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Role Of Anti-TPO And Some Other Biochemical Parameters In Serum Of Hypothyroidism Patients

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Abstract

Hypothyroidism (HPT) is a frequent disorder of the endocrine system in humans. The prevalence of HPT has increased to affect a significant portion of the global population. HPT is a medical term that describes the inability of the thyroid gland to compete with the requirement of the body for thyroid hormone (T3 and T4). The main two etiological factors of HPT are iodine deficiency and Hashimoto's disease. In some groups, thyroid peroxidase antibodies (anti-TPO) have been discovered to correlate with TSH concentrations and to foretell the onset of thyroid impairment. We have aimed to investigate the level of anti-TPO in hypothyroidism patients with respect to their body mass index (BMI), gender, and age in the Iraqi population. Also, lipid profile parameters including, choles.terol, triglycerides (TGs), high-, low-, and very low-density lipoprotein (HDL, LDL, and VLDL) were investigated in the patients. α-L-Fucose is a six-carbon deoxy-hexose, a monosaccharide. (6-Deoxy-L-galactose) or a methyl pentose sugar with a general formula C6H12O5 and a molecular weight of 164.2 g/mol. Alkaline phosphatase (EC 3.1.3.1) belongs to the class of hydrolases, is a membrane-bound glycoprotein. The results have shown a significant increase in the level of anti-TPO in HPT patients compared to healthy people. This increase in the anti-TPO level was observed in obese and lean, females and males, and on different scales of age. Obesity and age have been shown to affect the level of anti-TPO in HPT patients. Moreover, the lipid profile parameters were significantly changed in HPT patients compared to control. Also, obesity has shown a significant influence on the levels of lipid profile parameters. The results of the study are leading to conclude that anti-TPO is a very important biomarker in the prognosis of HPT patients.

Keywords: TSH, T3, T4, anti-TPO, lipid profile, BMI.
Aim: To understand the role of thyroid disorders on thyroid peroxidase, fucose and ALP.

1. Introduction

The human body contain the thyroid gland as part of the endocrine system to maintain healthy regular metabolism [1]. The thyroid takes the signal from the anterior loop of the pituitary gland in form of thyroid stimulating hormone (TSH) to stimulate the thyroid for releasing triiodothyronine (T3) and thyroxine (T4) [2, 3]. In most target cells, T4 is followed a conversion through enzymatic deiodination into T3, which is the active hormone of the thyroid gland [4, 5]. Hypothyroidism (HPT) is a medical term describes the inability of thyroid gland to compete with the requirement of the body for thyroid hormone [6, 7]. HPT has been classified as overt HPT and subclinical HPT [8]. The first describes the medical condition when the thyroid gland release insufficient amounts of T4 under the stimulation of overload of TSH, while the second term describes the medical condition when the thyroid release sufficient amounts of T4 under the stimulation of overload of TSH [9]. The prevalence of HPT has been increased rapidly in the world, becoming one of the most endocrine disorders in human [10]. Females are more likely to develop HPT compared to males [11].

The main two etiological factors of HPT are iodin deficiency and Hashimoto's disease [12]. The latter is a chronic inflammatory autoimmune disease of the thyroid gland [13], which considered as the most common cause of thyroid diseases in children and adolescents and it is also the most common cause of acquired hypothyroidism with or without goiter [14]. Thyroid dysfunction predominantly manifests as hypothyroidism in up to 50% of antibody positive subjects. The presence of thyroid autoantibodies has a high predictivity (up to 50%) for the development of thyroid dysfunction [15]. In some groups, thyroid peroxidase antibodies (anti-TPO) have been discovered to correlate with TSH concentrations and to foretell the onset of thyroid impairment [16]. We have aimed to investigate the level of anti-TPO in hypothyroidism patients with respect to their body mass index (BMI), gender, and age in Iraqi population. Also, lipid profile parameters including, cholesterol, triglycerides (TGs), high-, low-, and very low-density lipoprotein (HDL, LDL, and VLDL) were investigated in the patients.

2. Materials and Methods

2.1. Subjects

In the province of Baghdad, 120 blood samples were obtained and obtained from the labs of the Hospitals of Medicine City (educational laboratories), the Baghdad Teaching Hospital, and the Al-Numan Teaching Hospital (October 2021 to February 2022). Whereas the other 60 participants in the trial were healthy controls, 60 persons were diagnosed with HPT. Equal numbers of individuals in each of the 2 groups were classified as obese and lean.

