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## Serum level of cytokine (interleukin-10 ( IL-10 )) and oxidative stress markers Malondialdehyde and super oxide dismutase(MDA and SOD) in Iraqi patients with thyroid cancer

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**Keywords :**Thyroid CA ,interleukine-10 ,malondialdehyde and superoxide dismutase.

### Abstract:

*Thyroid cancer is the most common endocrine malignancy and its incidence continues to rise yearly .Thyroid carcinoma, in most cases, presents clinically as a solitary nodule or as a dominant nodule within a multinodular thyroid gland. it is very important to identify one or more markers to distinguish thyroid cancer from different types of thyroid disease., Oxidative stress is considered to be involved in the pathophysiology of all cancers.Interleukin-10 is a pleiotropic cytokine with both anti-inflammatory and anti-angiogenic properties that may be involved in the pathogenesis of autoimmune thyroid diseases. Thyroid cancer cells of all histological variants produce considerable amounts of IL-10, which might be important in the pathological features of thyroid cancer and/or outcome. Our study aimed to evaluate IL-10 and oxidative stress markers, Malondialdehyde and super oxide dismutase (MDA&SOD) levels in sera of patients with CA thyroid and their close relatives compared to healthy control.and to study the correlation of these markers before and after the thyroidectomy operation compared with 1<sup>st</sup> to 2<sup>nd</sup> relatives and healthy controls. This study includes Thirty (30) subjects as the study sample is made of: 1) 15 diagnosed cases of CA thyroid before surgery and post-surgical treatment, 2) 15 cases of their 1<sup>st</sup>, or 2<sup>nd</sup> degree relatives; and 30 healthy subjects (control) would be recruited from surgical clinic in Al-Imamain Al- kadhmain city hospital from October 2014 up to December of 2016.Serum IL-10 was estimated using an Enzyme-Linked Immunosorbant Assay ( ELISA ) technique. Serum MDA levels were assessed using thiobarbituric acid (TBA) method of Buege and Aust , while SOD was measured by Burtis and Ashwood, using modified photochemical Nitroblue Tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitor.Analysis of serum levels demonstrated significantly increased levels of IL-10 in cancer patients between pre-thyroidectomy and post-thyroidectomy( $p<0.01$ ) and significantly increased compared with control groups ( $p<0.001$ ) , SOD activities of pre-thyroidectomy, post-thyroidectomy and control groups were not different ( $p>0.05$ ).In post- thyroidectomy MDA levels decreased compared to prethyroidectomy levels(  $p<0.01$ ).also thyroid cancer significantly decreased compared with control groups ( $p<0.001$ ). Although post-thyroidectomy MDA levels significantly decreased, but still higher than the healthy control group.its concluded that cytokine profile (IL-10) is reflective of an inflammatory immune response. These cytokines may increase the pathogenicity of thyroid CA and may be useful as biomarkers or targets for therapy. SOD does not seem to change with thyroid cancer and thyroidectomy but MDA change.IL-10 &*

*MDA may be a more useful for monitoring the clinical status of thyroid cancer patients.*

مستوى المصل من المدورات الخلوية (انترلوكين- 10) وعلامات الإجهاد التأكسدي مالونديالديهيد وأكسيد سوبر ديسموتاز (SOD) في المرضى العراقيين الذين يعانون من سرطان الغدة الدرقية

**الكلمات الرئيسية:** الغدة الدرقية، انترلوكين 10، مالونديالديهيد والسوبر اوكسايد ديسميوتاز.

## الخلاصة

سرطان الغدة الدرقية هو من الامراض الخبيثة الأكثر شيوعا في الغدد الصماء و حدوثه متوازا في الارتفاع سنويا. سرطان الغدة الدرقية، في معظم الحالات، ويعرض سريريا كعقيدات الانفرادي أو كعقدة مهيمنة داخل الغدة الدرقية متعدد الغدد الدرقية. من المهم جدا تحديد واحد أو أكثر من العلامات لتمييز سرطان الغدة الدرقية من أنواع مختلفة من مرض الغدة الدرقية، ويعتبر معامل الإجهاد التأكسدي له دور في الفسلفة المرضية لجميع أنواع السرطان. Interleukin-10 هو مدور خلوي متعدد المظاهر مع كل من المضادة للالتهابات ومكافحة الخصائص التي يمكن أن تشارك في التسبب في المناعة الذاتية لأمراض الغدة الدرقية. تنتج خلايا سرطان الغدة الدرقية من جميع المتغيرات النسيجية كميات كبيرة من الانترلوكين-10، والتي قد تكون مهمة و / أو كنتيجة في السمات المرضية لسرطان الغدة الدرقية.

