European Science Methodical Journal
ISSN (E): 2938-3641
Volume 2, Issue 7, July - 2024

# THE ROLE OF PENTRAXIN 3, KLOTHO-PROTEINS, ANTI- TPO AND MAGNESIUM IN SERUM OF SUBCLINICAL HYPOTHYROIDISM WOMEN PATIENTS

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**Abstract**:
Thyroid hormones, plays an important and essential functions in metabolic processes. Subclinical hypothyroidism happens when someone have exposure to elevated thyroid-stimulating hormone levels with normal values of thyroxine. Pentraxin 3 is an important endogenous mediator for defenses innate immunity, and its analogous molecule of inflammation C-reactive protein molecule. Klotho protein plays an essential multiple physiological and pathological functions in several organs and tissues of the body. Magnesium is a cofactor for many enzymes

**Materials and methods:** The sixty five women patients suffering have Subclinical hypothyroidism women were participated, their ages ranged from 25 to 40 years. Biochemical measurements of thyroid hormones levels by Minividas technique, while Anti TPO protein measured by kit from mybiosource company, PTX3, Klotho protein from Sun Long Biotech – China, and Mg2+ measured with of Biomaghreb kit.

**Results**: There was no significant different (P>0.05) in T3, T4 and Klotho protein levels women patients group when compared to women control group. There was : a highly significant increase (<0.01) in TSH level, a significant increase (p<0.05) in the serum levels of PTX3 , highly significant increase (p<0.001) in Anti-TPO and a highly significant deceased (≤ 0.01) in the serum levels of Mg2+ mg/dl in Subclinical hypothyroidism group when compared with control group.

**Conclusion**:PTX3 may be relatively independent marker for prognosis of subclinical hypothyroidism.

**Keywords**: PTX3, subclinical, Klotho protein , magnesium.

European Science Methodical Journal
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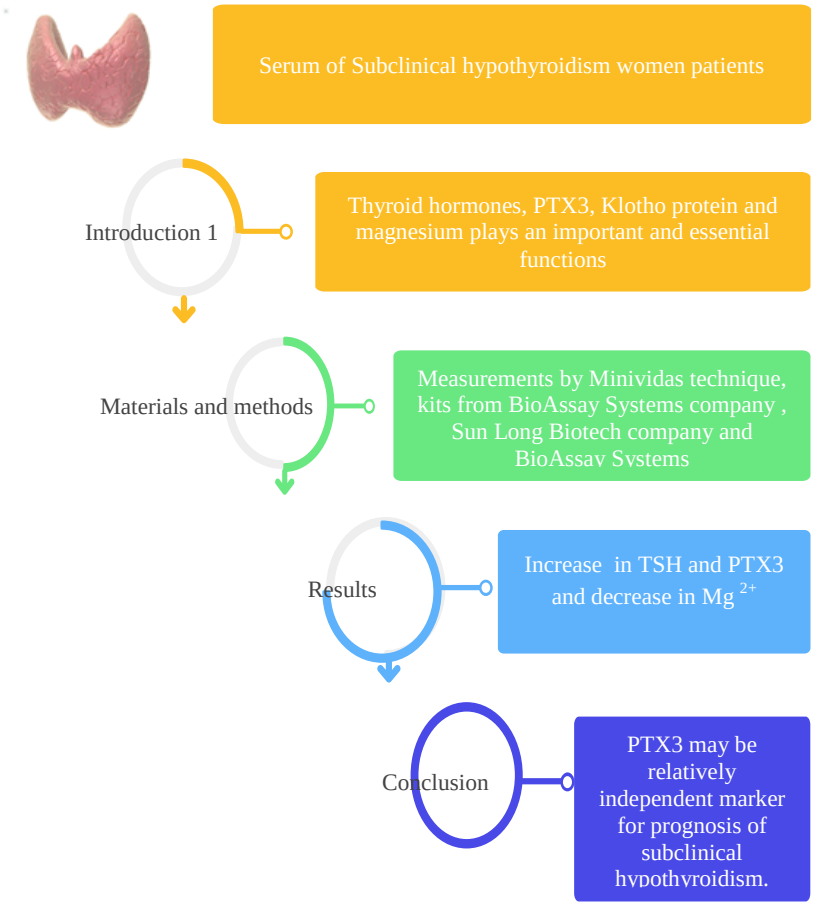
## Graphical Abstract