2.2. Methods

The serum of each individual was used to analyze the level of TSH, T3, and T4 by using Biomerieux (France) kits for Vidas analyzer, the level of anti-TPO by using ELSA kit based on sandwich methodology from Sunlong (China), and the levels of lipid profile parameters from Linear (Spain). ELISA microplate reader (Paramedical, Italy), UV-Vis spectrophotometer (Optima, Japan), and Vidas analyzer (Biomerieux, France) were used in the experimental part of the study. The data were analyzed statistically for mean comparison by using independent sample t-test, where the results are expressed in mean ± standard deviation.

3. Results

In Table 1, obese and slim HPT patients have shown significant ($P<0.05$) higher levels of anti-TPO compared to the corresponding categories of control. Also, the level of anti-TPO in obese HPT patients and obese control were significantly ($P<0.05$) lower compared to the slim HPT patients and slim control. Moreover, TSH was significantly increased in obese and slim HPT patients compared to the corresponding categories, while T3 and T4 were significantly reduced.

Table 1. *The results of clinical parameters in HPT and control according to BMI.*

Parameter	HPT		Control	
	(G4)Obese, N=30	(G3)Slim, N=30	(G2)Obese, N=30	(G1)Slim, N=30
Anti-TPO (pg/mL)	6171.2±506.85	17866 ±1800.9 *	1062.96±101 [a]	2829.47±279.5 [b]
TSH (mIU/mL)	48.212±3.16	9.51±1.093 *	1.86 ±0.204 [a]	1.96±0.076 [b]
T3 (nmol/L)	0.982±0.074	1.304±0.179 *	1.688±0.120 [a]	1.735±0.042 [b]
T4 (nmol/L)	58.310±4.85	65.016±4.42	94.911±5.8 [a]	97.509±8.7 [b]
Cholesterol (mg/dL)	236.3±22.25	203.13±21.8 *	197.06±12.7 [a]	177.66±6.25 #[b]
TGs (mg/dL)	159.6±13.7	124.833±13 *	124.66±17.0 [a]	113.33±14.0
HDL (mg/dL)	39.633±1.75	40.666±1.93	40.133±0.99	39.733±2.91
LDL (mg/dL)	164.74±10.9	137.45±22.0 *	132±11.668 [a]	115.266±8.0 #[b]
VLDL (mg/dL)	31.92±3.358	25.013±3.04 *	24.933±2.0 [a]	22.666±1.8
Fucose (mg/dl)	14.8 ± 1.11	17.9 ± 1.08 *	11.4 ± 0.91	12.2 ± 1.07
ALP (U/L)	169.1± 15.9*	136.6± 12.5	110± 7.3	78.1 ± 5.6

* significant between obese and slim HPT, # significant between obese and slim control, [a] significant between obese HPT and obese control, [b] significant between slim HPT and slim

control.

Table 2. *The results of clinical parameters in HPT and control according to gender.*

Parameter	HPT		Control	
	(F2)Female, N=32	(M2)Male, N=28	(F1)Female, N=30	(M1)Male, N=30
Anti-TPO (pg/mL)	12168.64±1033	11639.28±980	1948.181±128 [a]	1933.478±183 [b]
TSH (mIU/mL)	46.26±4.04 *	33.39±3.01	1.898±0.05 [a]	2.022±0.11 [b]
T3 (nmol/L)	1.142±0.19	1.143±0.12	1.715±0.31	1.687±0.20
T4 (nmol/L)	61.880±6.2	61.112±4.1	96.42±9.7 [a]	94.83±5.1 [b]
Cholesterol (mg/dL)	218.209±20.86	223.529±20.25	186.653±13.71	192±16.71
TGs (mg/dL)	137.654±18.36	158.064±13.47 *	120.923±13.26 [a]	106.5±5.07 #[b]
HDL (mg/dL)	40.441±1.74	39.411±2.15	40.038±1.93	39.25±3.59
LDL (mg/dL)	150.609±12.66	152.341±16.00	122.430±12.66 [a]	131.45±11.12 #[b]
VLDL (mg/dL)	27.158±3.06	31.776±3.071 *	24.184±6.66 [a]	21.3±1.013 [b]
Fucose(mg/dl)	15.4± 1.41 [a]	17.0± 1.32 [b]	11.7± 1.01	12.0± 0.99 b
ALP (U/L)	146.1± 16.9	156.6± 12.5	80.1± 9.2	106.1± 7.2 # b

* significant between male and female HPT, # significant between male and female control, [a] significant between female HPT and female control, [b] significant between male HPT and male control.

