Clinical And Immunological Assessment Of Activity In Graves' Ophthalmopathy

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Abstract: Graves' disease is an autoimmune disorder, which is characterized by hyperthyroidism, goiter and in some cases ophthalmopathy. Patients with serious inflammatory Graves' ophthalmopathy should be treated with anti-inflammatory drugs or radiotherapy to prevent complications, while those with extraocular muscle dysfunction at the stage of fibrosis and proptosis does not generally respond well to corticosteroid, thus treated surgically. The aim of this work is to know whether a patient has active or quiescent Graves' ophthalmopathy as it is often difficult however to distinguish inflammatory from non inflammatory Graves' disease. The present study compromised 40 patients with Graves' ophthalmopathy selected from Out Patient Clinic in Mansoura University Hospital, and 15 healthy volunteers as control. All control subjects and patients were subjected to the following: Complete history taking, complete general examination, thyroid examination and neurological examination, Clinical activity scoring, Orbital ultrasonography. Thyroid function test: T3, T4, TSH, sICAM-1, γIFN, IL-2 serum level, CD3, CD4, CD8 in the peripheral blood. Using orbital U.S. 70% of our patients with active Graves' ophthalmopathy have a low extraocular muscle reflectivity. Also there were significant elevation of CAS, sICAM-1, IL-2 and γIFN in patient with positive U.S. compared to those with negative U.S. in active Graves ophthalmopathy. Serum levels of sICAM-1 were significantly high in patients with Graves' disease than controls. And it is higher in patients with active Graves' ophthalmopathy. In addition, there was significant high level of both IL-2 and γIFN in patients with active Graves' ophthalmopathy than inactive one. Also they positively correlated with sICAM-1 level and CAS. CAS is a good tool for differentiation between active and inactive Graves' ophthalmopathy and can be strengthened by orbital U.S. as well as sICAM-1, IL-2 and γIFN. On the other hand, T cell subsets were found to be of little help in differentiation between active and inactive disease status.

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Introduction

Graves' ophthalmology is one of the major presentation of Graves' disease[(2)]**.** It is an organ specific autoimmune disorder resulting from inflammatory effect on retro-orbital connective tissue and extraocular muscles with Th1 rather than Th2 dominance within the thyroid gland[(3)]**.** Autoimmune reaction has been assumed because orbital tissue is usually infiltrated by immunocompetent T cells**.** Increased de novo adipogenesis causing a rise in adipose tissue volume[(4)]**.** Despite recent progress in the understanding of this pathogenesis, treatment is not often satisfactory. In severe forms of the disease, aggressive measures are required. If the disease is active, high dose of corticosteroids, immunosuppressive therapy and/or orbital radiotherapy is the main line of treatment[(5)]**.** If the disease is severe but not active orbital decompression is preferable**.** To detect the activity of the disease, a clinical activity score was designed by **Mourits et al.,**[(1)]**.** This score is highly correlated with response to steroid, immunoglobulin and immunosuppressive therapy**.** However this clinical activity score depends upon ten factors: some of them are subjective and others need recurrent assessment on 3 months interval**.** For this reason other indicator of this activity is needed. Graves' ophthalmopathy being inflammatory condition could be reflected on adhesion molecules**.** Recently, it has been shown that serum level of sICAM-1 is correlated to the clinical activity score in active Graves' ophthalmopathy with or without hyperthyroidism suggesting that sICAM-1 serum level could reflect the degree of ocular inflammatory activity[(6)]**.** This leads to development of new treatment strategies able to hindering immune cell adhesion with ultimate goal of preventing clinical retro-orbital endothelial and fibroblast activation in Graves' ophthalmopathy. Switching of the autoimmune reaction inducing the active phase perhaps by altering the cytokine milieu in the orbit[(7,8)]**.** Graves'

