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The Role of Tenascin-X, Paraoxonase 1 and Some Other Biochemical Parameters in Infertility Women

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Abstract : The thyroid interact with the hypothalamic-pituitary reproductive axis. Tenascins (TN) are a group of versatile glycoproteins found in the extracellular matrix (ECM) that have distinct expression patterns in different tissues and stages of development, as well as in homeostasis tissue balance and during infections and diseases. Furthermore there are four types of family members that are referred as tenascin (-C, -R, -X, -W). Tenascin-X and tenascin-C have significant involvement in human diseases. The paraoxonase family consists of three genes: paraoxonase 1, paraoxonase 2, and paraoxonase 3. These genes have around 70% genetic sequence similarity and 60% same sequence of amino acid. **Materials and Methods:** 120 Sample of the current study were collected from married women aged 20 and 45 years after taking their information (age, height, weight, and health status in detail) to obtain suitable samples. **Results :** The anti-mullerian hormone showed significant decrease at ($P<0.01$) in hypothyroidism and increase significant in infertility at ($P<0.01$). Prolactin showed highly increase significant in hypothyroidism at ($P<0.01$) and in infertility women patients compared with women control. Luteinizing hormone level showed increase significant in hypothyroidism and infertility at ($P<0.01$) and follicle-Stimulating hormone level showed decrease significantly. The tenascin-X showed a high significant increase at ($P<0.01$) in hypothyroidism patient compared with control group. The optimal cut-off value tenascin-X was (10.382 pg/ml as cut-off value). The tenascin-X showed a high significant increase at in infertility with hypothyroidism women patient compared with control group. The optimal cut-off value for tenascin-X was (8.904 pg/ml). Albumin level showed decrease significant in hypothyroidism at ($P<0.01$) and increase significant in hypothyroidism and infertility patients at ($P<0.01$) with control group. **Conclusions:** Hypothyroidism is associated with changes in the level of sex hormones, which is one of the main causes of infertility. tenascin-X were related with hypothyroidism. Hypothyroidism is associated with oxidative stress, PON-1 considered anti-oxidant and activity is changes in oxidative stress of different etiology.

Key words : hypothyroidism , infertility , Tenascin-X, Paraoxonase 1 .

1. INTRODUCTION

The thyroid interact with the hypothalamic-pituitary reproductive axis. The main components of the reproductive system receive input from: (a) fuel energy transmitters among includes insulin, insulin-like growth factor 1, glucose and leptin; (b) Substances associated with stress, such as glucocorticoids and corticotrophin releasing hormone (CRH); and (c) THs act as regulatory homeostasis. Most of these indications are earned by the kisspeptin-neurokinin B-dynorphin neurons that triggers the cascade of hypothalamic-pituitary-gonadal (HPG) axis signaling which producing kisspeptin to induces the pulsatile generating of GnRH from GnRH neurons [1]. Additional signals are acquired directly by the GnRH neurons itself. Afterwards, GnRH stimulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from gonadotrophs in the anterior pituitary gland. These hormones then act on the theca and granulosa cells in the ovary, Facilitating the synthesis of gonadal

steroids and peptides that as well as the growth of follicles in the ovaries. Thyroid hormones, including TRH, TSH, T3, and T4, have a facilitating function at every level of the HPG axis[2].

Tenascins (TN) are a group of versatile glycoproteins found in the extracellular matrix (ECM) that have distinct expression patterns in different tissues and stages of development, as well as in homeostasis tissue balance and during infections and diseases. Furthermore there are four types of family members that are referred as tenascin (-C,-R,-X, -W).Tenascin-X and tenascin-C have significant involvement in human diseases. Tenascin-X is extensively synthesized throughout various loose connective tissue types and its plays a role in ensuring the steadiness and management of collagens' network. Insufficient levels of the TNX protein caused by mutations or inadequate production (haploinsufficiency) may culminate in a rare illness termed classical-like Ehlers-Danlos syndrome [3]. Tenascin-X commonly known as hexabrachion-like protein or flexillin, is a 450kDa glycoprotein found in connective tissues. It belongs to the tenascin family. The TNXB gene is responsible for encoding it in humans [4]. The preliminary identification of Tenascin-X protein occurred while investigating human steroidogenesis and related problems, specifically among individuals with 21-hydroxylase deficiencies in contrast to in the context of exploring connective tissue illnesses[5, 6].