The levels of cholesterol, TGs, LDL, and VLDL were significantly increased in obese HPT patients compared to obese control. While in slim HPT patients, only cholesterol and LDL were increased significantly. There was a highly significant increase level of fucose between G1/G3, and a significant increase between G3/G4, and G2/G4. While there were no significant differences between G1/G2. Also, there was a highly significant increase in the serum activity of ALP between G1/G3, and G2/G4, while there was a significant increase between G1/G2, and G3/G4 (Table 1).

In Table 2, The males and females with HPT disease have shown significant increase in the level of anti-TPO compared to the corresponding categories of control. The level of anti-TPO was not changed significantly between males and females neither in control nor in HPT patients. Males have shown significant higher levels of TGs and VLDL compared to females in HPT patients, while they have shown significant higher levels of TGs and LDL compared to females in control. There were no significant differences in the serum levels of fucose between M1/F1 and M2/F2 Patients. There was highly significant increase between M1/M2, and significant increase F1 /F2. There was no significant differences in the serum activity of ALP between M1/F1 and between M2/F2. While, there is a highly significant increase in the serum activity of ALP between M1 /M2, and between F1 /F2.

In Table 3, the patients and the control were divided into two categories based on their age, which were A (21-40 years old), and B (41-65 years old). The level of anti-TPO was increased significantly in A and B categories of HPT patients compared to the corresponding

categories of healthy control. Moreover, the level of anti-TPO in B category of HPT patients was significantly higher than anti-TPO level in A category of HPT patients. Also, TSH level was increased significantly in A and B categories of HPT patients compared to the corresponding category of healthy control, while T4 level was reduced significantly. Nevertheless, T3 level did not changed significantly between A or B categories of HPT patients compared to the corresponding category of healthy control. Additionally, TSH level was significantly higher in B category of HPT patients compared to A category of HPT patients. The levels of TGs and LDL were increased significantly in HPT patients compared to the corresponding groups of control. Moreover, VLDL was increased in the A category of HPT group compared to the A category of control significantly. There was no significant differences in the serum levels of fucose between A1/A2 and A3/A4, a highly significant increase between A1/A3, and a significant increase between A2/A4. Also, There were no significant differences in the serum activity of ALP between A3/A4. While, there are a highly significant increase in the serum activity of ALP between A1/A3 and between A2/A4, and a significant increase between A1/A2.

Table 3. *The results of clinical parameters in HPT and control according to age.*

Parameter	**HPT**		**Control**	
	(A4) B, N=34	**(A3) A, N=26**	**(A2) B, N=32**	**(A1) A, N=28**
Anti-TPO (pg/mL)	2998.68±301.9	2300.3±218.6 *	1916.67±142.4 [a]	1968.81±144.66 [b]
TSH (mIU/mL)	52.429±3.225	33.744±2.91 *	1.758±0.40 [a]	2.0347±0.35 [b]
T3 (nmol/L)	1.156±0.2133	1.127±0.26	1.783±0.09	1.656±0.173
T4 (nmol/L)	60.943±5.76	62.29±4.62	93.72±8.9 [a]	98.10±9.24 [b]
Cholesterol (mg/dL)	221.892±20.904	217.812±22.356	184.538±13.39	189.529±14.37
TGs (mg/dL)	168.321±11.051	150.875±21.828 *	121.615±11.15 [a]	117±9.99 [b]
HDL (mg/dL)	40.035±1.8555	40.25±1.967	39.384±2.93	40.352±1.22
LDL (mg/dL)	152.192±11.997	150.143±11.437	120.830±12.39 [a]	125.776±13.01 [b]
VLDL (mg/dL)	29.664±1.821	27.418±1.033	24.85±3.029	23.4±2.18 [b]
Fucose (mg/dl)	15.9± 1.32 [a]	16.4± 1.17 [b]	11.8± 0.87	11.9± 1.22
ALP (U/L)	152.0± 13.3 [a]	141.0± 13.2	112± 10.1 # [b]	77.2± 8.0

* significant between B and A HPT, # significant between B and A control, [a] significant between B HPT and B control, [b] significant between A HPT and A control.