ophthalmopathy is considered to be chronic, autoimmune inflammatory disorder that impacts all orbital tissue sections and results in various eye features, including lid retraction (class I), soft tissue inflammation (class II), proptosis (class III), extraocular muscle dysfunction (class IV), corneal involvement (class V) and sight loss (class VI). Even weak to moderately severe ophhtalmopathy greatly impacts on and reduces the quality of life in affected patients. The course of graves' ophthalmopathy is unpredictable and a rapid worsening of Graves' ophthalmopathy can occur at any time. As the treatment of graves' ophthalmopthy is often inadequate there is a need to recognize potential predisposing factors and to develop sensitive and specific diagnostic procedure to identify Graves' disease patients at high risk of developing ophthalmopathy. Both endogenous such as genetic factors, increasing age, sex and exogenous factors including cigarette smoking, thyroid dysfunction and radioiodine treatment, may contribute to the development and/or severity of Graves' ophthalmopathy. Of these the greatest risk factor for developing clinically evident eye signs in patients with Graves' disease is smoking. The role of genetic factors in development of Graves' ophthalmopathy so far remains unknown. Although a genetic predisposition to the development of Graves' disease is well established by twin studies and several susceptibility loci have been identified, the significance of genotype-phenotype correlations in Graves' disease remains speculative. So far, twin studies concerning Graves' ophthalmopathy have not been carried out and the results of family-based studies are conflicting. Most recently, the segregation ratio for severe Graves' ophthalmopathy requiring 'active treatment' such as orbital surgery, orbital irradiation, steroids or other immunosuppressive drugs was reported to be zero[(9)]**.** Our aim of the work was detection of other indicators of active Graves' ophthalmopathy which could correlate with clinical activity score and also to detect if sICAM-1 serum levels help identification of subgroups prone to develop severe clinical ophthalmopathy.

Patients and Methods

Two subject groups were enrolled in the present study, group of patients with Graves' ophthalmopathy and control group. The group of patients with Graves' ophthalmopathy was chosen randomly from patients suffering from Graves' disease attending The Endocrine Outpatient Clinic in Mansoura University Hospitals. They were diagnosed by: clinical assessments, thyroid function tests, thyroid ultrasound, and radioactive iodine uptake and thyroid autoantibodies as TSHR Ab, antithyroglobulin, thyroid peroxidase antibody) in some patients. Forty patients with Graves' opthalmopathy were included 18 males and 22 females. Their ages ranged from 15 to 55 years. This group was subdivided according to clinical activity score into: Patients with active Graves' ophthalmopathy (10 patients), CAS>3, Patients with inactive Graves' ophthalmopathy (30patients), CAS<3. Exclusion of any associated disease. Patients were receiving conventional therapy; Carbimazole 5mg, by average daily dose three times, Propranelol 40mg, twice daily, Benzodiazepin 5mg, once daily. The patients with Graves' ophthalmopathy were subjected to the following: *Complete history taking with particular stress on the following:* age and sex; parity, menses**;** special habits**;** age of onset and duration of Graves' disease**;** type of treatment received**;** Clinical presentation with particular stress on the symptoms of thyrotoxicosis **,** family history of Graves' or other autoimmune disease**,** past history of surgical operation**.** *Complete general examination with particular stress on:* complexion: jaundice, pallor**;** weight and height, upper and lower measurements, body mass index**;** pulse, blood pressure**;** edema lower limbs**;** C.V examination with stress on size of the heart**;** chest examination**;** abdominal examination**;** thyroid examination for size, surface, consistency**;** neurological examination. *Clinical activity scoring:* was done by the same ophthalmologist by slit lamp (Z.E.I.S.S Germany). Ten factors, based upon the classic symptoms and signs of inflammation are considered in a Clinical Activity Score (CAS). Other factors, seven may be assessed on a single occasion and three require two examination at 1-3 months apart (decrease movement, decrease visual acuity and increase proptosis). Two factors are symptoms(pain and impaired vision) and three are signs somewhat difficult to assess consistency (lid redness, lid swelling, conjunctival redness), two are more readily assessed (chemosis and caruncular swelling) and three are based upon fairly reproducible numerical assessments[(1)]**.** Ten points to include: painful, oppressive feeling on or behind the globe; pain on attempted up, side, or down gaze; redness of the eye lids; diffuse redness of conjunctiva; chemosis; swollen caruncule; edema of the eye lids; increase of proptosis of 2mm or more during a period between 1 and 3 months (by Hertle exophthalmometer.); decrease in visual acuity of 1 or more lines on the Snellen chart (using a pinhole); decrease of eye movements in any direction equal to/or more than 5 degrees during a period of time between 1 and 3 months. For each of the signs presents one point. The sum of these points defines the activity score. A score of four or more on the CAS was classified as active ophthalmopathy[(10)]**.** *Orbital Ultrasonography:* using Humphery Instrument Inc. A/B Scan 835. to Assess eye muscle reflectivity,

