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An Investigation into the Levels of Asprosin Hormone and Some Immunological Variables in a Number of Obese Women in the City of Kirkuk

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ABSTRACT
The current study involved examining the level of Asprosin hormone and some immunological variables, including TNF-α, IL-1, and IL-6, in 100 samples from women. These samples were divided into 50 blood samples from obese women, 30 from lean women, and 20 from women with ideal weight, all ranging in age from 25 to 45 years. The study was conducted from November 2021 to the end of April 2022. Blood samples were collected in the early morning after a minimum fast of 6-8 hours, excluding pregnant women and patients. The statistical analysis results showed a significant increase ($P \leq 0.01$) in Asprosin concentration in obese women (5.82 ± 0.83) ng/ml, compared to the control group (3.27 ± 0.40) ng/ml. However, there were no significant differences in Asprosin concentration in lean women (3.10 ± 0.22) ng/ml compared to the control group (3.27 ± 0.40) ng/ml. As for the immunological variables, the statistical analysis showed a significant increase ($P \leq 0.05$) in the concentration of TNF-α in the serum of obese women (225.13 ± 6.71) ng/L, and in the serum of lean women (155.75 ± 3.04) ng/L compared to the control group (148.41 ± 5.18) ng/L. The results also showed a significant increase at ($P \leq 0.05$) in the concentration of IL-6 in the serum of obese women (2.28 ± 3.10) pg/ml compared to the control group (0.33 ± 2.19) pg/ml, while there were no significant differences in the serum of lean women (0.92 ± 2.69) pg/ml compared to the control group. However, the statistical analysis showed a significant decrease ($P \leq 0.01$) in the concentration of IL-1 in the serum of obese women (168.06 ± 19.8) pg/ml, and in the serum of lean women (309.25 ± 13.47) pg/ml compared to the control group (420.45 ± 29.47) pg/ml.

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INTRODUCTION

Obesity is one of the health problems that has attracted the attention of many researchers, as it is currently one of the causes of a number of serious diseases, including: heart disease, vascular disease, arteriosclerosis, diabetes, high blood pressure, among others (1). Obesity is defined as a metabolic disorder that causes an increase in fat storage and an increase in cholesterol concentration and is one of the causes leading to heart and vascular disease (2). The adipose tissue is an active organ in the endocrine glands made up of mature fat cells that produce certain hormones. Adiponectin and Asprosin are produced by the fat cells of white adipose

tissue during hunger, and these hormones affect obesity (3). Excessive accumulation of adipose tissue in obesity causes an imbalance in the balance of pro-inflammatory and anti-inflammatory adiponectin secretion, leading to hyperinsulinemia, insulin resistance, and other obesity-related disorders. Individual fat cell storage capacity deficiency, therefore, leads to fat deposition in organs including visceral fat tissues, liver, and muscles (4). It was also found that the release of Asprosin may be due to hyperlipidemia, and by binding to Toll-like receptor (TLR4) receptors and activating the pathway, it may lead to a malfunction in pancreatic beta cells, thereby reducing insulin secretion and intensifying the inflammatory response in a dose-dependent manner. This helps B cells also secrete Asprosin in cases of hyperlipidemia through the TLR4/INK pathway to increase the production of oxidative factors and pro-inflammatory cytokines, thus enhancing the inflammation of B cells and cell death (Lee.et al, 2019). Inflammation is one of the major complications of obesity. Obesity is associated with an increase in macrophages in adipose tissue (ATMs), which are the main source of cytokines. ATM infiltration is positively correlated with inflammatory factors, including Tumor Necrosis Factor Alpha (TNF-α) (5). Activated macrophages are characterized by an increase in the production of pro-inflammatory cytokines such as IL-6, TNF-α, IL-12, IL-23, and a decrease in anti-inflammatory IL-10 synthesis (6). Based on numerous studies, it has been proven that adipose tissue is an active organ involved in many metabolic, hormonal, and immune processes that affect other organs and systems that play an important role in overall body balance. There are many proposed mechanisms to explain how excessive adipose tissue can cause metabolic disorders. One of the most illustrative concepts is the development of local and systemic inflammation characterized by an increase in fat cells in adipose tissue by immune cells and an increase in the production of widespread inflammatory factors. On this basis, our current study aims to evaluate the concentration of Asprosin hormone in the serum of obese and lean women compared to a group of healthy women, and to evaluate the concentrations of TNF-α, IL-1, and IL-6 in the studied groups' samples.