4. Discussion

The results of the study have shown significant change of anti-TPO in HPT patients, in which the level of anti-TPO has been increased significantly as a consequence of the presence of hypothyroidism. In the study of Mutlu and Mutlu (2021), the authors have indicated an association between the increase of BMI and anti-TPO in people with euthyroid function and those with hypothyroidism. Furthermore, they have reported that a reduction in the weight of individuals would cause a reduction in the level of anti-TPO [17]. Another study was performed by Song *et al.* (2019) has mentioned a strong influence of obesity on thyroid function. The

authors have reported that obesity increases the risk of hypothyroidism and has a strong association with the elevation of anti-TPO based on meta-analysis according to previous studies [18]. González-Mereles *et al.* (2021) have examined the relationship between thyroid function and obesity in children. The workers have indicated a strong relationship between hypothyroidism and obesity, in which most of the hypothyroidism patients were obese. Moreover, a high percentage of obese children have shown positive anti-TPO compared to overweight and normal-weight children [19]. Ateş *et al.* (2015), have reported a significant increase in the level of anti-TPO in hypothyroidism patients compared to people with normal thyroid function. Additionally, they have reported a higher percentage of obese individuals in the hypothyroidism group compared to the control group of their study [20].

The data in Table 2 indicate that gender did not affect the level of anti-TPO in control or hypothyroidism patients. But it is a high effect significant in the patient group and healthy people. Nevertheless, Zynat. et al. (2020) reported that the males in their study exhibited a significantly higher percentage of positive anti-TPO compared to the females. Yet the male participants of their study had a higher BMI than the females, and the reason was probably attributed to the BMI [21]. Another study by Nino Turashvili. et al. (2021) found a higher percentage of positive anti-TPO found more in females than in males [22]. The data in Table 3 indicate that age affects the level of anti-TPO in hypothyroidism patients (but not in control). Wiersinga *et al.* (2004) have reported that the level of anti-TPO rise in women significantly with the increase of age, but age does not affect the level of anti-TPO in males [23]. Moreover, lipid profile has been confirmed to be significantly changed in obesity [24], and hypothyroidism [25] in previous studies. The increased serum glycoprotein content in hypothyroid patients could be attributed to membrane disruptions. Depleted glycoproteins in hyperthyroidism may be due to an increase in glycoprotein depolymerisation and accelerated glycoprotein secretion (like protein-bound fucose). Hypothyroidism increased oxidative stress, whereas hypothyroidism decreased oxidative stress and caused significant changes in T3, T4, and TSH levels during the thyroid state when all stressors were present[26],[27].The current study supports Yuan Li's (2021) discovery of a high level of fucose in thyroid diseases[28].

Thyroid hormones regulate the rate of metabolism of tissues, so when the action of these hormones changes, various organs and enzyme levels are affected[29]. The current study confirmed that thyroid disorders have a significant impact on the metabolism of various cells in the body, as evidenced by increased serum enzyme levels to varying degrees. The heart and liver are the organs most affected by thyroid dysfunction, which alters the levels of hepatic enzymes (ALP and AST) [30]. The findings of this study were consistent with the findings of other studies[31]. According to these studies, the levels of liver enzymes (ALP, ALT, and AST) are elevated in hypothyroid patients. Thyroid hormones are required for the growth and regulation of all body cells' metabolic rates, so any abnormality in the thyroid gland will affect cellular metabolism. Because the liver is the most affected organ by any defect in the cellular metabolism process, the effectiveness of liver enzymes suffers as a result [30]. The current study's finding of decreasing serum ALP levels in hypothyroid patients contradicts *Mane and Bhagwat* [32], *Al-Hindawi et al.* [33], and *Al-Janabi G et al.* [34], which stated that ALP levels in hypothyroid patients were significantly lower than in controls. Pandey et al. conducted yet another study [35]، showed hypothyroidism, serum ALP levels increased significantly. Elevated ALP levels in hypothyroid and hyperthyroid patients may indicate a change in the rate of bone turnover [36].

Previous studies on ALP levels in normal subjects discovered that ALP levels in obese people are higher than in non-obese people [37] , [38]. Other studies, however, found that body

weight has no effect on ALP levels in healthy people [39]. Because adipocytes contain an ALP isozyme, it is possible that adipose tissue is a source of serum ALP. Furthermore, higher serum ALP concentrations in obese versus non-obese subjects may indicate additional ALP release from adipose tissue in obesity. Obese subjects had significantly higher serum ALP levels, according to the findings [40].Obesity increases the risk of liver injury, and increases in liver enzymes are thought to be the most sensitive biochemical indicator of hepatic steatosis [40],[41].

Conclusions

The results of the study have shown that anti-TPO is greatly affected in HPT patients. The presence of positive anti-TPO was observed in the full number of samples in the patients. This makes anti-TPO as very important marker in the prognosis of autoimmune hypothyroidism. Moreover, obesity has shown significant influence on the level of anti-TPO making obesity as important risk factor in hypothyroidism.