using standardized A-mode ultrasonography of both orbits. The sound beam was directed perpendicularly to the axis of the rectus muscle, and reflectivity was determined was at the muscle belly. The eye muscle reflectivity was calculated by measuring the distance from the baseline to the mean of the tops of all spikes within the anterior and posterior muscle sheaths, and expressed as a percentage of the initial scleral spike, which was set at 100%. Low reflectivity at least in one eye muscle indicates active Graves' ophthalmopathy. *Laboratory Assessment***:** Thyroid function tests (T3, T4, TSH)**;** sICAM-1 serum level**;** CD3, CD4, CD8**;** γIFN, IL-2 serum level. Immunoenzymetric assay for the quantitative measurement of human sICAM-1(CD54) provided by Medgenix. Determination of IL-2 using Enzyme-Linked Immuno Sorbent Assay (ELISA). The kit was supplied from Diaclone Research, France. Determination of γIFN: By using sandwich enzyme immunoassaying kit supplied by titerzyme Company USA. The detection limits of γIFN was 0.1 pg/ml. Assessment of T cell subsets (CD3, CD4, CD8) using direct immunoflourescent technique: Separation of mononuclear cells were done using Ficol-Hypaque Centrifugation Method according to ***Boyum***[11] and then labeling using specific monoclonal antibodies against CD3, CD4, CD8 measuring T cell subsets using fluorescent microscopy. *Separation of lymphocytes as follows:* 5ml of heparinized blood was diluted with the same volume of RPMI 1640**.** The diluted blood was layered over 5ml of Ficol 1077, centrifuged for 20 minutes at 2500rpm at room temperature. The mononuclear cells were collected aseptically by sterile pasteure pipettes and transferred into another sterile conical tube and washed by phosphate buffer. The mononuclear cells centrifuged for 10 minutes at 2000rpm at room temperature. The supernatant was discarded and the pellet was resuspended in RPMI and washed twice, the supernatant was discarded and 1ml of RPMI medium was added to the pellet of lymphocytes. The lymphocytes were counted by using freshly prepared trypan blue as a test for viability and the count was adjusted.

Direct immunofluorescence using fluorescent microscopy (clonal Mab) for CD3, CD4, and CD8: Lymphocytes was separated according to ***Boyum***[11]. 100µl of lymphocytes containing 5 million were mixed with 10 µl Mab. They were incubated in ice for 30 minutes. Washing 3 times in PBS and discarding the supernatant. The pellet resuspended in 20µl of washing solution. Examination under fluorescent microscopy.

Statistical analysis

Data was analyzed using SPSS (statistical package for social sciences) version 10. Qualitative data was presented as a number and %. Comparison between groups was done by Fisher's exact test, as appropriate. Quantitative data was presented as mean ± SD. Student's T test was used for comparison between groups. Spearman correlation coefficient (r) was used to test correlation between variables. $P<0.05$ was considered to be statistically significant.

Results

Table (1): Demonstrate some basic data of the studied groups. Group I "active Graves' ophthalmology" represents 18.2%, group II "inactive Graves' ophthalmology" 54.5%, group III "control" represents 27.3% (15 healthy subjects). In group I, group II and group III. There were no significant difference ($P>0.05$) in the mean age. There were a high significant mean duration ($P<0.01$) of the disease in group II "inactive Graves' ophthalmology" than group I "active Graves' ophthalmology". CAS mean values were significantly higher in active Graves' ophthalmology patients than inactive are ($P<0.001$).