**Key words** : PTX3, subclinical, Klotho protein , magnesium.

## Introduction

The thyroid hormones (THs) perform a variety of metabolic processes, including the promotion of the oxidation of nutrient molecule (carbohydrates, lipids, and proteins) in a variety of tissues. Thyrotropin/Thyroid-stimulating hormone, (TSH) referred to glycoprotein hormone that pituitary gland releases to trigger thyroid gland to produce and release its own hormones—thyroxine (T4) and triiodothyronine (T3) [1]),[2]. Thyroid peroxidase (TPO) is a singular enzyme located in the apical membrane of follicular cells in thyroid that responsible for the iodine incorporation and the joining of mono-and diiodotyrosine in order to make manufacture tri-iodo thyronine (T3) and thyroxine (T4). TPO (EC.1.11.1.8) contains active sites (two) that worked with dual oxidase and hydrogen peroxide to tyrosine residues and iodinate in thyroglobulin (Tg), followed by simultaneously incorporating two intramolecular iodotyrosine residues to make thyroid hormones [3-5].

Subclinical hypothyroidism (SCH) happens when persons or patients have elevated TSH levels with don't changes levels T4. These persons don't technically have hypothyroidism, but it has

the potential to develop into overt hypothyroidism(6). The World Health Organization (WHO) has recommended body mass index (BMI) for identifying overweight and obese adults. Height in meters was squared by divided weight in kilos, in the time a person's BMI is measured in ($kg/m^2$). In adults if their BMI is ≥ 30 are considered overweight or obese. Excess especially visceral fat (in adipose tissue) in female increases the risk of infertility [7].

The long pentraxin 3 (PTX3) is an important endogenous mediator for defenses innate immunity, and its analogous molecule of inflammation C-reactive protein molecule (CRP). Klotho protein plays an essential multiple physiological functions and pathological role in several organs and tissues of the body. The PTX3 expression by myeloid cells and by stromal, in many cells, especially endothelial and epithelial, in response to signals by primary proinflammatory, Toll-like receptors engagement, microorganisms or microbial recognition and tissue Injury [8]. PTX3 is a promising prognostic biomarker due to its ability to be produced locally by various cell types at the site of infection or tissue damage, and its quick or rapid rise in circulating levels in inflammatory or infectious conditions. PTX3 plasma levels are elevated and generally correlated with severity in a variety of pathological conditions, from infections and sepsis to cardiovascular diseases [9–13]. PTX3 is for important complement activators [like., C1q, ficolin1,2, mannan binding lectin, and complement component 3] and inhibitors [like a regulator : factor H] , and modulates complement pathways. Also, PTX3 regulates the extravasation of leukocytes via its interaction with P-selectin at sites of inflammation , thus reduce and control the inflammatory response *via* complement-system mechanisms [14],[15].

Klotho protein is a marker aging-related closely associated with a number of diseases. Dietary related factors and routine habits can largely effect on Klotho levels in serum. The effect of dietary fiber, a healthy diet essential factor on the serum Klotho levels has not fully clarified (16). The Klotho protein encodes by *Klotho* gene, a pleiotropic protein that includes three isoforms: α-, β-, and γ-Klotho. Klotho have multiple physiological roles and pathological functions in several organs and tissues of the body [17-19].

Magnesium ($Mg^{2+}$) is a cofactor for many enzymes and fourth most prevalent essential mineral in the body and that regulate of metabolic activities and biochemical processes, such as deoxy/ribonucleic acid synthesis, biosynthesis of protein, and catabolism od carbohydrate [20].

**Aim:**

The aim of current study is to discern whether plasma PTX3 , Klotho protein and $Mg^{2+}$ levels may have role as a potential biochemical markers for Subclinical hypothyroidism.