MATERIALS AND METHODS

5 ml of venous blood was drawn using disposable syringes and placed in a non-anticoagulant containing Gel Tube for the purpose of performing serum tests. Then, the blood components were separated in the non-anticoagulant tubes using a centrifuge at a speed of 3000 RPM for 15 minutes. The remaining serum was drawn with a micropipette and transferred to an Eppendorf tube, and stored at a temperature of -20°C for subsequent hormonal testing.

Body Mass Index (BMI) Calculation

This measurement includes the weight and height of all samples to extract the BMI, the result of dividing the weight in kilograms by the square of the height in meters (kg/m^2), using a tape measure for height and a scale for weight as mentioned by (Aminian et al, 2018).

Asprosin Hormone Concentration in Blood Serum

The basic principle for estimating the concentration of Asprosin hormone is to use the ready-made BT-LAB test kit. This test relies on the Enzyme-Linked Immuno

Sorbent Assay (ELISA) method. The plate was pre-coated with antibodies to human Asprosin. Asprosin present in the sample was added, binding to the coated antibodies in the wells. Then the human Asprosin antibody, supplemented with protein, was added and binds with the Asprosin in the sample. Afterwards, Streptavidin-HRP was added and binds with the Biotinylated Asprosin antibody. After incubation, Streptavidin-HRP was washed and the Substrate Solution was added, changing the color proportionally to the amount of human Asprosin. The reaction ended by adding the Stop Solution to the reaction wells to stop the reaction, then the reading was taken at a wavelength of 450 nm using a microplate reader.

Tumor Necrosis Factor Concentration in Blood Serum

The test relies on the Enzyme-Linked Immuno Sorbent Assay (ELISA) method. The plate was pre-coated with antibodies to human TNF-α. TNF-α present in the sample was added, binding to the coated antibodies in the wells. Then the human TNF-α antibody, supplemented with protein, was added and binds with TNF-α in the sample. Afterwards, Streptavidin-HRP was added and binds with the Biotinylated TNF-α antibody. After incubation, Streptavidin-HRP was washed and the Substrate Solution was added, changing the color proportionally to the amount of human TNF-α. The reaction ended by adding the Stop Solution to the reaction wells to stop the reaction, then the reading was taken at a wavelength of 450 nm using a microplate reader.

IL-1β Concentration in Blood Serum

The test relies on the Enzyme-Linked Immuno Sorbent Assay (ELISA) method. The plate was pre-coated with antibodies to human IL-1β. IL-1β present in the sample was added, binding to the coated antibodies in the wells. Then the human IL-1β antibody, supplemented with protein, was added and binds with IL-1β in the sample. Afterwards, Streptavidin-HRP was added and binds with the Biotinylated IL-1β antibody. After incubation, Streptavidin-HRP was washed and the Substrate Solution was added, changing the color proportionally to the amount of human IL-1β. The reaction ended by adding the Stop Solution to the reaction wells to stop the reaction, then the reading was taken at a wavelength of 450 nm using a microplate reader.

IL-6 Concentration in Blood Serum

This test uses the Enzyme-Linked Immuno Sorbent Assay (ELISA) sandwich immunodetection method. Blood serum from the sample was added to the wells in the microtitration plate, which was pre-coated with monospecific antibodies for IL-6. After an incubation period, the plate was washed to remove unbound proteins in the study sample, a detection antibody solution was added, and the conjugated antibody was bound. Then the microtitration plate was washed to remove unbound conjugated antibodies, and the substrate solution (solution A and B) was added. The enzyme associated with the molecules of the substrate forms a colored complex, the intensity of its color density is directly proportional to the concentration of the studied antigen. The reaction ended by adding the Stop Solution to the reaction wells to stop the reaction, then the reading was taken at a wavelength of 450 nm using a microplate reader.

Statistical Analysis

The results were statistically analyzed using the Minitab statistical program. One-

way Analysis of Variance (ANOVA) test was used to compare the arithmetic means using Duncun Multiple Range Test at a probability level (P≤0.01) and (P≤0.05) to determine the significant differences between the groups (7).

RESULTS AND DISCUSSION

• Asprosin Hormone Concentration in Blood Serum

The results shown in Table 1 reveal a significant increase (P≤0.01) in the concentration of asprosin hormone in the blood serum of obese women (5.82 ± 0.83 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml). However, the results did not show any significant difference in the concentration of asprosin hormone in the blood serum of thin women (3.10 ± 0.22 ng/ml) compared to the control group (3.27 ± 0.40 ng/ml).

