatemeh Sadat Razavinia[2], Enayatollah Noori[3]

[1]Department of Thoracic Surgery, Qom University of Medical Sciences, Qom, Iran.
[2]Department of Student Research Committee, Qom University of Medical Sciences, Qom, Iran.
[3]Department of Research Committee, School of Medicine, Qom University of Medical Sciences, Qom, Iran.

Correspondence: Seyed Jalal Eshagh Hosseini, Department of Thoracic Surgery, Qom University of Medical Sciences, Qom, Iran. **E-mail:** mohsen_eshraghi@yahoo.com

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ABSTRACT

Objective: To evaluate the level of inflammatory factors of erythrocyte sedimentation rate and reactive protein C in benign and malignant thyroid nodules. **Material and Methods:** In this case-control study, patients who were referred because of an enlarged thyroid gland were selected, patients who had undergone surgery for the thyroid nodule were included in the study. Erythrocyte sedimentation rate and reactive protein C were measured before surgery in patients who were candidates for thyroid surgery. The histopathological records of patients were retrospectively reviewed. Relevant cases had a cytological evaluation of thyroid nodules by fine-needle aspiration cytology (FNAC). The mean of ESR / CRP in both groups was compared using an independent t-test ($p>0.05$). **Results:** In malignant tumor type, in all patients, with Pill (PTC), analyzes in the malignant group showed a significant difference between the mean ESR / CRP in both groups with and without thyroid history. Sub-analyzes in the malignant group were significantly different between the mean ESR / CRP in both groups with and without thyroid histories ($p=0.009$) (40.16 ± 28.81). The association between ESR and CRP, ESR / CRP and tumor size, ESR / CRP and age in each group as well as in the whole patients were evaluated using Pearson correlation test, which showed a positive association between ESR age and ESR ($p=0.024$, $r=0.375$). In the malignant group, a negative correlation was found between the age and the CRP rate ($p=0.027$, $r=-0.441$), and in the total patients between the age and the rate ($ES=0.043$, $r=-0.256$). **Conclusion:** Factors such as ESR and CRP, which are considered acute phase reactors and their levels increase in acute inflammatory conditions, may not have a significant increase in chronic inflammatory conditions and malignancies.

Keywords: Pathology, Oral; Thyroid Neoplasms; Thyroid Nodule.

Introduction

The development of thyroid nodules (overgrowth of normal thyroid tissue, thyroid cyst, chronic inflammation of the thyroid, multinodular goiter, thyroid cancer, iodine deficiency) is a common clinical problem and is found on physical examination and in more than 25% of cases using diagnostic methods such as sonography [1]. These nodules are important for malignancy. Thyroid malignancies are the most common endocrine malignancies, and 5 to 10 percent of medically considered thyroid nodules are malignant. On the other hand, in most studies performed on operated nodules, 10% of them have been malignant in pathology examination [2].

A higher incidence of malignancy in about 20 patients treated with surgery indicates a preoperative effort in selecting patients and does not show an accurate picture of the normal course of all thyroid nodules in the general population [3]. With a 5 to 1 chance for benign nodules, there is a greater need for preoperative differentiation between benign and malignant nodules. Therefore, to properly select patients for surgery, it is necessary to differentiate between benign and malignant nodules using different methods [2].

Among the various diagnostic methods, including radioisotope scanning, ultrasonography, and thyroid suppressant therapy response evaluation, the fine needle aspiration (FNA) method has been widely accepted as a simple, safe, and accurate method for evaluating thyroid nodules [4].

The palpable thyroid nodules are found in 5% of adults [5]. Most palatable nodules have a diameter greater than 1 cm. But the ability to touch the nodule is affected by factors such as the location of the nodule in the thyroid (superficial or deep), the neck of the anatomy, and the observer's experience. Susceptible methods such as computed tomography (CT), ultrasonography studies show thyroid nodules in more than 50% of people over 50 [6,7].

Most patients with thyroid nodules have normal thyroid function tests. If the thyroid-stimulating hormone (TSH) is low, a radionuclide scan is done indicating whether the nodule is hot or not because the nodules increase the excessive removal of thyroid ultrasonography due to the following reasons [8]: ultrasonography confirms that the palpable nodule is a nodule. About 15% of the palpable nodules are not confirmed by imaging, so further evaluation is not required [9]. If there are more palpable nodules, based on their imaging findings and their size, ultrasonography will assess the need for fine-needle aspiration (FNA) [10]. Ultrasonography facilitates decision-making about FNA by determining the shape and size of the nodule [11].