**Materials and methods**

The sixty fifty six women patients suffering from subclinical hypothyroidism were participated, their ages ranged from 25 to 40 years. Samples were collected during the period from January to August 2022. All patients were diagnosed by a specialist doctor. Samples, grouped in to two categories:- 25 from healthy subjects (control) whose age ranged between 24 to 40 years , and 65 from patients. Biochemical measurements of hormones levels by Minividas , while Anti TPO protein measured by kit depend on sandwich enzyme-linked immune-sorbent assay

technology (ELISA) from mybiosource company , PTX3, Klotho protein from Sun Long Biotech – China, and $Mg^{2+}$ measured with of BioAssay Systems kit (Figure 1).

**Statistical Analysis**: Data were analyzed by XLSTATE statistical package software.

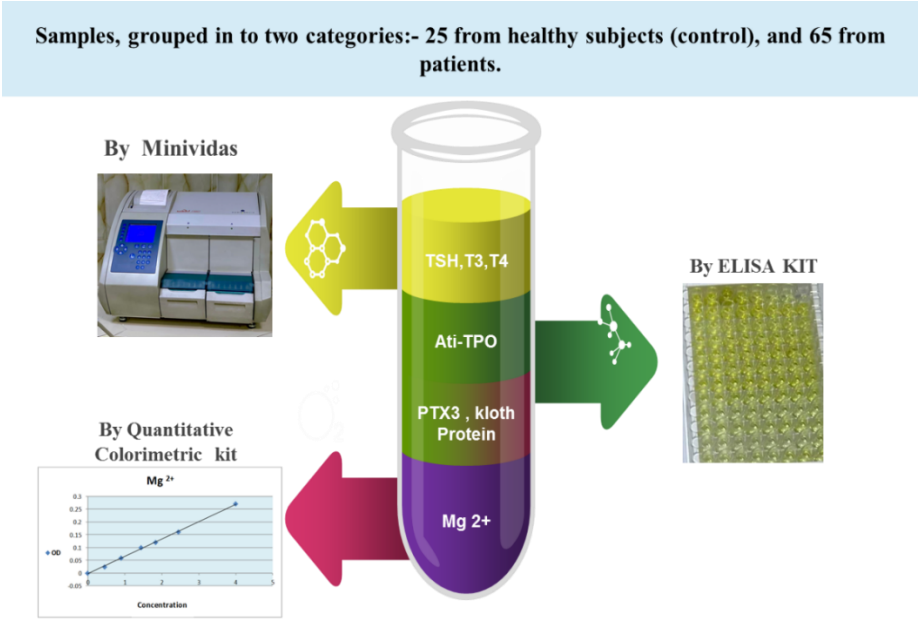



**Figure (1) : Graphic summary of study of methods**

## Results & Discussion

The graphic summary of all study results were illustrated in Figure (2)

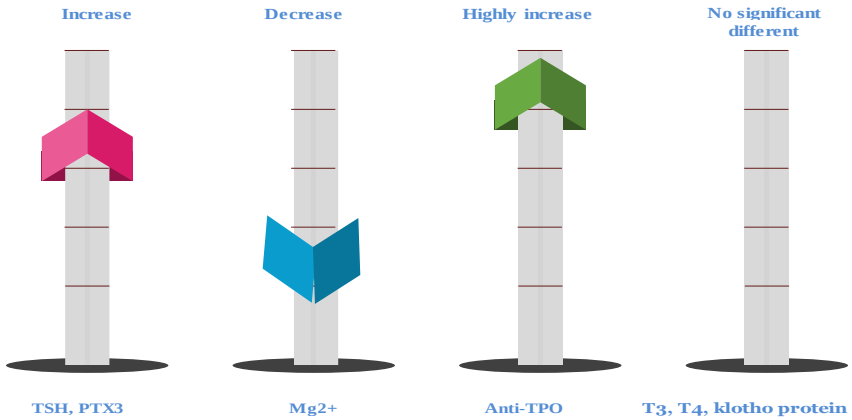



**Figure (2) : Summary of the results**

### A- Thyroids hormones

The mean (±SD) of T3,T4,TSH ng/ml concentration in serum of women control group and subclinical hypothyroidism women patients group according to BMI are illustrated in table (1) and figure (3),(4),(5) .