Table (1) demonstrates the concentration of Asprosin hormone in the serum of the studied groups.

Variable	Control	Obese	Thin	P-Value @0.05
ASP (ng/ml) Mean ± Std.	3.27 ± 0.40b	5.82 ± 0.83a	3.10 ± 0.22b	0.001

- Different letters denote a significant difference among the studied groups.
- Similar letters denote no significant difference among the studied groups.

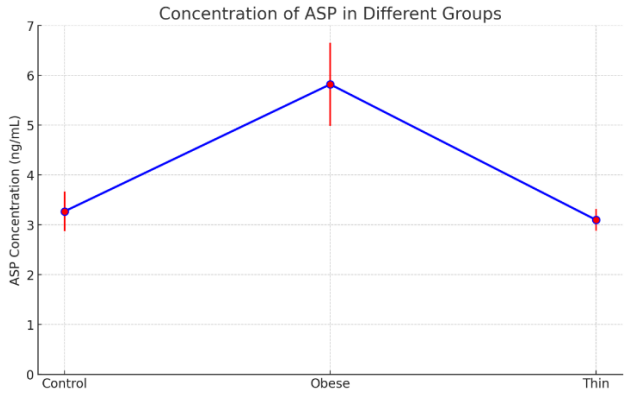

Figure 1: Line Plot of ASP Concentration (ng/mL) in Serum Among Control, Obese, and Thin Groups

Hormonal variables are among the most important physiological indicators of the normal physiological and environmental balance of the body, i.e., the internal stability of the body in terms of energy balance and maintenance of body weight. On this basis, the current study revealed a significant increase (P≤0.01)) in the concentration of Asprosin hormone in the serum of obese women when compared with the control group, as shown in Table (1). Obesity can be attributed to either disease conditions such as hormonal imbalances, genetic causes, excessive food intake (poor diet) without physical exertion like exercise to burn excess calories which lead to weight gain, or due to depression as some cases of depression cause excessive appetite.

The results of our study (8), which indicated that Asprosin increases significantly, which in turn leads to increased appetite and changes in energy balance between the consumed and lost

energies, leading to an increase in body weight and insulin resistance. Asprosin works directly on the neurons in the hypothalamus, which gradually activate, leading to a gradual increase in appetite and food consumption. Neurons are essential for regulating food intake and energy stability.

Similarly, the current study's results agree with (9), which established that the relationship between obesity and Asprosin is direct. Asprosin levels rise in people suffering from obesity. The level of Asprosin fluctuates according to the biological clock rhythm. After overnight fasting, its level rises significantly in humans, mice, and rats then decreases after eating. Injecting recombinant Asprosin also leads to an increase in blood glucose and hyperinsulinemia (10). The study also agrees (11), which was conducted on patients with morbid obesity. The results of Asprosin hormone and immune factors were elevated in them, and when they underwent laparoscopic sleeve gastrectomy to treat morbid obesity, blood Asprosin levels decreased significantly after 6 months from the bariatric surgery. Therefore, Asprosin hormone levels in adipose tissue are a potential risk factor in causing obesity.

As for the concentration of Asprosin hormone in the serum of thin women, as shown in Table (1), no significant difference was observed. The decrease was slight when compared to the control group. This coincides with (12), which explains that a decrease in Asprosin hormone causes functional disturbance in adipose tissue with decreased fat mass.

The reason for thin women might be consuming a small amount of food with increased physical activity or it might be genetic reasons or psychological changes that may affect metabolic food. As Asprosin is primarily secreted by adipocytes from white adipose tissue during hunger, it is a regulator in metabolic balance. We find that thin women do not have a high concentration of Asprosin since adipocytes are lower than normal, unlike obese women where the Asprosin ratio increases due to a higher ratio of adipocytes.

• The concentration of tumor necrosis factor-alpha in serum

The results of the current study, shown in Table (2), indicate a significant increase ($P \leq 0.05$) in the concentration of TNF-α in the serum of obese women (225.13 ± 6.71) ng/L and thin women's serum (155.75 ± 3.04) ng/L when comparing both groups with the control group (148.41 ± 5.18) ng/L.