The paraoxonase (PONs) family consists of three genes: paraoxonase 1 (PON1), paraoxonase 2 (PON2), and paraoxonase 3 (PON3). These genes have around 70% genetic sequence similarity and 60% same sequence of amino acid [7]. The genes that encode PONs in humankind are situated throughout the long arm of chromosome 7, and are located in region 7q21.3–22.1. Paraoxonases, specifically PON1, are a type of hydrolase enzyme that have a wide range of substrates. They exhibit different sets of operations, including the lactonase (LACase), which includes thiolactonase and paraoxonase (POase), also known by the terms phosphotriesterase likewise arylesterase (AREase). Paraoxonase name is derived from the capability of enzymes to break down paraoxon, which is the very poisonous active metabolite of parathion. However, current studies suggest that PON1 is not very effective in breaking down this substrate in living organisms. PON1 and PON3 are enzymes found in the bloodstream fluid whereas PON2 is located inside cells. Paraoxonase 1 remains the most extensively researched member of the family, while PON2 appears to be the earliest ancestor from which PON3 and PON1 developed [8,9].

Albumin (ALB) is the main protein the plasma found in a bloodstream, with levels range from (35-50 g/l) in humans constituting 55-65% of total protein. Additionally, albumin is a significant result of liver synthesis, with around (0.7 mg/h) produced for each gram in liver tissue, Exhibiting have half-life of 19 days. Albumin is composed of a single chain of 585

amino acids and has a molecular weight of 65,000. It is organized into multiple domains and has distinct binding abilities to various ligands [10].

2. MATERIALS AND METHODS

Sample of the current study were collected from married women aged 20 and 45 years after taking their information (age, height, weight, and health status in detail) to obtain suitable samples and accurate results. There were 120 patients in the current investigation. The study was carried out at Higher Institute for the diagnosis of infertility and assisted reproduction techniques in Baghdad during the period between December 2023 and March 2024. One hundred and twenty individuals were enrolled in this study and all of them were females, They were classified as four groups: G1= Sample in first group (control group), G2= Sample in second group (hypothyroidism group), G3= Sample in third group (infertility group), G4= Sample in fourth group (hypothyroidism +infertility). The body mass index (BMI) is calculated by using specific equation, and standard levels. Tenascin-X and Paraoxonase-1 determined by Elisa kit. LH, FSH, AMH, prolactin by VIDAS assay. The measurement of albumin relies on spectrophotometer kit from Cromatest Company.

3. RESULTS AND DISCUSSION

Level of hormones Anti-Müllerian Hormone, Prolactin, Luteinizing hormone, and Follicle-Stimulating Hormone

The value (mean \pm SD) of AMH, PRO, LH and FSH levels in serum of the control groups and patient were clarified in the table (1) and the figure (1). The results showed the levels of AMH between (G1/G2) and between (G3/G4) there are significant decrease at $p < 0.01$, while the groups (G1/G3, G1/G4, G2/G3, G2/G4) there are significant increase at ($p < 0.01$). Prolactin levels results showed the a significant increase between groups (G1/G2, G1/G3, G1/G4, G2/G3) respectively at ($P < 0.01$), while between groups (G2/G4) there was no significant at $p > 0.05$, and between groups (G3/G4) there was a significant decrease at ($P < 0.01$). While the result of FSH levels showed the significant decrease in all groups except in (G2/G3) showed no significant difference at $p > 0.05$. The results of LH level showed that significant increase in all groups at ($P < 0.01$) when comparison between them, except in (G3/G4) showed no significant difference ($P > 0.05$).

Table 1: Serum Level of AMH, PRO, FSH, and LH

GROUPS		AMH	PRO	FSH	LH	
Control (G1)	Mean	1.63	15.11	12.49	3.03	
	SD	0.19	3.14	8.28	0.6	
Hypothy. (G2)	Mean	0.77	24.03	8.85	4.15	
	SD	0.41	6.43	2.82	3.09	
Infertility (G3)	Mean	3.37	35.59	7.97	6.29	
	SD	0.08	7.99	1.73	1.78	
Hypothy. &Infertility (G4)	Mean	2.07	27.06	4.38	5.89	
	SD	0.57	4.22	0.7	0.34	
<i>P value</i>						
Parameters	G1/G2	G1/G3	G1/G4	G2/G3	G2/G4	G3/G4
AMH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PRO	<0.01	<0.01	<0.01	<0.01	>0.05	<0.01
FSH	<0.01	<0.01	<0.01	>0.05	<0.01	<0.01
LH	<0.01	<0.01	<0.01	<0.01	<0.01	>0.05