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ISSN (E): 2938-3641
Volume 2, Issue 7, July - 2024

**Table (1) : The Mean of T3,T4 and TSH for studied Groups**

| Parameters/Groups | | Mean ± SD | |
|---|---|---|---|
| | | Control | Patients |
| T3 ng/ml | Total | 49.04± 3.25 | 46.22± 2.67 |
| | A1 (BMI =23-25) | 50.11± 5.13 | 47.56± 2.28 |
| | A2 (BMI=25-30) | 48.77± 1.43 | 45.24± 2.52 |
| T4 ng/ml | Total | 233.1± 14.11 | 223.2± 13.20 |
| | A1 (BMI =23-25) | 241.1± 13.23 | 239.7± 18.24 |
| | A2 (BMI=25-30) | 224.1± 18.11 | 227.6± 10.33 |
| TSH µIU/ml | Total | 1.532± 0.12 | 2.06± 0.18 |
| | A1 (BMI =23-25) | 1.499± 0.10 | 2.49± 0.20 |
| | A2 (BMI=25-30) | 1.581± 0.15 | 1.69± 0.16 |

*P value*

| Parameters | Control / Patients | A1 Control /A2 Control | A1 Patients /A2 Patients | A1 Control /A1 Patients | A2 Control /A2 Patients |
|---|---|---|---|---|---|
| T3 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |
| T4 | >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |
| TSH | ≤ 0.001 | <0.05 | <0.01 | <0.01 | <0.01 |