هدفت دراستنا هو لتقييم مستويات المدور الخلوي-10 وعلامات الإجهاد التأكسدي، مالونديالديهيد وأكسيد سوبر ديسميوتاز (SOD) في مصل المرضى الذين يعانون من الغدة الدرقية وأقاربهم من الدرجة الاولى والثانية مقارنة مع السيطرة السليمة. و لدراسة علاقة هذه العلامات قبل وبعد عملية استئصال الغدة الدرقية مقارنة مع الدرجة الاولى والثانية من الأقارب. تضمنت هذه الدراسة ثلاثين (30) مريضا حيث تم إجراء عينة الدراسة من: (1) 15 حالة تم تشخيصها من الغدة الدرقية قبل الجراحة والعلاج بعد الجراحة، (2) 15 حالة من أقاربهم من الدرجة الاولى والثانية. و 30 مريضا حيين (سيطرة) تم تعيينهم من عيادة الجراحة في مستشفى مدينة الامامين الكاظمين (ع) الطبية للفترة من أكتوبر 2014 حتى ديسمبر من عام 2016. وقد تم تقدير مصل الانترلوكين-10 باستخدام تقنية الفحص المناعي المرتبط بالانزيم (ELISA). وكذلك تم تقييم مستويات مصل الدم باستخدام طريقة حمض ثيوباربيتوريك باستخدام طريقة بويج و أوست، في حين تم قياس السوبر اوكسايد ديسميوتاز (SOD) بواسطة طريقة بيرتس و آشود، وذلك باستخدام طريقة نيتروبلو تيترازوليوم الضوئية المعدلة (NBT) باستخدام سيانيد الصوديوم كمثبط البيروكسيداز. أظهر تحليل مستويات المصل مستويات زيادة كبيرة من المدور الخلوي-10 في مرضى السرطان بين استئصال الغدة الدرقية قبل وبعد استئصال الغدة الدرقية ( $p > 0.01$ ) وزيادة كبيرة مقارنة مع مجموعات السيطرة ( $p > 0.001$ )، وأنشطة (SOD) من استئصال ما قبل الغدة الدرقية، في حين ما بعد الغدة الدرقية ومجموعات السيطرة لم تكن مختلفة ( $p < 0.05$ ). في مستويات ما بعد الغدة الدرقية انخفضت مستويات المالونديالديهيد (MDA) مقارنة مع مستويات ما قبل الغدة الدرقية ( $p > 0.01$ ). كما انخفض سرطان الغدة الدرقية بشكل ملحوظ مقارنة مع مجموعات السيطرة ( $p > 0.001$ ). على الرغم من أن مستويات الملونديالديهيد ما بعد الغدة الدرقية انخفض انخفاضاً كبيراً، ولكن لا يزال أعلى من مجموعة الاقارب كمجموعة سيطرة. كاستنتاج من هذه الدراسة تبين أن المدور الخلوي (الانترلوكين-10) يعكس استجابة مناعية التهابية. هذه المدورات الخلوية قد تزيد من إمراضية حدوث سرطان الغدة الدرقية وقد تكون مفيدة كمؤشرات حيوية لأهداف العلاج. SOD لا يبدو أن يتغير مع سرطان الغدة الدرقية واستئصال الغدة الدرقية ولكن كان هناك تغيير في مستوى الملونديالديهيد MDA. الانترلوكين-10 و المالونديالديهيد قد يكونا أكثر فائدة لرصد الحالة السريرية لمرضى سرطان الغدة الدرقية.

## 1.Introduction

Thyroid cancer is the most common endocrine malignancy and its incidence continues to rise yearly. Thyroid carcinoma, in most cases, presents clinically as a solitary nodule or as a dominant nodule within a multinodular thyroid gland. The challenge to clinicians is to discriminate between the minority of thyroid nodules (5–15%) that harbor malignancies and the majority of cases that can be managed conservatively[1]. Thus, it is very important to identify one or more markers to distinguish thyroid cancer from different types of thyroid disease. Oxidative stress can participate in the pathogenesis and complications of many diseases (including cancer; [2]. Thyroid hormones regulate oxidative metabolism and thus play an important role in free radical production[3]. Thyroid hormones regulate the synthesis and degradation of enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase, and non-enzymatic antioxidants, such as vitamin E and C, glutathione, uric acid, ferritin, transferrin, and ceruloplasmin. Undoubtedly, the changes in these enzymes and non-enzymatic substances affect the redox balance in the body and, in turn, enzymatic feedback regulates thyroid function. One of the major effects of thyroid hormones is to increase mitochondrial respiration, which results in upregulation of Reactive oxygen species (ROS), leading to oxidative damage to membrane lipids [3].