Table (2) shows the concentration of TNF-α in the blood serum of the studied groups

Variable	Control	Obese	Thin	P-Value @0.05
TNF-α (ng/L) Mean± Std.	148.41±5.18b	225.13±6.71a	155.75±3.04a	0.001

- Different letters indicate a significant difference in the studied groups

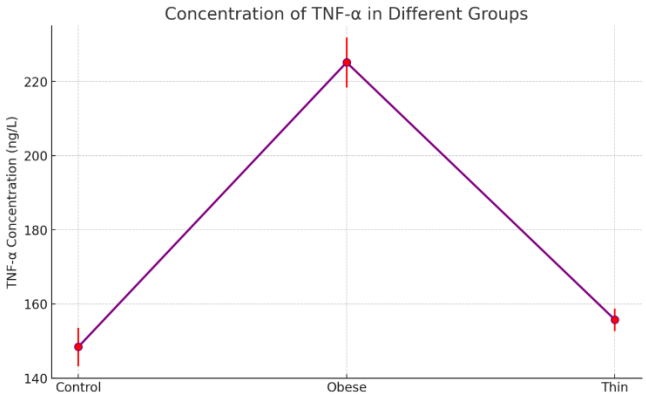

Figure 3: Line Plot of TNF-α Concentration (ng/L) in Serum Among Control, Obese, and Thin Groups

• **Interleukin-6 concentration in blood serum**

The current study results shown in Table (3) indicate a significant increase at (P≤0.05) in the IL-6 concentration in the blood serum of obese women (2.28 ± 3.10) pg/ml when compared to the control group (0.33 ± 2.19) pg/ml. However, no significant difference was observed in the blood serum of thin women (0.92 ± 2.69) pg/ml when compared to the control group.

Table 3 illustrates the concentration of IL-6 in the blood serum of the groups studied.

Variable	Control	Obese	Thin	P-Value @0.05
IL-6 (pg/mL)	2.19 ± 0.33b	3.10 ± 2.28a	2.69 ± 0.92ab	0.035

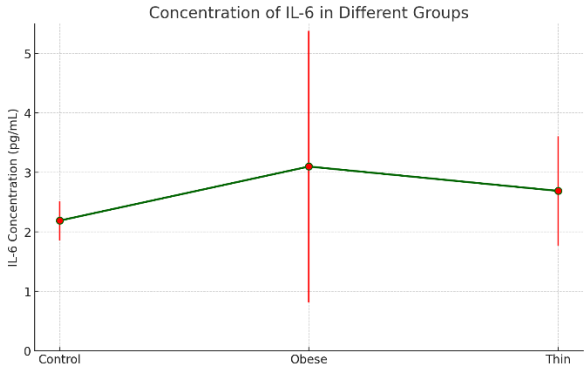

Figure 3: Line Plot of IL-6 Concentration (pg/mL) in Serum Among Control, Obese, and Thin Groups

The current study's results reveal a significant increase in both tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in obese women when compared to the control group. There was also an increase in TNF-α in slim women compared to the control group, but this increase was lesser in obese women. The rise is attributed to the development of inflammation due to an increase in the number of adipose cells or a decrease, which stimulates an increase in the cytokines responsible for the immune response. Both weight gain or loss have an effect on the body's ability to resist inflammations resulting from metabolic imbalance. This study aligns with the research of (13), who demonstrated that an

increase in IL-6 occurs due to chronic inflammatory conditions resulting from metabolic dysfunction and energy production, leading to body weight changes. Such changes may cause diseases including arteriosclerosis, heart diseases, and cardiovascular diseases, indicating a close relationship between metabolic pathways and inflammations.

In a study by (14), adipocyte death was considered very common in individuals suffering from obesity, possibly attributed to the lack of oxygen in fat cells due to the expansion of adipose tissue. This oxygen deficiency might actively contribute to the development of obesity-related inflammation by increasing adipose cell production and enhancing the expression of genes causing inflammation. This, in turn, increases the levels of TNF-α and IL-6. Our current study also agrees with a study by (15), who pointed out that obesity is a significant cause of chronic diseases and is also a condition of low-grade chronic inflammation. Excessive fat accumulation in adipose tissue leads to an increase in the production of several cytokines and chemokines that promote inflammation, including TNF-α and IL-6, which can attract inflammatory cells.