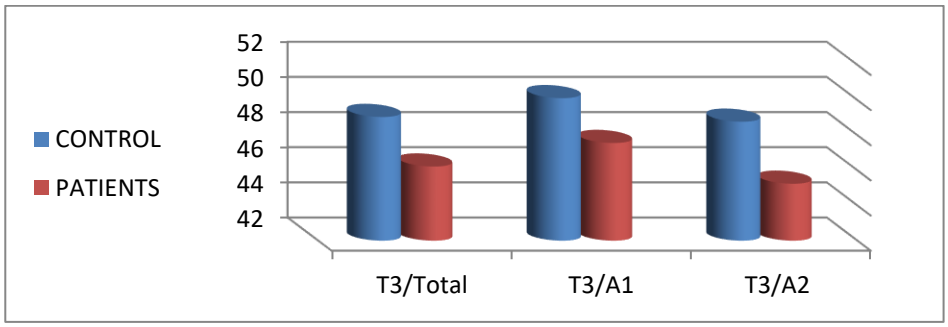


**Figure (3) : The Mean of T3**

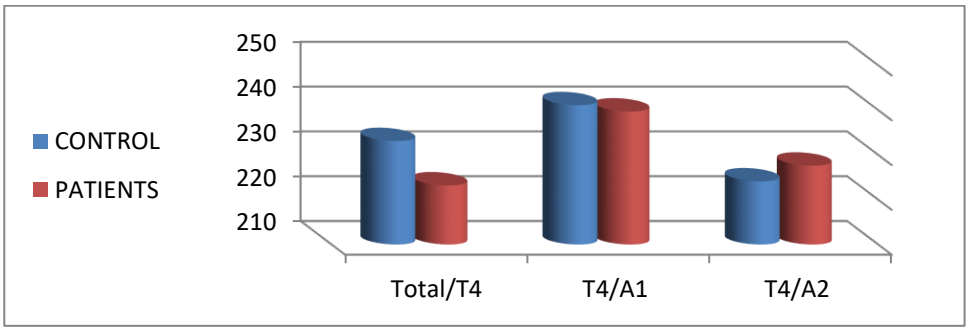


**Figure (4) : The Mean of T4**

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ISSN (E): 2938-3641
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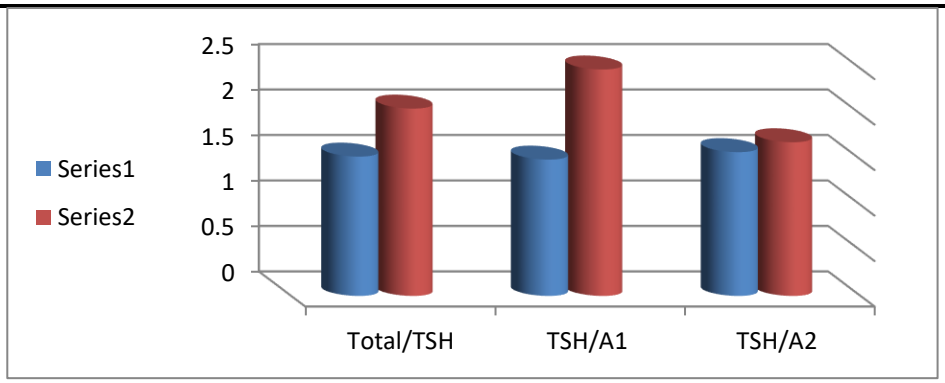


**Figure (5) : The Mean of TSH**

There was no significant different (P>0.05) in T3 and T4 levels in women patients group when compared to women control group and there was no significant differences (P>0.05) in BMI groups. There was: a highly significant increase (<0.01) in TSH level in women patients group compared to women control group, a significant increase (<0.05) between A1 and A2 women control groups, a highly significant increase (<0.01) between A1 and A2 women patients groups, a highly significant increase (<0.01) between A1 women control group and A1 women patient groups, and a highly significant increase (<0.01) between A2 women control group and A2 women patient groups.

There is a pleiotropic effect of thyroid disorders on female reproduction, with both direct and indirect effect on female reproductive axis. By altering binding proteins, the TH influences the biological availability of sex steroids. Euthyroid status promotes the healthy growth and operation of the female reproductive system and controls the development of the fetus and placenta during pregnancy. This complex interplay of thyroid and female reproduction deserves a comprehensive review to recognize the nuances that impact the clinical management of thyroid and reproductive disorders [21].

After diabetes, thyroid conditions are the second most common disorders worldwide. Normal ranges of thyroid hormone are necessary for proper reproductive physiology and behavior. The body's mechanisms for controlling thyroid hormone levels can alter with age, especially in females. This can lead to variations in TSH levels [22],[23].

**B- Anti- TPO**

The mean (±SD) of Anti- TPO (IU/mL) concentration in serum of control group and subclinical hypothyroidism women patients group according to BMI are illustrated in table (2) and figure (6). There are a highly significant increase (p<0.001) in the serum levels of Anti-TPO in women subclinical hypothyroidism group when compared with women control group, a highly significant increase (p<0.001) in serum Anti- TPO (IU/mL) levels in A2 women patients groups when compared with A1 women patients groups , and between A1 women control and A1 women patients and A2 women control and A2 women patients.

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**Table (2) : The Mean of Anti- TPO (IU/mL)**

| Groups | Mean ± SD of Anti- TPO (IU/mL) | |
|---|---|---|
| | Control | Patients |
| Total | 1.666± 0.212 | 22.843±0.200 |
| A1 (BMI =24-25.5) | 1.643± 0.144 | 18.630±0.198 |
| A2 (BMI=25.6-30) | 1.68± 0.132 | 29.301±0.22 |
| *P value* | | |

| Control/ Patients | A1 Control /A2 Control | A1 Patients /A2 Patients | A1 Control /A1 Patients | A2 Control /A2 Patients |
|---|---|---|---|---|
| ≤0.001 | >0.05 | ≤0.001 | ≤0.001 | ≤0.001 |