In recent years, interest has grown in studying the role played by oxidative stress in thyroid carcinogenesis by investigating one or more of the antioxidant markers, including SOD[4,5,6], catalase [5], GPx[5,6], vitamin E [7], vitamin C [8], glutathione[9], and/or uric acid[10]. However, the measurement of different antioxidant molecules separately was not only impractical but also held no clinical significance. Oxidative stress is common in the thyroid tissue during utilization of H<sub>2</sub>O<sub>2</sub> for thyroxine (T<sub>4</sub>) synthesis, the inflammation produces ROS, and when tumor has active proliferation[11]. Therefore, oxidative stress has been reported to be consistently associated with hyperthyroidism or hypothyroidism [12]. Oxidative stress, which is characterized as a misbalance between free radicals and antioxidants in favour of radicals, participates in the pathogenesis of many diseases and their complications. Reactive oxygen species (ROS) consisting mainly of superoxide, hydrogen peroxide and hydroxyl radical, have been conventionally considered to have carcinogenic potential<sup>2</sup> and to promote invasiveness [12].

Cytokines promote the expression of a variety of genes involved in the survival or death of different target cells[13-16]. Three functionally distinct subsets of T-helper cells have been characterized on the basis of cytokine production [17]. TH1 cells secrete IFN- $\gamma$  and other cytokines associated with inflammation and cell-mediated immune responses. In contrast, TH2 cells promote the humoral response and produce IL-4, IL-5, and IL-10, whereas TH3 cells inhibit the immune response through the release of IL-10 and transforming growth factor-1[17-19].

Interleukin-10 is a pleiotropic cytokine with both anti-inflammatory and anti-angiogenic properties that may be involved in the pathogenesis of autoimmune thyroid diseases[20]. It is normally produced by activated T cells, monocytes, B cells, and thymocytes, contributing to antigen or mitogen-driven B cell differentiation,

acting as a growth factor, and stimulating the humoral immune response[21]. The IL-10 gene is located on chromosome 1q31-q32. Thyroid cancer cells of all histological variants produce considerable amounts of IL-10, which might be important in the pathological features of thyroid cancer and/or outcome[22]. A polymorphism at codon -1082 has been associated with increased IL-10 protein production.[23] Hence, this study was carried out to evaluate the oxidative stress markers(MDA & SOD) and cytokine (IL-10) in sera of patients with CA thyroid and their close relatives compared to healthy control. and to study the correlation of these markers before and after the thyroidectomy operation compared with 1<sup>st</sup> to 2<sup>nd</sup> relatives and healthy controls.

## **2. Subjects , Material and Methods**

### **2.1. Subjects**

This study comprised thirty(30) consecutive patients of thyroid cancer were recruited from surgical clinic in Al-Imamain Al- kadhmain city hospital from October 2014 up to December of 2016. patients group with thyroid cancer is made of: 1) 15 diagnosed cases of CA thyroid before surgery and post-surgical treatment, 2) 15 cases of their 1<sup>st</sup>, or 2<sup>nd</sup> degree relatives; and 30 healthy subjects (control) age and sex matched volunteers with no family history of thyroid cancer .

### **2.2.Diagnostic criteria:**

1. History and clinical examination: exaggeration of previous symptoms like weight loss, difficult swallowing, fatigue, recent rapid increase in size & on examination: solid solitary or dominant nodules  $\pm$  lymph node enlargement, multinodular enlargement of thyroid.
2. Histopathological report of their fine needle aspiration reveals malignant changes.
3. Histopathological report of their thyroid gland biopsy reveals malignant cytology.

### **2.3. Exclusion Criteria**

Patients to be excluded if they are on treatment for any autoimmune diseases, Pregnant ladies, Other autoimmune diseases like, (rheumatoid arthritis, diabetes mellitus, autoimmune hypothyroidism).

### **2.4. 2.4. Inclusion Criteria**

Every patient with thyroid nodule proved to be malignant on fine needle aspiration (FNA) and or Histopathological study.