Table (2): Shows different clinical presentation of Graves' ophthalmology patients. The patients represented by different symptoms and signs as anxiety, bone ache, exophthalmos, easy fatigue diplopia, intolerance to hot weather, insomnia, tremors, sweating, psychic trauma, loss of weight, palpitation and polyphagia.

Table (3): Demonstrate thyroid function tests mean values of T3, T4 and TSH in different groups of Graves' ophthalmopathy versus control. Serum T3 and T4 were significantly higher in total, active and inactive Graves' patients than the control ($P1<0.001$), while the comparison between active and inactive Graves' ophthalmopathy, serum T3 and T4 mean values were non significantly different ($P2>0.05$). Serum TSH mean value was significantly lower in studied groups than the control ($P<0.001$). However among active and inactive Graves' ophthalmopathy there was no significant statistical difference ($P>0.05$).

Table (4): Demonstrate serum concentration of sICAM-1 in patient with Graves' ophthalmopathy versus control. sICAM-1 mean value was significantly higher in studied than the control ($P1<0.001$). Also sICAM-1 serum concentrations were significantly higher in active Graves' ophthalmopathy compared to inactive one ($P2<0.001$).

Table (5): Demonstrate serum concentration of IL-2 in Graves' ophthalmopathy patients versus the control. IL-2 mean value was significantly higher in studied groups compared to control group ($P1<0.001$). Also there was significant increase of IL-2 mean values compared to inactive Graves' ophthalmopathy ($P2<0.001$).

Table (6): Demonstrate serum concentration of γIFN in Graves' ophthalmopathy patients versus the control. γIFN mean value was significantly higher in different studied groups compared to control (P1<0.001). Also there was significant increase of γIFN in active compared to inactive Graves' ophthalmopathy (P2<0.001).

Table (7): Shows the results of immunophenotyping of T cells subsets in active and inactive Graves' ophthalmopathy. CD3, CD4 and CD8 mean values showed no significant statistical difference among the 2 groups (P>0.05).

Table (8): Shows the results of orbital U.S. In studied groups 70% of patients with active Graves' ophthalmopathy have low extraocular muscles reflectivity. While normal extraocular muscles reflectivity was found in all patients with inactive Graves' ophthalmopathy.

Table (9): Shows the results of measured parameters in patients with low extraocular muscles reflectivity (+ve US) versus patients with normal extraocular muscles reflectivity (-ve US). The mean value of CAS, IL-2 and γIFN showed significant elevation in patients with +ve US compared to –ve US patients. While the mean value of sICAM1 was elevated but statistically non-different, in contrast to the T3, T4, TSH, CD3, CD4 and mean values showed non-significant statistical difference between +ve and –ve US.

Table (1): Some basic data of the studied groups

Group	CAS		Age		Disease duration	
	Mean ± SD	P1	Mean ± SD (years)	P	Mean ± SD (months)	P1
Active Graves' ophthalmopathy N=10 (M:6 and F:4)	5.0 ± 0.81		37.2 ± 12.6	>0.05	16 ± 5.7	
Inactive Graves' ophthalmopathy N=30 (M:12 and F:18)	1.56 ± 0.62	<0.001	31.5 ± 11	>0.05	32 ± 14	<0.001
Control N=15 (M:6 and F:9)			32.4 ± 10.6			

Table (2): Different clinical presentation of Graves' patients

Symptoms & signs	Number (n=40)	%
Anxiety	32	80
Bone ache	15	37.5
Exophthalmos	40	100
Dyspnea	28	70
Easy fatigue	19	72.5
Diplopia	18	45
Intolerance to hot weather	19	47.5
Insomnia	28	70
Tremors	18	45
Sweating	24	60
Psychic trauma	19	47.5
Hot & sweaty hand	19	47.5
Loss of weight	37	92.5
Palpitation	29	72.5
Polyphagia	15	37.5