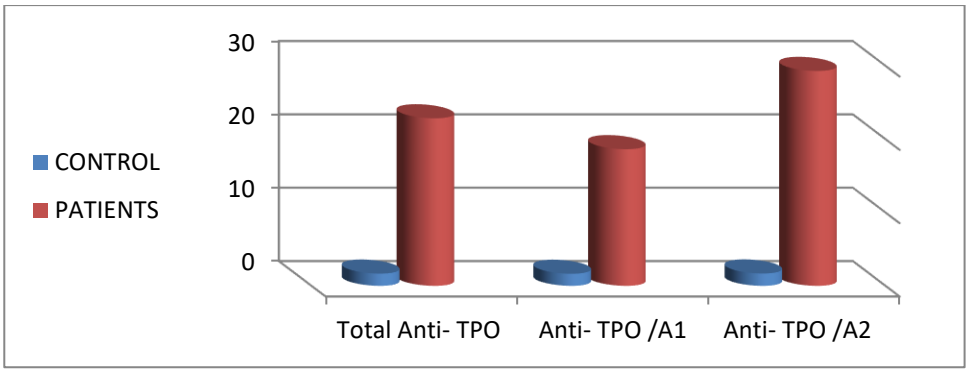


**Figure (6) : The Mean of Anti- TPO**

The most frequent cause of SCH, which is characterized by an elevated TSH level with normal free T4 level, is autoimmune thyroid disease (autoimmune thyroiditis). Due to a negative feedback loop, individuals with decreased levels of free T4 have higher TSH levels because high levels of free T4, prevent the pituitary gland from producing TSH. In these circumstances, increased thyroid hormone production is stimulated by elevated TSH, which raises T4 [24]. Patients with autoimmune thyroiditis produce more TSH than people without given the feedback system that controls these hormones [25],[26].

**C- PTX3**

The mean (±SD) of PTX3 ng/ml concentration in serum of control group and Subclinical hypothyroidism women patients group according to BMI are illustrated in table (3) and figure (7). There are a significant increase ($p<0.05$) in the serum levels of PTX3 in Subclinical hypothyroidism group when compared with control group, and no significant increase ($p>0.05$) in PTX3 serum levels in BMI groups.

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**Table (3): Mean ±SD of PTX3 ng/ml concentration**

| Groups | Mean ± SD of PTX3 (ng/ml) | |
|---|---|---|
| | Control | Patients |
| Total | 1.03± 0.151 | 2.422±0.130 |
| A1 (BMI =24-25.5) | 1.654± 0.149 | 2.132±0.130 |
| A2 (BMI=25.6-30) | 2.405± 0.167 | 2.737±0.130 |

| *P value* | | | | |
|---|---|---|---|---|
| Control/ Patients | A1 Control /A2 Control | A1 Patients /A2 Patients | A1 Control /A1 Patients | A2 Control /A2 Patients |
| ≤0.001 | ≤0.01 | ≤0.01 | ≤0.001 | ≤0.001 |

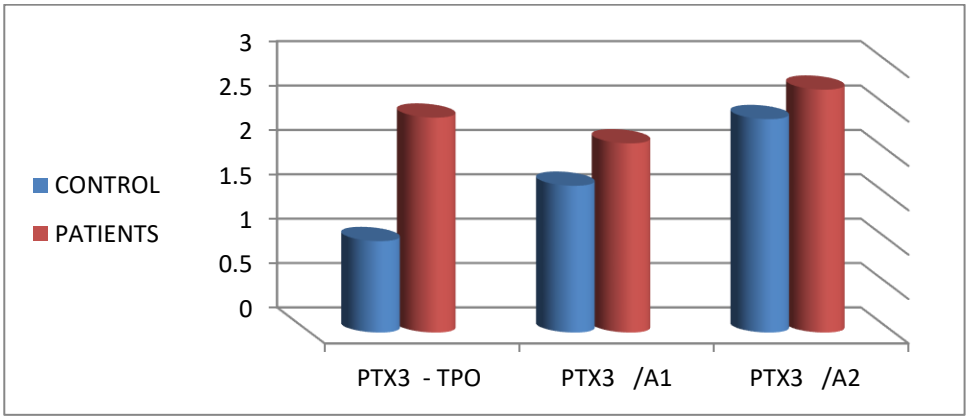


**Figure (7): Mean ±SD of PTX3**

They revealed that because PTX3 released by cell compartments involved in the onset and progression of cardiovascular disease (CVD), serum PTX3 serves as a marker linking to inflammation condition [27].