### **2.5. Blood Sampling**

Blood samples (10 ml) were collected from patients and control subjects in serum separator vacutainers ( BD Vacutainer Systems, Plymouth, UK). Sera were separated and immediately stored at  $-20^{\circ}\text{C}$  until analysis.

### **2.6. Serum Cytokine Measurement**

The quantitative determination of IL-10 level was conducted by an Enzyme-Linked Immunosorbent Assay ( ELISA ) technique, using a commercial available kit (IL-10 Elisa Kit , Genway GWB), Every sample was run in duplicate, measurements differed by less than 10 %, and the mean value was calculated and used for statistical analysis.

## 2.7. Serum Oxidative Stress Measurment

Serum levels were assessed for MDA using thiobarbituric acid (TBA) method of Buege and Aust[24], SOD was measured by using modified photochemical Nitroblue Tetrazolium (NBT) method utilizing sodium cyanide as peroxidase inhibitor[25].

## Statistical analysis

All data were coded and entered using the program statistical package for social sciences (SPSS) version 20 under windows XP. Descriptive data was summarized using mean, standard error (SD). Linear regression analysis was done to test for significant predictors for thyroid cancer. P values < 0.05 were considered statistically significant.

## 3. Results:

Serum Oxidative stress markers (MDA&SOD), and Cytokine profile (IL-10) levels were estimated in 30 patients with thyroid cancer, (15 thyroid cancer & 15 relative 1<sup>st</sup> & 2<sup>nd</sup> degree to patient with thyroid cancer) compared with 30 healthy control group, age and sex matched.

As expected, the patients with thyroid cancer had significantly higher level of IL-10 & Total Lipid peroxidation (MDA) levels than the healthy controls and relative. and a significant difference within thyroid cancer patients (between pre & post thyriodectomy), as shown in table (1-1) and figures (1-1) & (1-3).

while decrease level in SOD but not a significant value in thyroid cancer as compared with normal & relative subjects, not significant difference within thyroid cancer (pre & post thyriodectomy). as shown in table (1-1), see figure (1-2).

The level of IL-10 and Oxidative stress (MDA&SOD) in normal healthy subjects and thyroid cancer subjects was depicted in Table (1-1).

**Table(1- 1): The Anthropometric and biochemical variables among the four studied groups.**

Parameters	Control	Prethyriodecto my	postthyriodectomy	1 <sup>st</sup> or 2 <sup>nd</sup> degree relative	P(ANOVA)-(T-Test)
NO.	30	15	15	15	.....
IL-10 (pg/ml)	58.99± 41.37	579.8± 288.31	169.38±177.18	110.36±65.65	Post thyriod CA x Pre thyriod CA : p < 0. 01 thyriod CA x C: P < 0.001 thyriod CA x relative: P < 0.001
SOD (U/ml)	10.48 ±0.37	9.18 ±0.13	7.31 ±0.51	10.31±0. 17	Post thyriod CA x pre thyriod CA : p> 0.05 thyriod CA x C: P>0.05 thyriod CA x relative: P > 0.05
MDA (µmol/l)	0.58±0. 35	2.89±0.73	1.99±0.66	0.86 ± 0.65	Post thyriod CA x Pre thyriod CA : p< 0.01 thyriod CA x C: P<0.001 thyriod CA x relative: P < 0.001

Values are Mean ± SD, X=VS.