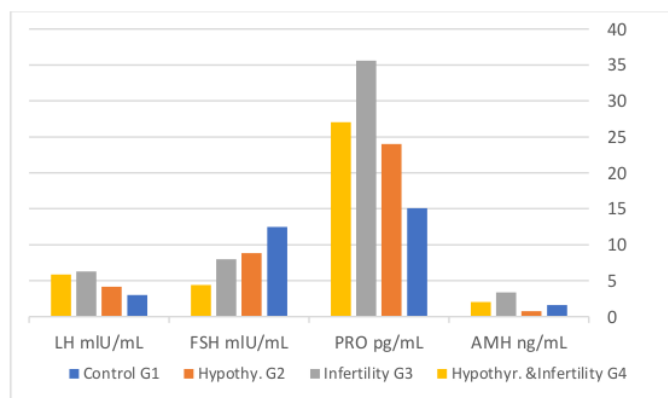


Figure 1: Serum Level of AMH, PRO, FSH, and LH

The current study which showed significant decrease of AMH with control in clinically hypothyroidism, and that consist with Yuko et al. who showed in many trials decrease level of AMH than control groups[11]. Therefore, thyroid dysfunction affects the regularity of menstrual cycles and the process of ovulation. Hypothyroidism can have an impact on the ovarian reserve. The contrasting impact of it on AMH levels based on age indicates that may have a role in the reduction of follicles in adults after significant stimulation of primordial

follicles during adolescence[12]. While the current study increase significant of AMH with control in infertility this study are consist with Acharya N et al.[13],who demonstrated during their studies AMH has been suggested as a method for predicting how the ovaries would respond to gonadotropin stimulation,in contrast others did not[14].

The production of AMH by the early and tiny follicles of the ovary seems to have a significant impact on the development of PCOS. Women with PCOS commonly have high levels of AMH in their blood. Elevated serum levels of AMH are strongly associated with PCOS, the presence of many ovarian cysts, excessive androgen production, and irregular or absent menstrual periods. Moreover, serum AMH exhibits a high level of diagnostic accuracy when used as a standalone indicator for PCOS or as a substitute for the presence of polycystic ovarian morphology. [15].

The present investigation, which demonstrated increase significant of PRO with control clinical hypothyroidism was consistent with Omar et al., who showed the hypothyroid state leads to an increase in the release of TRH in the central hypothalamus, which in turn stimulates the production of prolactin, the mechanism is that hypothyroidism lowers the inhibitory action of dopamine on the release of prolactin from the pituitary gland. This results in a significant rise in prolactin levels, frequently reaching 2-3 times the usual range[16]. Infertility is frequently associated with PRO with increase significant in the current study that consistent with Iancu et al .[17] and Lee et al.[12] , who found evidence of an inverse correlation between the levels of prolactin in the follicular fluid and the competence of oocytes .

The study showed decrease significant of FSH with control in hypothyroidism consistent with Acharya N et al.[13] and Brown EDL et al. [6],while the LH with control in hypothyroidism showed increase significant this study consistent with the Shi D et al.[14],who showed Hypothyroidism patients frequently have insulin resistance, resulting in increased levels of serum LH and the stimulation of the ovaries to create excessive androgens. This process can induce or worsen PCOS.

The level of Tenascin-X

The (mean \pm SD) of TNX of both patients and the control groups levels in the serum were shown in table (2), figure (2) based on BMI.

Table 2: Serum Level of TNX based on BMI

Mean ± SD of TNX					
GROUPS		TOTAL	BMI1 (22.2-25)	BMI2 (25.2-31)	<i>P value</i> BMI1/BMI2
Control (G1)	Mean	3.25	2.37	3.92	<0.01
	SD	0.75	0.41	0.86	
Hypothy. (G2)	Mean	27.8	19.594	33.77	<0.01
	SD	5.7	4.99	7.77	
Infertility (G3)	Mean	4.91	3.53	6.57	<0.01
	SD	0.85	0.84	1.38	
Hypothy.& Infertility (G4)	Mean	22.69	19.75	27.01	<0.05
	SD	5.54	4.81	6.56	
<i>P value</i>					
Total G1/G2	Total G1/G3	Total G1/G4	Total G2/G3	Total G2/G4	Total G3/G4
<0.01	<0.05	<0.01	<0.01	>0.05	<0.01