References

Nilsson, M. and H. Fagman, Development of the thyroid gland. Development, 2017. 144(12): p. 2123-2140.

1. Voutsadakis, I.A., The TSH/thyroid hormones axis and breast cancer. Journal of Clinical Medicine, 2022. 11(3): p. 687.
2. Hulbert, A., Thyroid hormones and their effects: a new perspective. Biological Reviews, 2000. 75(4): p. 519-631.
3. Pitt-Rivers, R. and J.R. Tata, The thyroid hormones. 2013: Elsevier.
4. Mondal, S., D. Giri, and G. Mugesh, Halogen Bonding in the Molecular Recognition of Thyroid Hormones and Their Metabolites by Transport Proteins and Thyroid Hormone Receptors. Journal of the Indian Institute of Science, 2019. 100.
5. Gaitonde, D.Y., K.D. Rowley, and L.B. Sweeney, Hypothyroidism: an update. South African Family Practice, 2012. 54(5): p. 384-390.
6. Chiovato, L., F. Magri, and A. Carlé, Hypothyroidism in context: where we've been and where we're going. Advances in therapy, 2019. 36(2): p. 47-58.
7. Calsolaro, V., et al., Overt and subclinical hypothyroidism in the elderly: when to treat? Frontiers in endocrinology, 2019. 10: p. 177.
8. Khandelwal, D. and N. Tandon, Overt and subclinical hypothyroidism. Drugs, 2012. 72(1): p. 17-33.
9. Vaidya, B. and S.H. Pearce, Management of hypothyroidism in adults. Bmj, 2008. 337.
10. Taylor, P.N., et al., Global epidemiology of hyperthyroidism and hypothyroidism. Nature Reviews Endocrinology, 2018. 14(5): p. 301-316.
11. Vanderpump, M.P. and W.M.G. Tunbridge, Epidemiology and prevention of clinical and subclinical hypothyroidism. Thyroid, 2002. 12(10): p. 839-847.
12. Hou, W., G. Xu, and H. Wang, CHAPTER 9 - Hashimoto's thyroiditis, in Treating Autoimmune Disease with Chinese Medicine, W. Hou, G. Xu, and H. Wang, Editors. 2011, Churchill Livingstone: Edinburgh. p. 157-172.
13. Lorini, R., et al., Hashimoto's thyroiditis. Pediatric endocrinology reviews: PER, 2003. 1: p. 205-11; discussion 211.
14. Muhame, R.M., et al., Thyroid autoimmunity and function among Ugandan children and adolescents with type-1 diabetes mellitus. Pan Afr Med J, 2014. 19: p. 137.
15. Roos, A., et al., Thyroid peroxidase antibodies, levels of thyroid stimulating hormone and development of hypothyroidism in euthyroid subjects. European Journal of Internal