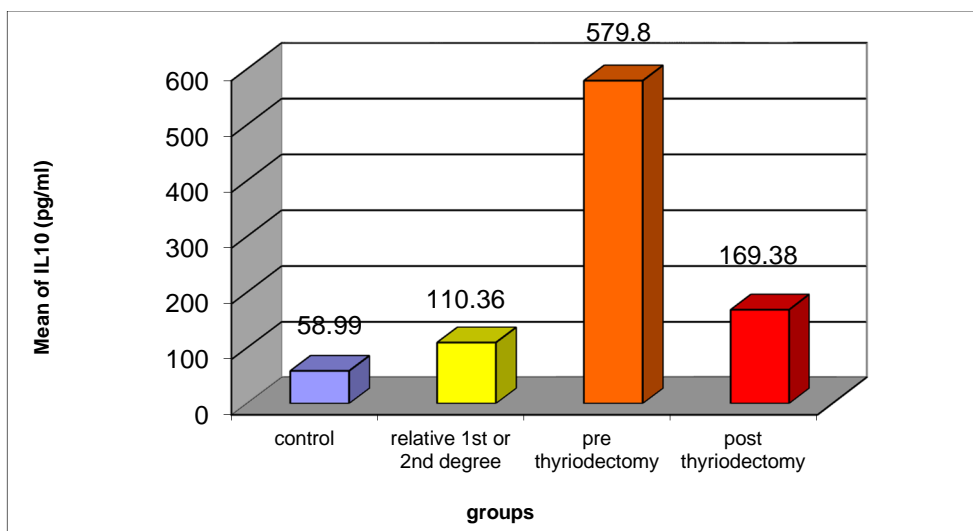


Figure (1-1): Serum levels of IL - 10 in patients with thyroid CA compared to healthy subjects

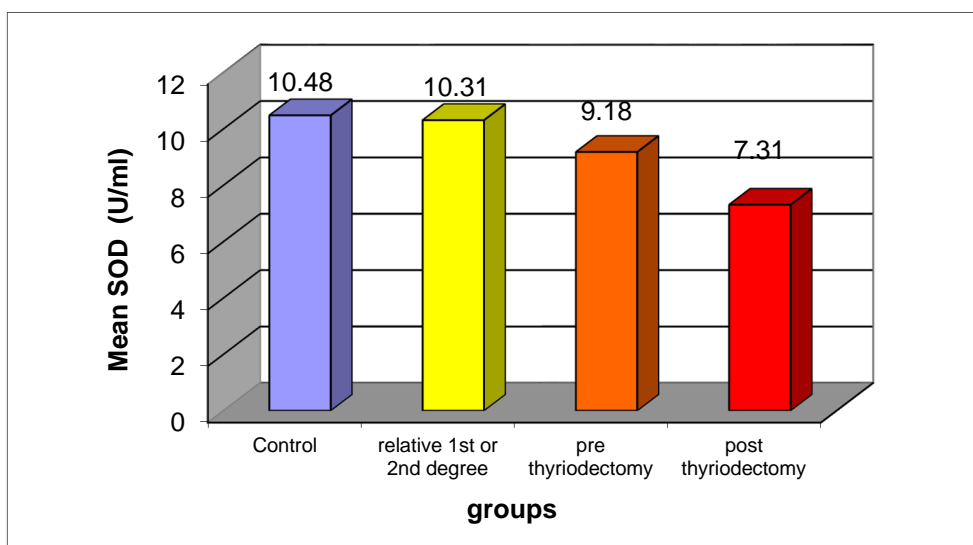


Figure (1-2):Serum levels of SOD in patients with thyroid CA compared to healthy subjects

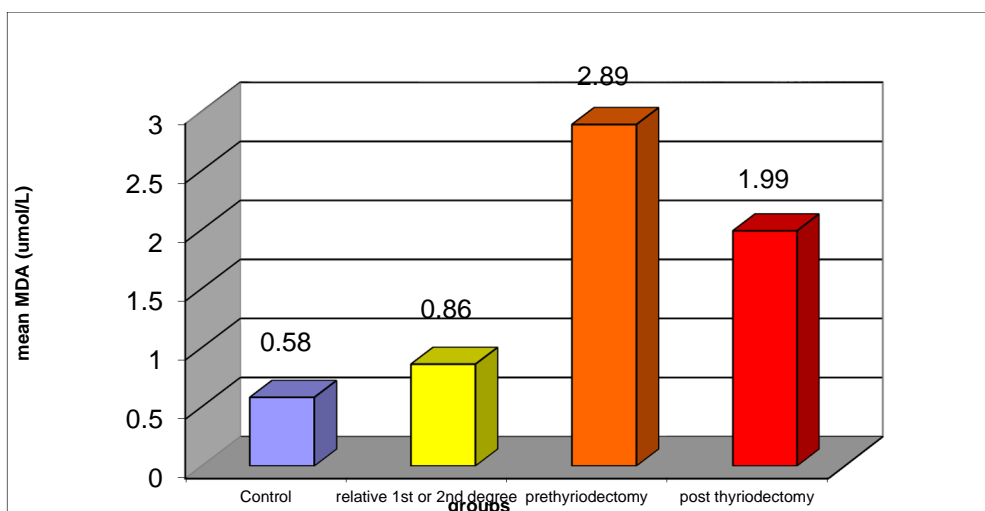


Figure (1-3):Serum levels of MDA in patients with thyroid CA compared to healthy subjects