Tissue homeostasis is regulated by the innate immune system's soluble pattern recognition receptor, PTX3 [28]. Acute myocardial infarction, small-vessel vasculitis, sepsis, and other diseases have all been linked to PTX3 as a potential biomarker in serum. In sepsis, PTX3 may also serve as an early indicator of the severity and prognosis of the illness.. Furthermore, It has been proposed that PTX3, an innate immune system component that is highly expressed in patients with systemic inflammation, serves as a sensitive indicator of vascular inflammation [29]. The innate immune response, inflammation, tissue remodeling, and the humoral soluble pattern recognition molecule PTX3 are all critically dependent on each other. Furthermore, new studies have suggested that PTX3 a related to the pathophysiology of several distinct autoimmune diseases[30]. Acute-phase protein PTX3 synthesis is necessary for the body's homeostatic reactions to inflammation and tissue damage, and it plays a significant role in the innate immune system. Coordinating the recruitment and trafficking of leukocytes,

encouraging the removal of autoantigens and dying cells, and safeguarding the vasculature are just a few of its many functions. Inflammation must be resolved and tissue repair requires PTX actions [31]. In conditions where the inflammation does not responding to treatment, PTX3 is still produced and is an important biomarker for stratifying patients and forecasting clinical outcomes. Novel therapies for controlling leukocyte migration and the resolution of inflammatory processes may result from an understanding of the mechanisms underlying PTX3's homeostatic role. PTX3 have a role in oncogenesis or tumorigenesis, promoting and inhibiting tumor growth. Its significance in cancer biology has been linked to its ability to influence and modulate the immune response and interact with the extracellular matrix [28].

**D- Klotho protein**

The mean (±SD) of Klotho protein ng/ml concentration in serum of control group and subclinical hypothyroidism women Patients group according to BMI are illustrated in table (4) and figure (8). There are no significant difference (>0.05) in the serum levels of Klotho protein in Subclinical hypothyroidism group when compared with control group, and between BMI groups.

**Table (4): Mean ±SD of Klotho protein ng/ml concentration**

| Groups | Mean ± SD of Klotho protein (pg/ml) | |
|---|---|---|
| | Control | Patients |
| Total | 5.191 ± 0.44 | 5.32 ± 0.33 |
| A1 (BMI =24-25.5) | 5.22 ± 0.39 | 5.49 ± 0.48 |
| A2 (BMI=25.6-30) | 5.03 ± 0.68 | 5.23 ± 0.38 |

| *P value* | | | | |
|---|---|---|---|---|
| Control /Patients | A1 Control /A2 Control | A1 Patients /A2 Patients | A1 Control /A1 Patients | A2 Control /A2 Patients |
| >0.05 | >0.05 | >0.05 | >0.05 | >0.05 |

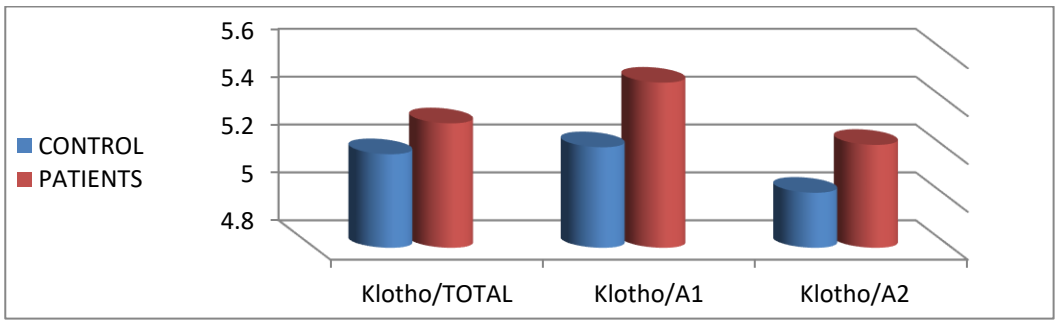


**Figure (8): Mean ±SD of Klotho protein**

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**E- Magnesium**

The mean (±SD) of $Mg^{2+}$ mg/dl concentration in serum of control group and Subclinical hypothyroidism women patients group according to BMI are illustrated in table (5) and figure (9). There were a highly significant deceased ($\leq 0.01$) in the serum levels of $Mg^{2+}$ mg/dl in subclinical hypothyroidism group when compared with control group, while there was no significant difference $\leq 0.01$ between BMI groups.