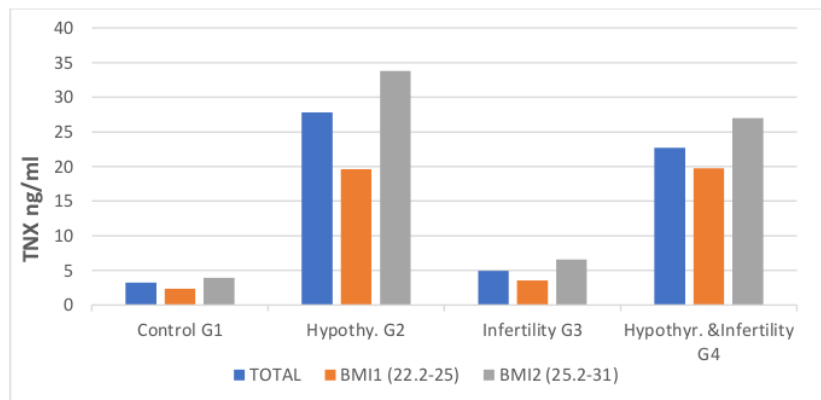


Figure 2: Serum Level of TNX based on BMI

The results showed the level of TNX in the groups (G1/G2) there are increase significant at ($P < 0.01$) when compared between the hypothyroidism patient and control. A significant increase in (G1/G3) at ($P < 0.05$), while the significant increase in the groups (G1/G2), (G1/G4) and (G3/G4) at ($P < 0.01$), but no significant difference ($P > 0.05$) in (G2/G4). the significant increase at ($P < 0.01$) between BMI groups in (G1,G2) and the G3 groups, the significant showed increase at ($P < 0.05$) between BMI groups in G4 group.

The table (3) and the Figure (3) shows the diagnostic validity criteria of (sensitivity, specificity, and accuracy). The ROC curve was produced for the TNX level, using a cut-off value of (10.382pg/ml). Based on these findings, the test is considered positive when the test value exceeds the threshold value for the respective categories.

Table 3: protective value of TNX in hypothyroidism group

Sensitivity	Specificity	Accuracy	AUC	CUT OFF
0.905	0.955	0.930	0.939	10.382

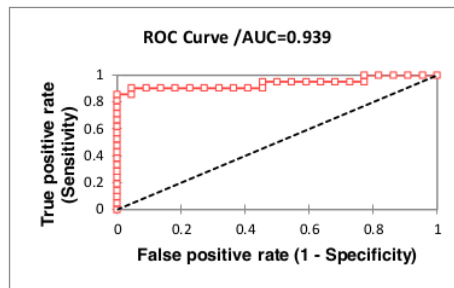


Figure 3: ROC curves of TNX in hypothyroidism

In the table (4) and the Figure (4) shows the diagnostic validity criteria of (sensitivity, specificity and accuracy) . The ROC curve was produced for the TNX level, using a cut-off value of (3.451pg/ml). Based on these findings, the test is considered positive when the test value exceeds the threshold value for the respective categories.

Table 4: Protective value of TNX in Infertility group

Sensitivity	Specificity	Accuracy	AUC	CUT OFF
0.524	0.682	0.605	0.571	3.451

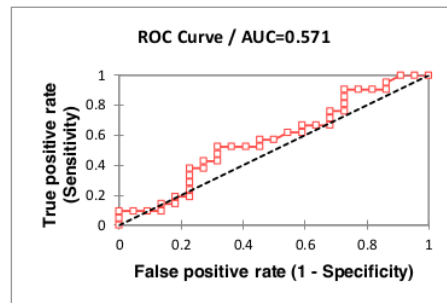


Figure 4: ROC curve of TNX in Infertility

Table (5) and figure (5) shows the criteria of diagnosis validity (Sensitivity, specificity, and accuracy) of TNX level by using 8.904 pg./ml as cut-off value. According to these results, Test is positive if test value > threshold value .