Table (3): Thyroid function tests in diseased patients versus controls

Groups	T3 (ng/ml)	T4 (µg/ml)	TSH (µIU/ml)
Control (n=15) **Mean ± SD**	1.5 ± 0.46	8.4 ± 1.99	2.0 ± 1.2
Active Graves' ophthalmopathy (n=10) **Mean ± SD**	1.8 ± 1.1	16.3 ± 3.2	0.036 ± 0.018
t_1	7.079	7.522	4.880
P_1	<0.001	<0.01	<0.001
Inactive Graves' ophthalmopathy (n=30) **Mean ± SD**	2.8 ± 1.3	15.0 ± 3.9	0.27 ± 0.03
T_1	3.787	6.020	6.430
P_1	<0.001	<0.001	<0.001
T_2	2.011	0.950	1.330
P_2	>0.05	>0.05	>0.05

P_1= comparison between active or inactive Graves' ophthalmopathy with control
* Significant P value <0.05
P_2= comparison between active and inactive Graves' ophthalmopathy

Table (4): sICAM-1(CD54) serum concentration in-patients with Graves ophthalmopathy and controls

Groups	Mean (ng/ml)	SD	t_1	t_2	P_1	P_2
Control (n=15)	10.4	3.08				
Graves ophthalmopathy (n=40)	40.4	5.0	21.499	<0.001		
Inactive Graves' ophthalmopathy (n=30)	38.4	3.4	26.514		<0.001	
Active Graves' ophthalmopathy (n=10)	46.20	4.6	23.136	5.588	<0.001	<0.001

t_1 and P_1: Comparison of active or inactive Graves' ophthalmopathy with controls
*Significant P value <0.05
t_2 and P_2: Comparison between active and inactive Graves' ophthalmopathy

Table (5): Serum IL-2 levels in studied groups

Groups	Mean (pg/ml)	SD	t_1	t_2	P_1	P_2
Control (n=15)	65.8	24.3				
Graves ophthalmopathy (n=40)	440.2	187.6	7.657	<0.001		
Inactive Graves' ophthalmopathy (n=30)	351	87.6	12.315		<0.001	
Active Graves' ophthalmopathy (n=10)	707	149	6.430	9.218	<0.001	<0.001

Significant P value <0.05

Table (6): The results of γIFN serum levels (pg/m;) in Graves' ophthalmopathy versus controls

Groups	Mean (pg/ml)	SD	t_1	t_2	P_1	P_2
Control (n=15)	116.4	24.0				
Graves ophthalmopathy (n=40)	479.5	160.3	8.684		<0.001	
Inactive Graves' ophthalmopathy (n=30)	404.2	86.5	12.569		<0.001	
Active Graves' ophthalmopathy (n=10)	705.3	108.2	20.522	8.945	<0.001	<0.001

Significant P value <0.05

Table (7): The results of T cell immunophenotyping in active versus inactive ophthalmopathy

Group	CD3	CD4	CD8
Inactive Graves' ophthalmopathy (n=30) Mean ± SD	71.8 ± 5.53	53.7 ± 7.26	14.8 ± 3.18
Active Graves' ophthalmopathy (n=10) Mean ± SD	75.5 ± 6.19	58.4 ± 4.62	14.8 ± 6.05

t	1.763	1.894	
P	>0.05	>0.05	>0.05

P: Comparison between active and inactive graves' ophthalmopathy
* Significant P value <0.05

Table (8): Results of orbital ultrasound in studied groups

Groups	Normal extraocular muscles reflectivity (-ve US)	Decreased extraocular muscles reflectivity (+ve US)
Active Graves' ophthalmopathy n=10	3 (30%)	7 (70%)
Inactive Graves' ophthalmopathy n=30	30 (100%)	0 (0%)
Total Graves' ophthalmopathy n=40	33 (82.5%)	7 (17.5%)

Table (9): Results of measured parameters in patients with low extraocular muscles reflectivity (+ve US) versus patients with normal extraocular muscles reflectivity (-ve US) in active Graves' ophthalmopathy