**Table (5): Mean ±SD of $Mg^{2+}$ mg/dl concentration**

| Groups | Mean ± SD of $Mg^{2+}$ (mg/dl) | |
|---|---|---|
| | Control | Patients |
| Total | 3.133 ± 0.31 | 2.32 ± 0.27 |
| A1 (BMI =24-25.5) | 3.10 ± 0.29 | 2.33 ± 0.21 |
| A2 (BMI=25.6-30) | 3.09 ± 0.28 | 2.36 ± 0.30 |

| *P value* | | | | |
|---|---|---|---|---|
| Control/ Patients | A1 Control /A2 Control | A1 Patients /A2 Patients | A1 Control /A1 Patients | A2 Control /A2 Patients |
| ≤0.01 | >0.05 | >0.05 | ≤0.01 | ≤0.01 |

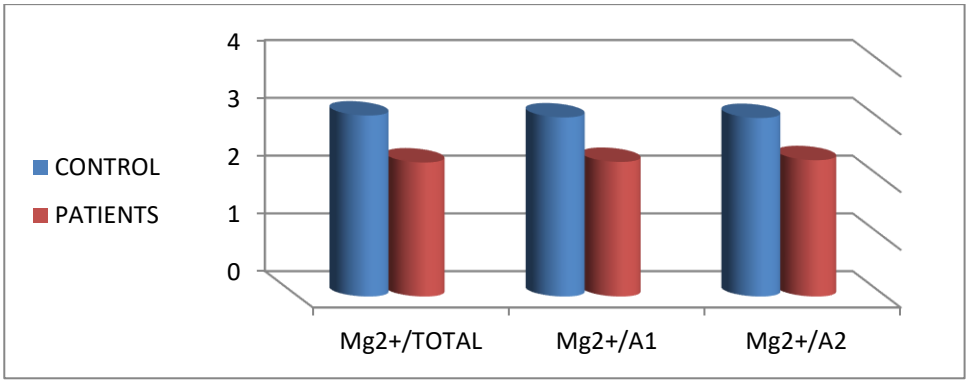


**Figure (9): Mean ±SD of $Mg^{2+}$**

Regulation and modulation of immunity effect exhibits by magnesium. $Mg^{2+}$ is a cofactor for the C3 convertase formation, which is engaged in the proteolytic action of complement components, antigen binding and the cytokine response, T-helper and B cell adhesion, immunoglobulin production, and IgM binding to lymphocytes. $Mg^{2+}$ functions as a secondary signaling messenger and aids in T cell activation (32),(33). serum patients with hyperthyroidism have lower magnesium levels than those with hypothyroidism; this difference may be due to thyroid hormones increasing the excretion of magnesium in the urine [34].

Hypothyroidism has been linked to lower serum magnesium levels in both animal models and clinical studies, and thyroid hormones' influence on the degree of urine concentration is primarily responsible for their impact on the magnesium urinary excretion rate [20],[35]. They

hypothesized that low blood magnesium levels might result in aberrant immune cell activation and a reduction in immunological tolerance [36]. Because magnesium is a coenzyme and is involved in several antioxidant metabolism pathways, including glutathione synthesis, low serum magnesium levels may impair cells' ability to respond to antioxidants and allow free radicals to accumulate, leading to oxidative stress and tissue damage [37],[38].

### F. Relationships between parameters

Table (6) showed the correlations between parameters in the women patients group. The results obtained in revealed significant related between parameters. There are stronger positive correlation between Anti-TPO and TSH, PTX3 , while there was negative correlation between Anti-TPO and $Mg^{2+}$.

**Table (6): Correlation of parameters between patients**

| Parameters | Anti-TPO | PTX3 | Klotho protein |
|---|---|---|---|
| T3 | -0.17 | 0.01 | 0.10 |
| T4 | -0.22 | 0.10 | 0.13 |
| TSH | 0.54 | 0.39 | 0.03 |
| Anti-TPO | - | - | - |
| PTX3 | 0.53 | - | - |
| Klotho protein | 0.01 | 0.12 | - |
| $Mg^{2+}$ | -0.61 | -0.58 | 0.23 |

**Conclusion**:

We concluded that there are significant higher level of PTX3 in patients with overt and subclinical hypothyroidism than in the healthy control. Thus, PTX3 may be relatively independent marker for prognosis of subclinical to overt cases or to metabolic complications in hypothyroidism patients as a potential risk factor for this disease.

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