Medicine, 2010. 21(6): p. 555-559.
16. Mutlu, H.H. and H.H. Mutlu, The impact of weight loss on thyroid autoimmunity-Weight loss decreases thyroid peroxidase antibody levels: a retrospective cohort study. The European Research Journal, 2021. 7(6): p. 635-644.
17. Song, R.-h., et al., The impact of obesity on thyroid autoimmunity and dysfunction: a systematic review and meta-analysis. Frontiers in immunology, 2019. 10: p. 2349.
18. González-Mereles, A.P., et al., Overweight and obesity in children and adolescents with chronic autoimmune thyroiditis. Boletín médico del Hospital Infantil de México, 2021. 78(5): p. 424-431.
19. Ateş, İ., et al., Circulating levels of irisin is elevated in hypothyroidism, a case-control study. Archives of endocrinology and metabolism, 2015. 60: p. 95-100.
20. Zynat, J., et al., Impact of Abdominal Obesity on Thyroid Auto-Antibody Positivity: Abdominal Obesity Can Enhance the Risk of Thyroid Autoimmunity in Men. International journal of endocrinology, 2020. 2020.
21. Turashvili, N., L. Javashvili, and E. Giorgadze, Vitamin D Deficiency Is More Common in Women with Autoimmune Thyroiditis: A Retrospective Study. International journal of endocrinology, 2021. 2021.
22. Wiersinga, W.M., University of Amsterdam, Amsterdam, The Netherlands. Encyclopedia of Endocrine Diseases: Po-Z, Index, 2004. 4: p. 453.
23. Taay, Y., et al. Determination of some biochemical parameters in sera of normotensive and hypertensive obese female in Baghdad. in Journal of Physics: Conference Series. 2021. IOP Publishing.
24. Murgod, R. and G. Soans, Changes in electrolyte and lipid profile in hypothyroidism. Int J Life Sci Pharma Res, 2012. 2(3): p. 185-194.
25. L. P. Keegan, A. Leroy, D. Sproul, and M. A. O'Connell, "Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes," *Genome Biol.*, vol. 5, no. 2, pp. 1–10, 2004.
26. D. R. M. M. H. AL-MURSHIDI, "RELATIONSHIP BETWEEN BLOOD GROUP AND THYROID DISEASES," 2012.
27. K. Flevaris and C. Kontoravdi, "Immunoglobulin G N-glycan Biomarkers for Autoimmune Diseases: Current State and a Glycoinformatics Perspective," *Int. J. Mol. Sci.*, vol. 23, no. 9, p. 5180, 2022.
28. B. F. Hasan, N. Al Hudak, and I. D. Abd, "Estimation of thyroid hormones and liver enzymes in hypo and hyperthyroidism in Iraqi women," Int. J. Pharm. Bio. Sci, vol. 7, no. 4, pp. B707-713, 2016.
29. Q. Samir and M. Hameed, "Influence of Thyroid Stimulating Hormone on Liver Enzymes Levels in Serum of Thyroid Disorder Iraqi Patients," Al-Nahrain J. Sci., vol. 22, no. 3, pp. 50–55, 2019.
30. A. Yadav, S. Arora, V. Saini, M. K. Arora, R. Singh, and J. Bhattacharjee, "Influence of thyroid hormones on biochemical parameters of liver function: a case-control study in North Indian population," Internet J. Med. Updat., vol. 8, no. 1, 2013.
31. A. Y. Mane and V. R. Bhagwat, "Assessment of some biochemical markers of bone turnover in thyroid dysfunction state," Int J Pharm Bio Sci, vol. 3, no. 3, 2012.
32. S. H. Al-Hindawi, N. M. Luaibi, and B. H. Al-Ghurabi, "Estimation of Alkaline Phosphatase level in the Serum and Saliva of Hypothyroid Patients with and without Periodontitis," Res. J. Pharm. Technol., vol. 11, no. 7, pp. 2993–2996, 2018.
33. G. Al-Janabi, H. N. Hassan, and A. Al-Fahham, "Biochemical changes in patients during hypothyroid phase after thyroidectomy," J. Med. Life, vol. 15, no. 1, p. 104, 2022.
34. R. Pandey, S. Jaiswal, J. P. Sah, K. Bastola, and S. Dulal, "Assessment of serum

enzymes level in patients with thyroid alteration attending manipal teaching hospital, Pokhara," Thyroid, vol. 10, p. 12, 2013.
35. S. Jubair, A. S. Nsaif, A. H. Abdullah, and I. H. Dhefer, "Vitamin D deficiency is associated with thyroid diseases," in Journal of Physics: Conference Series, 2021, vol. 1853, no. 1, p. 12036.
36. A. T. Ali, J. E. Paiker, and N. J. Crowther, "The relationship between anthropometry and serum concentrations of alkaline phosphatase isoenzymes, liver enzymes, albumin, and bilirubin," Am. J. Clin. Pathol., vol. 126, no. 3, pp. 437–442, 2006.
37. F. Schiele, J. Henny, J. Hitz, C. Petitclerc, R. Gueguen, and G. Siest, "Total bone and liver alkaline phosphatases in plasma: biological variations and reference limits.," Clin. Chem., vol. 29, no. 4, pp. 634– 641, 1983.
38. F. A. Rashid, S. Mahdi, S. A. Mahdy, and A. T. Salim, "Effect of Obesity on Plasma Alkaline Phosphatase Activity in Breast Cancer," Reports Biochem. Mol. Biol., vol. 10, no. 2, p. 307, 2021.
39. A. R. Khan, F. R. Awan, S. Najam, M. Islam, T. Siddique, and M. Zain, "Elevated serum level of human alkaline phosphatase in obesity," Age, vol. 48, no. 8.8, pp. 42–45, 2015.
40. E. Elfaki, A. Hamza, M. Ahmed, M. Ibrahim, E. KAbdalhabib, and T. Karar, "Assessment of Liver Enzymes Levelamong Obesesudanese Individuals in Khartoum State-Sudan," Biomed. Pharmacol. J., vol. 13, no. 4, pp. 1893–1897, 2020.