Furthermore, the study results showed that IL-1, TNF-α, and IL-6 enhance lipid breakdown, inhibit fat synthesis, and reduce blood lipid levels. Hence, the levels of IL-1, TNF-α, and IL-6 in blood plasma are significantly increased in patients suffering from obesity or osteoporosis. IL-1, TNF-α, and IL-6 act as regulators of articular cartilage. Additionally, IL-1, TNF-α, and IL-6 bridge lipids and joint cartilage either directly or indirectly ((16)).

The concentration of interleukin-1 (IL-1) in blood serum is depicted in Table 4. The current study results indicate a statistically significant decrease at \(P \leq 0.01 \) in the concentration of IL-1 in the blood serum of obese women (\(168.06 \pm 19.8\) pg/ml) and slim women (\(309.25 \pm 13.47\) pg/ml) when comparing both groups with the control group (\(420.45 \pm 29.47\) pg/ml).

Table 4 illustrates the concentration of IL-1 in the blood serum of the groups studied.

Variable	Control	Obese	Slim	P-Value @0.05
IL-1 (pg/mL)	420.45 ± 29.47a	168.06 ± 19.8b	309.25 ± 13.47ab	0.004

Different letters indicate the presence of a statistically significant difference in the groups examined.

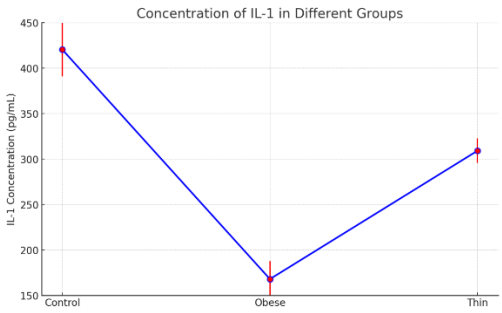

Figure 4: Line Plot of IL-1 Concentration (pg/mL) in Serum Among Control, Obese, and Slim Groups

Our current study reveals a significant decrease in IL-1 concentration in obese women and a significant increase in slim women when compared to the control group. These results align with the study by (17), which showed on experimental animals that IL-1 and other cytokines mediate both acute and chronic disease processes. Various forms of IL-1 beta or IL-1 alpha reduce food intake in experimental animals. In rats fed meals, a single injection of IL-1 leads to a 40% decrease in food intake, while daily injections slow down the natural weight gain. The appetite loss response to IL-1 is prevented by inhibitors of cyclic oxidase enzymes.

Recent studies have highlighted interactions and relationships between metabolism and activation of inflammatory particles. Research indicates that metabolism is a crucial factor in attracting plasma and inflammation, as well as differentiation of muscular fibrosis and fibrosis. Several molecular pathways are involved in metabolic regulation and inflammation, including sugar breakdown, citric acid cycle, amino acid metabolism, and fatty acid metabolism. Most of these are found to be unregulated, providing a mechanistic possibility of participating in inflammation activation and, consequently, the release of IL-1β, thus increasing its blood concentration when metabolism is unregulated (18). Our current study agrees with the study by (19), who pointed out that adipose cytokines, including IL-1β, induce inflammation from the adipose tissue of people suffering from morbid obesity. Therefore, a decrease in IL-1β concentration in adipose tissue reduces the inflammatory response, consequently reducing the risk of various diseases resulting from morbid obesity or severe slimness.

Another reason for the decrease in IL-1 concentration is that the groups mentioned in our current study are not diabetic, and an increase in IL-1 is associated with a high glucose concentration that stimulates IL-1 production in beta cells, leading to decreased insulin secretion, reduced cell proliferation, and programmed cell death. It improves the secretion function of beta cells, blood sugar levels, and reduces systemic inflammation signs, thereby protecting the cells from glucose-induced functional impairment and programmed cell death. This study does not agree with the study by (20), which indicates that people suffering from overweight and obesity have high IL-1 levels compared to people with normal weight, and it is significantly high in metabolic syndrome.(21) also proved in their study the relationship between body mass and depression and cytokines. Plasma IL-1 levels were significantly low and varied between low body mass index vs. overweight and obesity categories of body mass index. IL-1RA levels were significantly higher among women who scored high for depression symptoms. In contrast, groups of slim and obese women with low depression symptom scores also had low IL-1 levels due to the absence of inflammation resulting from stress caused by depression.