Nodular malformation is a disruption of thyroid cells' growth, often associated with gradual fibrosis. How to treat and control goiter based on its cause is different in patients. Therefore, after recognizing the thyroid gland's size in a physical examination, further assessment is needed to diagnose the cause. The term goiter is called the thyroid gland. Biosynthesis defects, iodine deficiency, autoimmune diseases and nodular diseases, each with different mechanisms, lead to goiter the creation. Graves' disease and Hashimoto's thyroids are also associated with goiter [9].

Thyroid carcinoma is the most common malignancy in the endocrine system. Malignant tumors derived from follicular epithelium are classified according to their histological characteristics. Distinct tumors like papillary thyroid carcinoma or follicular thyroid carcinoma are often curable and curable, and their prognosis is good if the disease is detected at an early stage. In contrast, anaplastic thyroid cancers are invasive and have a poor response to treatment. The prognosis is also weak [12].

Recently, some of the mechanisms that make up the relationship between inflammation and tumor are known. The small inflammatory environment of neoplastic tissues is characterized by leukocytes in the

supporting stroma and tumor cells, macrophages, dendritic cells, mast cells, and T cells distributed distinctly [13]. Several cytokines, tumor necrosis factor and chemokines produced by tumor cells and tumor-associated leukocytes and platelets can produce an aggressive phenotype [14].

Tumor-associated macrophages (TAMs) are major leukemia infiltrations that are initially called inflammatory chemokines {e.g., CC chemokine ligand 2 (CCL-2), and then held by cytokines present in a small tumor environment [13]. For example, Tumor-associated macrophages, vascular endothelial growth factor A, colony-stimulating factor-1, in response to cytokines such as interleukin-10, transforming growth factor β1, and macrophage colony-stimulating factor initiates tumors and progresses in the formation of stroma, and in times the density of tumor-associated macrophages increases in advanced thyroid cancers [8].

Previous authors investigated the link between blood CRP and cancer risk and whether increasing CRP could be a factor in cancer and found that 97% of participants had a CRP level below 10 mg / L. The mean plasma CRP levels (IQR 1.14-2.51) were 1.53 mg / L and 34% of participants had CRP > 2 mg / L [15].

The strong link between blood CRP levels and cancer risk may be due to 1) Increased CRP levels cause cancer, 2) Cancer increases CRP levels, and 3) Inflammation increases both CRP levels and cancer risk. Epidemiological studies of genetics (randomized controlled trials) examining the relationship between genetic polymorphism and cancer risk by considering blood CRP levels suggest that high CRP levels do not cause cancer. The lack of causality between elevated CRP levels and increased cancer risk does not invalidate the clinical application of mild CRP levels to predict cancer risk and treat cancer patients [16].

Growing evidence suggests inflammation is the seventh most important cancer characteristic that inflammation is associated with many malignancies' progression and prognosis. Red blood cell deposition and reactive protein C, as two indicators of inflammation, have often been reported in the development of solid cancers, but little research has been done on thyroid disease [17]. In this study, the level of inflammatory factors of Erythrocyte Sedimentation Rate and reactive protein C, which are commonly measured in clinical practice and are indicative of inflammation, in benign and malignant thyroid nodules were investigated.

Material and Methods

Research Design

In this cross-sectional analytical study, 100 patients with cold thyroid nodules referring to Shahid Beheshti Hospital of Qom were studied and the result of pathology in FNA and surgery in patients was compared.

Subjects

Patients who were referred to the Thyroid Gland Department were selected using a purpose-based sampling method. ESR / CRP was measured before surgery in patients who were candidates for thyroid surgery. According to the pathology report, thyroid nodules were divided into benign (control) and malignant nodules (the case group). The required number of samples in each group was 30, and the standard deviation in each group was considered to be 5.7 according to the previous study [18], but the two groups were matched in terms of age, sex, and finally, the levels of ESR and CRP were compared in two groups.

The criteria for entry in this study were patients with a history of cold thyroid nodules patients who had undergone surgery due to thyroid nodules, and their CRP, ESR was measured before surgery. Exclusion criteria included metabolic diseases, rheumatic diseases such as rheumatoid arthritis, SCL, microbial thyroiditis.

Data Collection

Ninety-four patients were chosen and divided into two groups of 47 people, and the history of thyroid disease was studied in two benign and malignant groups.

