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Assessment of Inflammatory and Morphological Changes in the Conjunctival Cells in Relationship to the Degree of ProptosiS in Thyroid – Associated Ophthalmopathy

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Abstract: Aim: to evaluate the inflammatory and morphological changes in conjunctival cells and the relationship to the degree of proptosis for helping early diagnosis of thyroid -associated eye disease. Subjects and methods: cross sectional observational prospective study that included 40 patients complain from proptosis of thyroid eye disease attended to Ain shams university, Cairo, from May 2019 to November 2020 with control 40 normal person. Exclusion criteria included patients with History of surgical correction of thyroid eye disease, auto immune disease, Previous Lasik surgery, recent Intraocular surgery, diabetic patients and patients under steroid or immune suppressive treatment. All patients were subjected to History taking of thyroid disease with laboratory investigations to assess the thyroid functions, visual acuity measurement, intraocular pressure, Hertel's exophthalmometry for measurement of the degree of proptosis, color vision using Ishihara's test, Funds examination and Conjunctival impression cytology. Images were taken using an Olympus CX31 microscope fitted with an Olympus E-620 camera. Results: The degree of inflammation in impression cytology showed that most of thyroid eye disease group is in moderate degree (80%) 32 cases, while the rest (20%) is between mild degree of inflammation (15%) 6 cases and severe degree of inflammation (5%) 2 cases. No statistically significant relation found between degree of proptosis and severity of inflammation of right and left eye with p-value = 0.650 and 0.331 respectively. Morphologically, most of thyroid eye disease group showed stage 2 in impression cytology results (65%) 26 cases, while the rest (35%) between stage 4, 3, 1 (5%, 15%, 15%, respectively). Conclusion: Detection of inflammatory and morphological changes occurring in conjunctiva of cases with thyroid –associated Ophthalmopathy by impression cytology can help in early diagnosis of thyroid-associated Ophthalmopathy patients even before appearance of other eye symptoms.

Keywords: Thyroid Disease, Proptosis, Inflammatory Changes, Impression Cytology

1. Introduction

Thyroid – associated Ophthalmopathy (TAO) is an autoimmune disorder, chronic in nature, that is usually associated with Graves' disease, it may also be rarely present in autoimmune hypothyroidism and euthyroid conditions [1]. The estimated incidence of thyroid eye disease is 16 women and three men per 100,000 population per year [2]. Upper eyelid retraction, conjunctival and caruncle injection and/or edema, eyelid oedema and/or erythema with diurnal variation, ocular motility disruption or strabismus and proptosis. Paradoxically, upper eyelid ptosis can also be a presenting sign of thyroid- associated Ophthalmopathy [3, 4]. The exact mechanism by which Grave's disease affects the eye is not fully understood. It can be explained by that the immune system produces an abnormal antibody called thyroid-stimulating immunoglobulin. This antibody mimics the function of normal thyroid-stimulating hormone. These abnormal antibodies affect the cells surrounding the eyes causing the symptoms associated with the disorder [5]. There

is also an overexpression of a protein called insulin-like growth factor 1 receptor (IGF-1R) that plays a significant role in TAO. There is an infiltration of the extraocular muscles by immunocompetent T-helper cells (type-1), B lymphocytes, macrophages and mast cells that can lead to restricted eye movements and proptosis [6].

Impression cytology had used to identify morphological changes, conjunctival goblet cell loss and changes to epithelial cells, also it can assess the infiltration of conjunctiva of patients with TAO by inflammatory cells [7] which suggests that the disease is active [8].

The degree of inflammation and the morphological changes in relation to the degree of proptosis in TED can be evaluated by impression cytology in our study.

2. Subjects and Methods

This study was conducted on patients attending the Ophthalmology outpatient clinic of Ain Shams University, Cairo, and the impression cytology were done in research institute of ophthalmology. A cross sectional observational study designed to analyze data of 40 patients complained of proptosis from TAO and control group of 40 normal people on the time interval between May 2019 and November 2020. Informed consents obtained from all participants. patients with History of surgical correction of thyroid eye disease, auto immune disease, had Lasik surgery, recent Intraocular surgery, diabetic patients and patients under steroid or immune suppressive treatment were excluded from the study. All patients subjected to History taking of thyroid disease with laboratory investigation to assess the thyroid functions, visual acuity, intraocular pressure, Hertel's exophthalmometry for measurement the degree of proptosis, color vision using Ishihara's test, Funds examination and Conjunctival impression cytology. Images were taken using an Olympus CX31 microscope fitted with an Olympus E-620 camera.

3. Impression Cytology

All the impression cytology specimens were obtained from temporal bulbar conjunctival surface of TAO patients and controls.

4. Type of Impression Cytology Filter Paper

Cellulose acetate filter paper Millipore type with pore size of 0.22um was used to take the impression cytology specimens [9]. The paper is originally circular in shape, 47mm in diameter and white in color.

5. Technique

The nitrocellulose paper was cut into strips of 5X15 mm. The rough surface was applied onto conjunctiva will always be downwards, while smooth surface was superficial, the rough surface was used for obtaining specimens to harvest broad sheets of cells.