REFERENCES

1. Ugur, K., & Aydin, S. (2019). Saliva and blood asprosin hormone concentration associated with obesity. International journal of endocrinology,
2. Abdulazeez, M. I., Hamdi, A. Q., Mohammed, H. Y., & Ahmed, M. (2020). Dental trauma of permanent incisor teeth in children/Kirkuk city. studies, 22, 23.
3. Al-Dori, Khaleda Khalil Abdullah Khader (2020) The role of the hormone hepcidin and copeptin in a number of biochemical and physiological variables in patients with hyperlipidemia in Salah al-Din Governorate. PhD thesis. Tikrit University / College of Education for Science
4. Ali, A. H., Ahmed, H. S., Jawad, A. S., & Mustafa, M. A. (2021). Endorphin: function and mechanism of action. Sci Arch, 2, 9-13.
5. Ali, S. H., Armeet, H. S., Mustafa, M. A., & Ahmed, M. T. (2022, November). Complete blood count for COVID-19 patients based on age and gender. In AIP Conference Proceedings (Vol. 2394, No. 1, p. 020044). AIP Publishing LLC.
6. Al-Rawi, Khasha'a Mahmoud (2000). Introduction to Statistics, second edition, Dar Al-Kutub for Printing and Publishing, University of Mosul
7. Aminian, A., Chang, J., Brethauer, S. A., & Kim, J. J. (2018). ASMBS updated position statement on bariatric surgery in class I obesity (BMI 30–35 kg/m2). *Surgery for Obesity and Related Diseases*, *14*(8), 1071-1087.
8. Barbier, L., Ferhat, M., Salamé, E., Robin, A., Herbelin, A., Gombert, J. M., ... & Barbarin, A. (2019). Interleukin-1 family cytokines: keystones in liver inflammatory diseases. *Frontiers in immunology*, *10*
.
9. Beutler, L. R., & Knight, Z. A. (2018). A spotlight on appetite. *Neuron*, *97*(4), 739-741.
10. Cantay, H., Binnetoglu, K., Gul, H. F., & Bingol, S. A. (2022). Investigation of serum and adipose tissue levels of asprosin in patients with severe obesity undergoing sleeve gastrectomy. *Obesity*, *30*(8), 1639-1646
11. Chooi, Y. C., Ding, C., & Magkos, F. (2019). The epidemiology of obesity. Metabolism, 92, 6-10.
12. Czech, M. P. (2017). Insulin action and resistance in obesity and type 2 diabetes. *Nature medicine*, *23*(7), 804-814.
13. Dinarello, C. A., Endres, S. T. E. F. A. N., Meydani, S. N., Meydani, M. O. H. S. E. N., & Hellerstein, M. K. (1990). Interleukin-1, anorexia, and dietary fatty acids. *Annals of the New York Academy of Sciences*, *587*, 332-338.
14. El-Mikkawy, D. M., EL-Sadek, M. A., EL-Badawy, M. A., & Samaha, D. (2020). Circulating level of interleukin-6 in relation to body mass indices and lipid profile in Egyptian adults with overweight and obesity. *Egyptian Rheumatology and Rehabilitation*, *47*(1), 1-7.
15. Gordon S, Martinez FO (2010): Alternative activation of

macrophages: mechanism and functions. Immunity 32: 593-604.

16. Govindarajan, S., Mustafa, M. A., Kiyosov, S., Duong, N. D., Raju, M. N., & Gola, K. K. (2023). An optimization based feature extraction and machine learning techniques for named entity identification. Optik, 272, 170348.
17. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K et al (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes. 56(4):901–911.
18. Kadham, S. M., Mustafa, M. A., Abbass, N. K., & Karupusamy, S. (2022). IoT and artificial intelligence–based fuzzy-integral N-transform for sustainable groundwater management. Applied Geomatics, 1-8.
19. Karupusamy, S., Mustafa, M. A., Jos, B. M., Dahiya, P., Bhardwaj, R., Kanani, P., & Kumar, A. (2023). Torque control-based induction motor speed control using Anticipating Power Impulse Technique. The International Journal of Advanced Manufacturing Technology, 1-9.
20. Ke, F.; Xue, G.; Jiang, X.; Li, F.; Lai, X.; Zhang, M.; Shen, Y.; Gao, L.(2020) Combination of asprosin and adiponectin as a novel markerfor diagnosing non-alcoholic fatty liver disease. Cytokine, 134, 155184. [CrossRef].
21. Lee, T.; Yun, S.; Jeong, J.H.; Jung, T.W.(2019) Asprosin impairs insulin secretion in response to glucose and viability through TLR4/JNKmediated inflammation. Mol. Cell. Endocrinol., 486, 96–104. [CrossRef] [PubMed].