Table 5: Protective value of TNX in hypothyroidism& Infertility group

Sensitivity	Specificity	Accuracy	AUC	CUT OFF
0.636	0.909	0.773	0.804	8.904

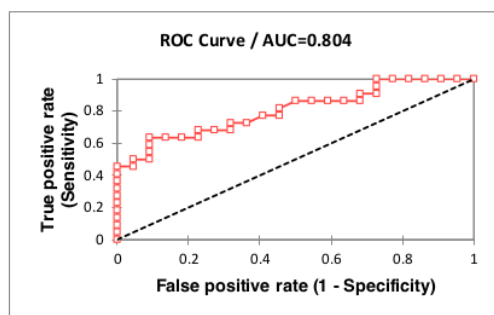


Figure 5: ROC curves of TNX in hypothyroidism& Infertility

Results indicate that the low AUC in infertility group (0.571) and the greatest AUC value (0.939) is found in hypothyroidism group and in hypothyroidism with infertility group AUC (0.804), and that means the TNX was related with hypothyroidism more than infertility. There are no studies about the relationship between TNX and disorders of the thyroid gland or reproductive system. For this reason, the current results will be interpreted on the basis of the correlation between the functions of both THs and TNX.

Thyroid hormones have an essential function in the formation as well as metabolism of various tissues and organs, both in the initial stages and in the adult life. This function is mostly carried out by T3, which controls gene expression via attaching to TH receptors. Thyroid hormones have been reported to modulate cells morphology, differentiation, and proliferation [18,19], and to regulate multifunctional extracellular matrix (ECM) organization and synthesis[20]. Theoretically hyperthyroidism may be accompanied by increased catabolism of both soluble and insoluble collagen. Hypothyroidism seems to be accompanied by decreased rates of catabolism of collagen[21,22]. The metabolism of collagen has been found to be influenced by THs [23,24]. However, any effect of THs on the expression of collagen in human tenocytes hasn't been previously documented [19]. The physiological functions of TNX in collagen deposition, collagen stability, physical property of collagen and collagen

fibrillogenesis. TNX and TNC have distinct roles in physiological and pathological conditions. In a physiological condition, TNX is involved in the structural integrity of collagen fibrils. The progress of fibrosis is attributable to an abnormal response determined by the accumulation of extracellular matrix proteins such as collagen and fibronectin[25].

The level of Paraoxonase 1

The value (Mean \pm SD) of PON1 (IU/mL) activity of the serum for patients and control groups were clarified in the table (6) and the figure (6) based on BMI.

Table 6: Serum Level of PON1 activity based on BMI

Mean ± SD of PON1 (IU/mL)					
GROUPS		TOTAL	BMI1 (22.2-25)	BMI2 (25.2-31)	<i>P value</i> BMI1/BMI2
Control (G1)	Mean	16.31	15.78	16.31	>0.05
	SD	2.25	2.93	3.04	
Hypothy. (G2)	Mean	11.21	11.16	11.30	>0.05
	SD	4.01	2.92	4.11	
Infertility (G3)	Mean	16.02	16.41	15.66	>0.05
	SD	4.01	2.92	4.11	
Hypothy.& Infertility (G4)	Mean	10.90	11.09	10.99	>0.05
	SD	0.55	0.45	0.51	
<i>P value</i>					
Total G1/G2	Total G1/G3	Total G1/G4	Total G2/G3	Total G2/G4	Total G3/G4
<0.01	>0.05	<0.01	<0.01	>0.05	<0.01

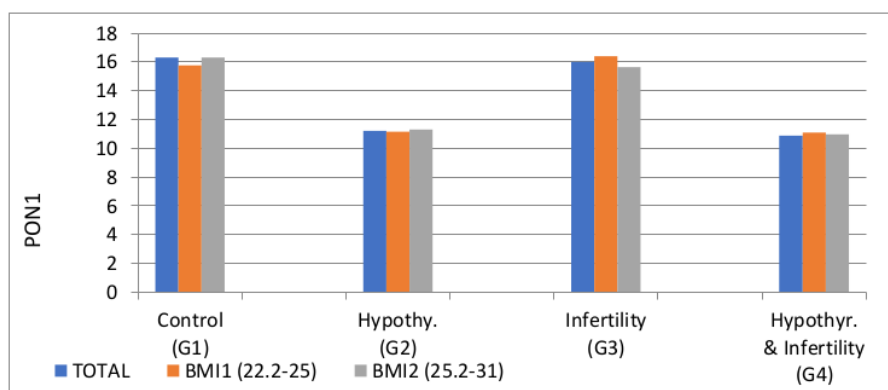


Figure 6: Serum Level of PON1 activity based on BMI

The results showed the level of PON1 activity in the groups (G1/G2), (G1/G4), (G2/G3) and (G3/G4) there are decrease significantly at ($P < 0.01$) when compared between groups. While showed no significant difference between (G1/G3) and (G2/G4) at ($P < 0.05$). There were no significant difference between BMI groups.