	+ve US (n=7) Mean ± SD	-ve US (n=3) Mean ± SD	t	P
CAS	7.1 ± 0.6	5.3 ± 0.5	3.951	<0.01
T3	3.9 ± 0.9	3.5 ± 1.7	0.570	>0.05
T4	17.5 ± 2.1	13.4 ± 4.1	2.197	>0.05
TSH	0.04 ± 0.02	0.03 ± 0.02	0.375	>0.05
sICAM-1	47.8 ± 4.5	42.3 ± 1.5	1.974	>0.05
IL-2	777 ± 96	543 ± 125	3.249	<0.01
γIFN	756 ± 82	586 ± 44	3.268	<0.01
CD3	73.7 ± 6.3	79.6 ± 3.9	1.467	>0.05
CD4	57.5 ± 5.4	60.3 ± 0.57	0.852	>0.05
CD8	13.7 ± 6.7	17.3 ± 4.0	0.853	>0.05

Discussion

Graves' disease is an autoimmune disease. It is may be a B cell mediated condition caused by TSH receptor antibodies [(12)]. Graves' ophthalmopathy appears to be an organ specific autoimmune disorder [(13)]. There is a pathogenic process that induces swelling, lymphocytic infiltration, later fibrosis and contructure that restrict the normal function of the extra ocular muscles. Two stages in the development of the disease are generally distinguished: the stage of active inflammation in which the eyes are red and painful and the quiescent stage in which the eyes are white and unchanging with a painless motility defect[(14)]. The present study aimed at evaluation of indicators of active Graves' ophthalmopathy and also to study if sICAM-1 serum levels help in identification of subgroups prone to develop severe clinical ophthalmopathy. In the present study there was no smoking history in about 80% of patients with inactive Graves' ophthalmopathy, while patients with active Graves' ophthalmopathy show only 50% with no smoking history and 50% with history of smoking. There was high incidence of smoking among male patients with active Graves' ophthalmopathy and this explains that the high incidence of active Graves' ophthalmopathy among male patients may be due to smoking. **Bartalena et al**[(15)] observed that cigarette smoking greatly increased the risk of developing the ophthalmopathy. In addition, cigarette smoking has been documented to have a negative influence on the effectiveness of orbital radiotherapy and high dose of systemic glucocorticoids[(9,16)]. Smoking might enhance