Topical anesthesia was used before application and after explanation of the procedure using 0.4% benoxinate hydrochloride, then a nitrocellulose strip was applied to the conjuntival surface using non toothed forceps to grasp the tapering end of the filter paper strip with gentle pressure over the filter paper using Q-tip to press the rough surface of the strip against the conjunctival surface and the tip was kept pointing outwards and upwards, after 10 seconds the strip was peeled off gently, the specimen was immediately placed in contact lens containers filled with 97% ethyl alcohol as fixation.

6. Staining Procedure

After removing the filter from the fixative it was put in a plastic fenestrated device to dip it in the different staining containers. Specimens were stained with hematoxylin and eosin stain, they were first rehydrated in consecutively decreasing concentration of ethanol, then stained with alum hematoxylin for 10 minutes followed by rinsing under tap water, subsequently they were differentiated with 1% acid alcohol. The specimens were counterstained with xanthenes dye eosin for 3 minutes and rinsed in running tap water, then dehydrated in absolute alcohol for 5 minutes and immersed in xylene, finally specimens were mounted in glass slide and drop of canada blasm was applied to the specimen, the slides were examined using a the slides were examined using a light microscope under magnification of 500 X.

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The comparison between groups regarding qualitative data was done by using Chi-square test. The comparison between groups with quantitative data and parametric distribution was done by using Independent t-test while data with non parametric distribution was done by using Mann-Whitney test. The relative proportions of inflammatory cells were quantified on a scale of three (few 1+, many 2+ and numerous 3+) = (mild, moderate, sever, respectively).

7. Results

The mean age of included patients with proptosis of Thyroid-associated ophthalmopathy was 44.55 years±11.35, 24 were females and 16 were males, and the mean age of control group was 44.85 years±12.85, 24 of them were females and 16 were males.

There was no significant difference between the two groups regarding age and gender.

There was significant increase in Hertel exophthalmometer measurement in diseased group than control group in right and left eye [table 1]. According to Tseng classification of stages [table 2] (figure 1) shows that there is statistically

highly significant difference in impression cytology stage between diseased group and control group with p-value < 0.001. most of thyroid eye disease group showed stage 2 in impression cytology results (65%) 26 cases, while the rest (35%) between stage 3 (15%) 6 cases, stage 1 (15%) 6 cases and stage 4 (5%) 2 cases. There was no statistically significant relation found between Hertel exophthalmometer measurement and impression cytology stage of right and left eye [table 3]. Most of TAO group showed moderate degree of inflammation in impression cytology results (80%) 32 cases, while the rest (20%) between mild degree of inflammation (15%) 6 cases and severe degree of inflammation (5%) 2 cases [table 4] (figure 2). There was no statistically significant relation found between Hertel exophthalmometer measurement and severity of inflammation of right and left eye [table 5].

Table 1. *Comparison between control group and diseased group regarding hertel exophthalmometer measurement.*

Hertel exophthalmometer measurement		**Control group_____ No. = 40_____**	**Diseased group No. = 40**	**Test value**	**P- value**
Right	Mean±SD Range	17.50 ± 0.89 16 – 19mm	23.45 ± 1.79 19 – 28mm	-11.072	0.000
Left	Mean±SD Range	17.50 ± 0.89 16 – 19mm	23.75 ± 1.41 20 – 29mm	-14.091	0.000

Independent t-test.

Table 2. *Comparison between control group and diseased group regarding impression cytology stage.*

Impression cytology stage		**Control group**		**Diseased group**		**Test value**	**P- value**
		No.	**%**	**No.**	**%**		
Eye	Right	20	50.0% 5	22	55.0%	0.100	0.752
	Left	20	0.0%	18	45.0%		
Stage	0	32	80.0%	0	0.0%	33.143	0.000
	1	8	20.0%	6	15.0%		
	2	0	0.0%	2	65.0%		
	3	0	0.0%	6	15.0%		
	4	0	0.0%	6	5.0%		
				2			

Chi-square test.

Table 3. *Relation between Hertel exophthalmometer measurement and impression cytology stage of the studied patients.*

Hertel exophthalmometer measurement		**Impression cytology stages**		**p-value**
		Stage (1-2) NO=32	**Stage (3-4) NO=8**	
Rt	Mean±SD	22.50 ± 1.71	22.25 ± 2.36	0.810 (NS)
	Range	19 – 24 mm	19 – 24 mm	
Lt	Mean±SD	22.56 ± 1.50	23.50 ± 0.58	0.244 (NS)
	Range	20 – 24 mm	23 – 24 mm	

Independent t-test.

Table 4. *Comparison between control group and diseased group regarding severity of inflammation.*

Severity of inflammation	**Control group**	**TAO group**	**P value**
	NO. %	**NO. %**	
Almost Nile	32 (80%)	0 (0%)	0.000
Mild	8 (20%)	6 (15%)	
Moderate	0 (0%)	32 (80%)	
Sever	0 (0%)	2 (5%)	

Chi-square test.