The present investigation demonstrated the adverse effects of hypothyroidism on PON - 1 levels compared to the control groups. The study found that patients with hypothyroidism experienced a significant decrease in paraoxonase-1 levels. This finding is supported by the studies conducted by Azizi et al. and Hasan et al., which also observed a reduction in PON 1 levels in individuals with hypothyroidism [26, 27] .

Primary hypothyroidism is linked to the presence of oxidative stress and inadequate antioxidant capacity PON 1 is produce by the liver and transport associated with HDL thought the plasma. It acts to prevent the oxidation of LDL, the formation of superoxide, and the peroxidation of HDL. Thus, it is considered a potential antioxidant that provides cytoprotection against lipid peroxidation. As a result, the activity of PON-1 decreases in oxidative stress caused by many causes. [28]. Torun et al., study demonstrated that increased oxidative stress in primary hypothyroidism may be due to insufficient antioxidant capacity and alterations in lipid metabolism [29] .

The Albumin Level

The (Mean \pm SD) of ALB levels of serum for patients and control groups were clarified in the table (7) and figure (7) based on BMI. The result showed the ALB levels in G2 group decrease significantly when compared G1,G3 and G4, while there was no significant difference in the groups G3 and G4 at ($P > 0.05$) when compared with G1, and there was no significant difference in the BMI groups.

Table 7: Serum Level of ALB based on BMI

Mean ± SD of ALB g/dl					
GROUPS		TOTAL	BMI1 (22.2-25)	BMI2 (25.2-31)	<i>P value</i> BMI1/BMI2
Control (G1)	Mean	4.22	4.13	4.3	>0.05
	SD	0.41	0.29	0.54	
Hypothy. (G2)	Mean	3.54	3.61	3.5	>0.05
	SD	0.42	0.43	0.44	
Infertility (G3)	Mean	4.12	4.00	4.3	>0.05
	SD	0.53	0.49	0.51	
Hypothy.& Infertility (G4)	Mean	4.19	4.09	4.32	>0.05
	SD	0.55	0.45	0.51	
<i>P value</i>					
Total G1/G2	Total G1/G3	Total G1/G4	Total G2/G3	Total G2/G4	Total G3/G4
<0.01	>0.05	>0.05	<0.01	<0.01	>0.05

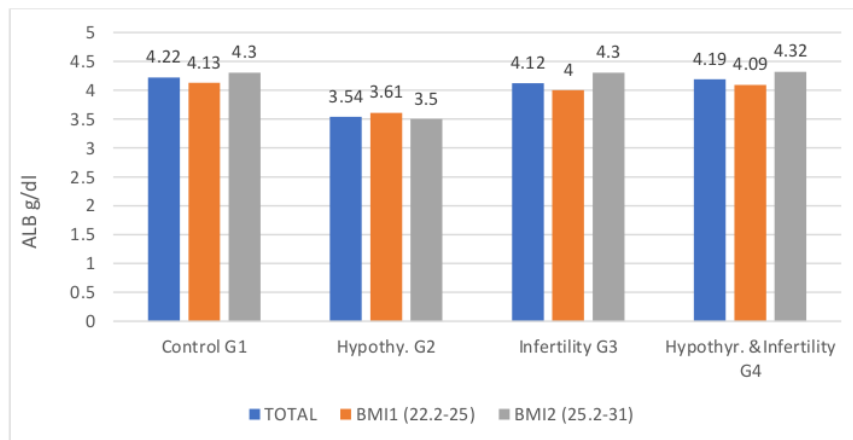


Figure 7: Serum Level of ALB based on BMI

In the current study of significant decrease between ALB and hypothyroidism was consistent with Muraleedhara et al.[16], thyroid dysfunction effected on albumin metabolism ,hypothyroidism extended catabolism of albumin , leded to low serum level (intake and synthesis) of albumin.while the current study referred to increase significant between Alb and hypothyroidism with infertility but not agree with Rahman et al. [30],who explained the albumin was low level with low level of thyroid hormone(T3,T4) but not hypothyroidism and not agree with Reinhardt W.[31].

4. CONCLUSIONS

Hypothyroidism is associated with changes in the level of sex hormones, which is one of the main causes of infertility . tenascin-X were related with hypothyroidism. Hypothyroidism is associated with oxidative stress, PON-1consider anti-oxidant and activity is changes in oxidative stress of different etiology

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