cytokine secretion and activity by causing hypoxia in retrobulbar space**.** In the present study the mean values of all measured parameters showed non-significant statistical difference among smokers and non-smokers. In the present study, exophthalmos was the most frequent sign in our patients. The second most frequent complaint is weight loss, followed by anxiety. Also palpitation and easy fatigue were common features in our patients. In addition sweating and diplopia are also frequent. While other symptoms as bone ache and polyphagia. However, in the present study, there was no significant difference in these clinical symptoms and signs between active and inactive Graves' ophthalmopathy (P>0.05). also there was no statistical difference in thyroid function tests between two groups of patients. In accordance with the results of this study **De Bellis et al**[17] found that, there were no significant differences between active and inactive Graves' ophthalmopathy in clinical, demographic and thyroid profile which could be explained by the fact that there is difference in the mechanism of pathogenesis of Graves' ophthalmopathy and Graves' disease[18]**.** In the present study using the CAS, we found that 10 patients have a score of 4 or more points "active" while 30 patients of score less than 4 points "inactive". Our patients with active Graves' ophthalmopathy complained of pain. Burning sensation, excessive lacrimation, photophobia, swelling of the eye lids, proptosis, double vision and visual impairment. These symptoms and signs are related to the classical signs of inflammation. In Graves' ophthalmopathy several kinds of pain can be distinguished. One kind of pain arises from stretching of the inflamed muscles, especially on attempted upgaze. Another sort of pain is caused by the rise in intraorbital pressure, when the orbital tissues expand through fluid accumulation and cellular infiltration. This is felt as a painful, oppressive feeling on or behind the globes. Both kinds of pain are the first symptoms to disappear after anti-inflammatory treatment. Therefore these kinds of pain are directly related to the inflammation in the orbit and thus useful in assessing inflammatory activity**.** Redness, as an expression of inflammation is caused by vasodilatation, and it is seen as redness of the eyelids and over the conjunctiva [19]. Swelling in Graves' ophthalmopathy is seen as chemosis and a swollen caruncule, both are signs of activity. Swollen eyelids can be caused by edema, fat prolapse through the orbital septum or fibrotic degeneration**.** Proptosis and eyelid swelling are not themselves signs of acute inflammation**.** Visual impairment in Graves' ophthalmopathy, resulting from dysfunction of the optic nerve, is caused by raised intraorbital pressure due to the inflammatory process. Therefore a decrease of visual acuity as a result of optic compression is a sign of disease activity. Also a decrease in eye movements implies that the extraocular muscle function is progressively impaired. It can therefore regard as sign of disease activity**.** In the present study, there was no significant correlation between the disease activity by CAS and thyroid hormone T3, T4 and TSH serum levels, this is in agreement with the results of **Ozata et al.,**[20]. Orbital U.S. is a reliable and inexpensive method with a promising capacity to differentiate patients who will respond to immunosuppressive treatment from the non-responding patients. In our study we found by the measurement of extraocular muscle reflectivity using orbital ultrasound, 70% of patients with active Graves' ophthalmopathy have low extraocular muscles reflectivity and 30% with normal extraocular muscles reflectivity, while normal reflectivity were found in all patients with inactive Graves' ophthalmopathy. Also, there were significant elevation of CAS, sICAM-1, IL-2 and γIFN in patients with +ve U.S. compared to those with –ve U.S. in active Graves' opthalmopathy. **Erickson et al**[21] demonstrated that, the role of A-mode ultrasonography in assessing disease activity is rather of poor negative predictive value precludes its use as sole activity parameter and it should be combined with other methods. This results in agreement with the results of **Gerding et al**[22] who also found, low eye muscle reflectivity in patients with Graves' ophthalmopathy and the treatment with corticosteroid and radiotherapy resulted in a response in 50% of patients and A-mode ultrasonography could identify 39% of these responders by detecting at least one eye muscle with low reflectivity. Adhesion molecules are known to be important for variety of interaction between immune competent cells, preadipocytes fibroblasts and adipocytes in addition, these molecules play a central role in lymphocyte activation and localization, facilitating antigen recognition T cell costimulation and various effector-target cell functions at the inflammatory sites, which result in amplification of the cellular immune process in active Graves' ophthalmopathy[23]. In the present study serum levels of sICAM-1 were significantly high in-patients with Graves' ophthalmopathy than controls. Moreover, sICAM-1 levels were positively correlated with the clinical activity score of Graves' ophthalmopathy suggesting that these levels may be considered as a useful marker of retro orbital endothelial and fibroblast activation in Graves' disease patients with clinical ophthalmopathy. However, no significant correlation was evidenced between sICAM-1 concentration and thyroid hormone levels in either group of patients. As T lymphocytes and local release of cytokines have been shown to play an important role in the pathogenesis in retro orbital connective tissue inflammation [24]. Moreover it has