#### 4. Discussion

According to our results, serum SOD does not seem to change with thyroid cancer and thyroidectomy but lipid peroxidation product MDA do. These preliminary findings may point out oxidant/antioxidant imbalance associated with thyroid cancer. Serum MDA level, as a marker for lipid peroxidation, is commonly used as an indicator for oxidative damage in cells and tissues. The MDA levels as the stable end product of lipid peroxidation. In our study, before thyroidectomy and post-thyroidectomy, blood MDA levels were significantly higher, compared to controls and relatives patients to thyroid cancer ( $P < 0.001$ ). However, in post thyroidectomy, the serum MDA level significantly decreased compared to prethyroidectomy levels ( $P < 0.001$ ). but was still higher significantly than the control group's level. Mano et al[26]. and Sadani et al[27]. showed that the lipid peroxide concentration, expressed as MDA concentration, was significantly higher in the specimens from papillary carcinoma than those in the normal thyroid tissue. The increase of free radicals in thyroid cancer condition is suggested to be due to the increased lipid peroxidation and the damage of antioxidant defence system[28] .

In our study, in thyroid cancer patients MDA levels before thyroidectomy were found to be higher than those in age matched controls indicating increased free radical generation. It is obvious that there are clues, there is an oxidant shift in thyroid cancer patients with normal hormone profile. At least we think that MDA level may have a value for control visits of thyroid cancer patients after thyroidectomy and there is no doubt that more investigations are needed.

In the current study there was a significant increase in MDA level in all groups of patients with thyroid cancer compared to the level of the control group. studied lipid oxidation in euthyroid and thyrotoxic tissue samples of the human thyroid gland. The authors observed that the content of TBA-active lipid peroxidation products was increasing in hyperthyroid tissue and the activity of antioxidant enzymes (catalase, GSHPx) was decreasing [29] . Similar studies by Sewerynek et al[30] have shown the ratio of CD/MDA decreased in the hyperthyroid patients, as compared to the controls. They believed that their results indicated a high rate of lipid peroxidation during hyperthyroidism[31] . Sugawara et al.[32] and Durak et al.[33] showed that endemic goiter tissues contained lower significantly SOD activity and concentration compared with those of normal tissues. In various other thyroid disorders including thyroid cancer SOD also found to be low. However in our study, both pre and post operative SOD activity was slightly lower than the control but it was not statistically different. SOD activity seems to play insignificant role in thyroid cancer development in our series but it is not possible to reach a certain decision as the number of patients is low and this must be investigated in larger series. Many previous studies have revealed increased oxidative stress in thyroid cancer[34-44] , Thus, in our study

In the present study, before thyroidectomy and post-thyroidectomy, serum IL-10 levels were significantly higher, compared to controls and relatives subjectss to thyroid cancer ( $P < 0.001$ ). However, in post thyroidectomy, the serum IL-10 level

significantly decreased compared to prethyroidectomy levels ( $P < 0.01$ ). but was still higher significantly than the control group's level, this results a greement with other results who domenstrate that Because IL-10 may be involved in leukocyte recruitment, [45,46] and the studied polymorphism increased IL-10 protein levels [47], our study could speculate that individuals with the inherited polymorphic variant would be more efficient at increasing their IL 10 levels, leading to thyroid autoimmunity. The IL-10 -1082A/G polymorphism has been associated with the development of various types of cancer, including thyroid cancer.

Cytokines promote the expression of a variety of genes involved in the survival or death of different target cells [48-51]. Three functionally distinct subsets of T-helper cells have been characterized on the basis of cytokine production [54]. Our study recently demonstrated that cytokines released by T-helper lymphocytes control autoimmune thyroid destruction through the modulation of proapoptotic and antiapoptotic proteins in target cells. IFN- $\gamma$  contributes to autoimmune thyrocyte destruction through the up-regulation of CD95, caspase-3, and caspase-8, whereas IL-4 and IL-10 promote thyrocyte survival during nondestructive autoimmunity through the up-regulation of cellular FLICE inhibitory protein (cFLIP) and Bcl-xL [52,53]. In this study we concluded that cytokine profile (IL-10) is reflective of an inflammatory immune response. These cytokines may increase the pathogenicity of thyroid CA and may be useful as biomarkers or targets for therapy. SOD does not seem to change with thyroid cancer and thyroidectomy but MDA change. IL-10 & MDA may be a more useful for monitoring the clinical status of thyroid cancer patients.

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