Histopathology

The histopathological records of patients were retrospectively reviewed. Relevant cases had a cytological evaluation of thyroid nodules by fine-needle aspiration cytology (FNAC). Aspiration was performed on an outpatient basis without local anesthesia using a 22-gauge disposable needle, a disposable plastic 10 ml syringe fitted in a 22-gauge disposable needle. The resulting smears were fixed in the air with alcohol and stained in the pathology laboratory using May-Grunewald-Giemsa, Hematoxylin-Eosin, and Papanicolaou methods were examined by a pathologist. The results of needle aspiration were classified according to the appropriate indicators mentioned in benign, suspicious, malignant, insufficient, or non-specific groups [19].

Biochemical Assays

ESR (mm/L) and CRP (mg/L) measurement methods were based on the standard laboratory methods as described elsewhere. In patients who were candidates for thyroid surgery, ESR / CRP was measured before surgery.

Statistical Analysis

ESR and CRP were measured before surgery and were compared by independent t-test in two groups of case and control. At a confidence level, 95% of the data were analyzed using IBM SPSS Statistics 20 software. Factors such as age, sex, and history of thyroiditis were also compared in two groups (chi-square). The independent t-test was used to compare age.

Ethical Clearance

This investigation was approved by the Research Ethics Committee (Protocol No. IR.MUQ.REC.1395.121) and informed consent was obtained from patients. The patients were assured that their information will be confidential and will be used only for research purposes.

Results

According to the obtained results and as shown in Table 1, the mean age of the patients in the benign (control) group was 42.28 ± 13.43 (minimum – 22 years and maximum 70 years) and in the malignant group (case) was 42.20 ± 16.32 (minimum – 20 years and maximum 85 years), which was not significantly different (p=0.350). There was no significant difference in the mean of ESR and CRP in benign and malignant groups (p=0.800) (Table 1).

Table 1. Clinic histopathological characteristics according to pathological results.

	Groups		
Variables	Malignant	Benign	**p-value**
Age (Years)	45.20 ± 16.32	42.28 ± 13.43	0.350
Gender - Female (N/%)	39 (83.0)	39 (83.0)	1.00
ESR	13.54 ± 8.09	13.03 ± 7.80	0.800
CRP	4.92 ± 5.88	4.92 ± 4.91	0.993

The rate of thyroiditis in both benign and malignant groups were evaluated and compared. In the benign group in the pathological report, 15 patients (31.9%) had a history of Hashimoto thyroiditis, while in the malignant group, a history of Hashimoto's thyroiditis was reported in 20 patients (42.6%), there was no significant difference between the two groups (p=0.216) (Table 2).

Table 2. Frequency of patients with a history of thyroiditis in benign and malignant groups.

History of Thyroiditis	Group		p-value
	Benign N (%)	Malignant N (%)	
Yes	15 (31.9)	20 (42.6)	0.216
No	32 (68.1)	27 (57.4)	

In the malignant group, 6 patients (12.8%) had capsular invasion, and 11 patients (23.4%) had vascular invasion and 9 (19.2%) patients had lymphatic metastases. The type of tumor was PTC in all patients in the malignant group. In the malignant group, the patients were divided into two groups according to the presence or absence of thyroiditis (Table 3).

Table 3. Comparison of ESR and CRP in the malignant group.

Variables	History of Thyroiditis		p-value
	Yes Mean (SD)	No Mean (SD)	
ESR	12.75 ± 8.19	14.13 ± 8.24	0.665
CRP	5.09 ± 5.26	4.80 ± 47.6	0.904

The mean ESR in the group reported in their pathological history of thyroiditis was 19.8 ± 7.12 and in the group with no history of thyroiditis, it was 24.8 ± 14.4. The mean ESR of the two groups showed no significant difference (p=0.665).

The mean CRP was also compared in the two groups: 5.09 ± 5.26 in the thyroid group and 6.47 ± 4.80 in the non-thyroid group. No significant difference between mean CRP was observed (p=0.904).

In the malignant group, the mean tumor size in the group with a history of thyroiditis was significantly less than that in the non-thyroid group (17.64 ± 9.31 *vs.* 40.16 ± 28.81, p=0.009).

The association between ESR and CRP, ESR / CRP and tumor size, ESR / CRP and age in each group were analyzed using Pearson correlation test. The association between ESR and CRP, ESR / CRP and tumor size, ESR / CRP and age in each group as well as in the whole patients were evaluated using Pearson correlation test, which showed a positive association between ESR age and ESR (p=0.024, r = 0.375). In the malignant group, a negative correlation was found between the age and the rate of CRP (p=0.027, r = -0.441), and in the total patients between the age and the rate (ES = 0.043 r = -0.256).