been suggested that sICAM-1 could be involved in crucial interaction among T-cells, macrophages, B-cells, endothelial cells and retro orbital fibroblast. **De Bellis et al**[13] found that, in active Graves' disease patients with or without hyperthyroidism sICAM-1 serum levels significantly higher than those demonstrated in hyperthyroid Graves' disease patients without ophthalmopathy. Moreover, sICAM-1 levels were positively correlated to the CAS. The origin of increased sICAM-1 levels in the sera of our patients may be mainly from retro orbital tissues as it is considered an important source even if other potential sources like the thymus or other lymphoid organs can't be excluded [25]**.** Because our Graves' disease patients did not suffer from other autoimmune, allergic or infectious disease so it is likely that subclinical and then clinically active inflammatory ophthalmopathy could be responsible for increased sICAM-1 levels. In agreement with the results of our study, **De Bellis et al**[6] evaluated the sICAM-1 serum level in Graves' disease patients during a 2 years follow up period and showed no significant correlation was evidenced between sICAM-1 concentrations and free thyroid hormone levels in either group of patients. Also in accordance with our assumption, **Heufelder and Bahn**[25] showed increase of serum level of sICAM-1 in marked inflamed retro-orbital connective tissue of Graves' ophthalmopathy. So a circulating form of ICAM-1 in Graves' ophthalmopathy can play a role in the ongoing immune process within the connective tissue in Graves' ophthalmopathy[26] even if other factors can be involved[27]**.** In particular, sICAM-1 may exert a cytokine like signaling effect similar to the costimulatory effect of surface bound adhesion molecules. In fact, Graves' ophthalmopathy sera with high sICAM-1 serum levels markedly increase the peripheral blood mononuclear cell adhesion to γIFN stimulated retroocular fibroblast monolayers[26]**.** Thus in Graves' disease a patient further significant increase of sICAM-1 serum concentration could be considered an early marker of subclinical mild retroocular inflammation in the ophthalmopathy, predictive of the clinically marked retroocular inflammation. **Li et al**[28] in study to detect the expression of ICAM-1 and HLA-DR on retroocular fibroblasts from patients with Graves' ophthalmopathy and the possible mechanism of humoral and cellular immunity in pathogenesis of Graves' ophthalmopathy, found that retroocular fibroblasts spontaneously expressed ICAM-1, but did not express HLA-DR. In the present study, there is significantly high level of γIFN in patients with Graves' ophthalmopathy as compared to controls. However there is significant increase of γIFN in active Graves' ophthalmopathy compared to inactive one. **Heufelder and Bahn**[25] found elevated levels of γIFN, TNF-α and IL-1. Moreover γIFN, TNF-α and IL-1 have shown to strongly enhance surface expression on both retroocular and endothelial cells of Graves' ophthalmopathy and to induce both improvement of periorbital inflammation and reduction of sICAM-1 serum levels in corticosteroid therapy**.** A diffuse infiltrate of mononuclear cells is apparent within the extraocular muscle and orbital fatty connective tissues of patients with Graves' ophthalmopathy[19]**.** Three subsets of CD4 + T cells (Th) classified according to the profiles of cytokines produced. CD4 + T cells that produce primarily γIFN, IL-2 and TNF-β are classified as Th1 and are involved primarily in cell mediated immune response**.** Whereas, IL-4, IL-5, IL-10 and IL-13 are the dominant cytokines secreted by Th2 cells involved in humoral immunity. Similarly CD8+ T cytotoxic cells can be divided into Tc1 and Tc2 subsets with comparable cytokines profiles**.** Recent research shows that an important factor in the pathogenesis of autoimmune disease is the change in the balance between Th1 cytokines and Th2 cytokines**.** In the present study, the possible roles of T cell subset in Graves' ophthalmopathy were investigated by analyzing lymphocyte subsets in peripheral blood mononuclear cells and their production of soluble factors and cytokines such as IL-2 and γIFN in patients with active and inactive Graves' disease[30]**.** There was positive correlation between CD4 T cell, sICAM-1 and γIFN. This results is in accordance with the results of **Jaroslaw et al**[31], they found that the predominant subtype of T cells are CD3+ CD4+ clone. Also **Massart et al**[32] studied the morphological and functional properties of T clones from peripheral blood lymphocytes and from intrathyroidal lymphocytes obtained from patients with Graves' disease. Predominance of CD4+ clones was observed whatever the origin of the lymphocytes or the autoimmune pathology[19]. Also they found γIFN in the majority of the tested clones. These data demonstrated that the large majority of T cells clones are principally CD4+ T cells and produced γIFN. We can conclude that CAS is a good tool for differentiation between active and inactive Graves' ophthalmopathy and can be strengthened by orbital U.S. as well as sICAM-1, IL-2 and γIFN. On the other hand, T cell subsets were found to be of little help in differentiation between active and inactive disease status.

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