Table 5. *Relation between Hertel exophthalmometer measurement and severity of inflammation of the studied patients.*

Hertel exophthalmometer measurement		**Severity of inflammation**		**p-value**
		Mild	**Moderate and Sever**	
		N0. 6	**NO. 34**	
Rt	Mean±SD Range	22.0 ±1.73 20 – 23 mm	22.53±1.84 19 – 24 mm	0.650 (NS)
Lt	Mean±SD Range	22.0±1.73 20 – 23 mm	22.88±1.36 20 – 24 mm	0.331 (NS)

Independent t-test.

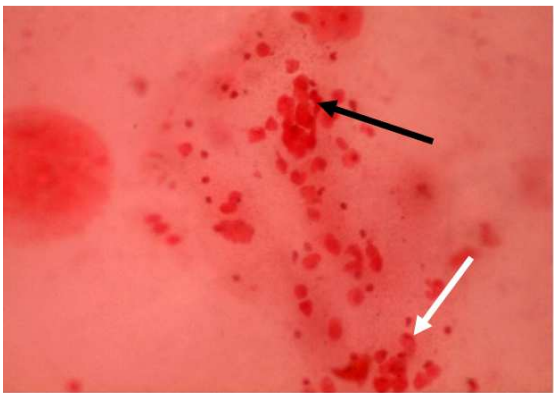

Figure 1. *Light micrograph of impression cytology specimen showing epithelial conjunctival cells (stage 2) with N/C ratio 1:4. (black arrow) Note keratinized cell with N/C ratio 1:6 with small pyknotic nucleus, squamous metaplasia (stage3) (white arrow). (H &E x500).*

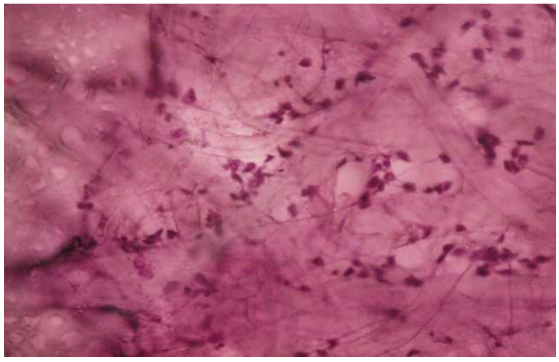

Figure 2. *Light micrograph of impression cytology specimen showing many inflammatory cells (lymphocytes) (H&E x 500).*

8. Discussion

Ocular surface, A functional unit that includes the corneal and conjunctival epithelium, lid margins and tear film. Is one of a frequent casualty of TAO. Classically, the widening of palpebral fissure and eyelid alterations caused by TAO have been implicated in the disruption of ocular surface homeostasis. This leads to exposure keratopathy, tear film instability [10], increased tear evaporation that can be accelerated by exophthalmos and high tear osmolarity. Eventually, ocular surface (OS) inflammation ensues, initiating a vicious cycle which eventually leads to dry eye syndrome (DES) [11].

TAO is frequently associated with Inflammation of the OS and dry eye which sometimes preceding ophthalmic changes [12]. A significant correlation was found between TAO activity, and OS damage and it can be detected by impression cytology on the bulbar conjunctiva in our study, IC shows complete loss of goblet cells with histological changes in the epithelial cells with mild to moderate keratinization, this matches with the results of Alves M et al, [13], whereas, the prevalence of dry eye was 65% in patients with TAO, and histopathologic changes in the conjunctiva were consistent with dry eye syndrome.

In one report, patients with occult TAO consistently reported symptoms of ocular irritation, foreign body sensation, redness, and excessive tearing [14]. These individuals were found to have OS inflammation in the absence of exophthalmos, lid retraction, dysmotility, and diplopia. Thus, the earliest forms of TAO may be confined to the OS, well in advance of lid retraction and lid lag. In our study demonstrate that all patients have a degree of inflammation verified from mild to sever with high percentage (80%) in a moderate degree of inflammation with increase infiltration of lymphocyte cells. thesis findings were in agreement with Gupta et al. [13], but did not clarify the degree of inflammation in each case. Although exophthalmos found in all of our TAO cases but our statistical results showed no relation between degree of exophthalmos and severity of inflammation and this demonstrated the effect of TAO as an autoimmune disease on the conjunctival surface damage. And this is in agreement with recent studies which reported that inflammation has a major role in ocular surface damage in TAO patients and exophthalmos, lid retraction other factors are not the main cause [15]. So our study demonstrated the effect of TAO on the conjunctiva which showed immunological changes and inflammatory cells infiltration, this clarify the difference in the immune state of TAO patients from normal person.

9. Conclusion

Detection of inflammatory and morphological changes occurring in conjunctiva of cases with thyroid-associated Ophthalmopathy by impression cytology, can help in early diagnosis of thyroid-associated Ophthalmopathy patients even before appearance of other eye symptoms.

10. Limitations of the Study

Although hematoxylin and eosin stain had used in impression cytology with satisfied results but need in the next study use lissamine green staining to detect the devitalized ocular surface areas in TED.

Conflicts of Interest

There are no conflicts of interest.

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