Discussion

Epidemiological studies link the relationship between chronic inflammation and thyroid cancer risk factors, such as Hashimoto's thyroiditis [20]. In our research, the factors of ESR and CRP were examined. According to the results, there was no statistically significant difference between the groups in the level of ESR and CRP factors. Our results did not find a link between levels ESR and CRP in patients with benign and malignant thyroid nodules, which contradicts the few studies previously investigating this association.

Despite our study results, previous authors showed a different ESR expression and CRP between PTC and nodular goiter and it was demonstrated that in patients with PTC, the mean ESR and CRP level were lower than nodular goiter [17]. The difference in the results of ESR / CRP can be due to the difference in the type and volume of the sample of the two studies and the genetic differences between the two populations [17].

The history of thyroiditis in the malignant group was higher than the benign group. So it seems that thyroiditis was a risk factor for thyroid cancer in our study. However, no significant difference between the two groups (p=0.216) was found, which is in line with the previous literature report [19]. However, in the study of Bradly et al., Hashimoto thyroiditis was higher (40%) in papillary cancer than benign thyroid nodules [21].

It was found that in the malignant group, sub-analyzes were performed and ESR / CRP was compared in a group with a history of thyroiditis or a group that did not. The mean of ESR of the two groups not show a significant difference. The mean CRP was also compared in two groups, with a history of thyroid and no significant difference was found. The relationship between ESR and CRP, ESR / CRP and ESR / CRP tumor size and age in each group were analyzed using Pearson correlation test. In the benign group, there was a significant correlation between ESR and ESR and in the malignant group, there was a significant difference between age and CRP and in the total number of patients between the age and ESR. In line with these results and in the study of Hou et al., a positive correlation between ESR and CRP was found [17].

In this study, in the malignant group, the type of tumor was PTC in all patients. It is suggested that in future studies, the association between the level of ESR / CRP and other inflammatory factors such as IL-1.6 with other types of thyroid cancers, such as follicular, modular, ana-plastic and thyroid lymphoma, should be considered. It is also suggested that further studies are needed to investigate the relationship between the level of anti-thyroid antibodies such as TgAb and TPOAb with the type of nodule (benign with malignant) and the size of the nodule and in the case of malignant nodules, the association of these antibodies with nodule invasion into thyroid capsules, blood vessels, lymph nodes, and prognosis and patient survival should be considered.

Factors such as ESR and CRP that are acute phase reactants and their levels increase in cases of acute inflammation may not increase significantly in chronic inflammatory conditions and malignancies. In this study, the malignant tumor type is PTC in all cases and it is suggested that in future studies, the relationship between ESR / CRP level and other inflammatory factors such as IL-1.6 with other types of thyroid cancers should be considered. It is suggested that further studies be conducted to investigate the relationship between the level of anti-thyroid antibodies, such as TgAb and TPOAb, with the type of nodule (benign with malignant) and the size of the nodule. In the case of malignant nodules, the association between these antibodies and the invasive behavior of the nodule and proptosis and the survival rate of the patients are examined.

The factors such as income level, alcohol consumption history, Metastasis, diagnostic, and treatment method affect the survival level, which can be a limitation in the present study. On the other hand, the tumor type in all patients was PTC in the malignant group, while the relation between ESR/CRP level and other inflammatory factors such as Il-1.6 and other thyroid cancers such as Follicular, modular, Anaplastic and also thyroid lymphoma can be studied.

The relation between anti-thyroid antibodies such as TgAb and TPOAb and nodule type (benign and malignant) was not studied. In malignant nodules, the relation between mentioned antibodies and nodule

invasion of the thyroid capsule and thyroid and blood vessels, lymph and nerves, as well as prognosis and survival rate of patients, were not considered.

Conclusion

Factors such as ESR and CRP that are considered acute phase reactors and their levels increase in acute inflammatory conditions may not increase significantly in chronic inflammatory and malignant conditions.

Authors' Contributions

ME	https://orcid.org/0000-0003-1231-1167	Investigation, Formal Analysis and Writing – Original Draft Preparation.
SJEH	https://orcid.org/0000-0003-3139-9512	Conceptualization, Methodology and Writing – Review and Editing.
FSR	https://orcid.org/0000-0002-4578-6209	Conceptualization, Methodology and Writing – Review and Editing.
EN	https://orcid.org/0000-0002-4457-129X	Writing – Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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