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Original Article

Comparison of spleen characterization and plasma levels of interleukin 6 and 10 in obese and healthy individuals

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Abstract

Background and aims: Spleen is one of the organs that has great importance in Iranian traditional medicine due to its relationship with obesity. Therefore, in this study, we aim to investigate spleen characterization and plasma levels of interleukin 6 (IL-6) and IL-10 in patients referred to medical centers affiliated with Golestan University of Medical Sciences during 2021-2022.

Methods: This cross-sectional study included 24 subjects with obesity and 24 subjects with normal body mass index (BMI). The length, thickness, and width of the spleen were determined for each subject. Spleen volume was calculated for each subject using the standard prolate ellipsoid formula (length x thickness x width x 0.523). Fasting blood samples were taken from the subjects to check IL- 6 and 10 using the ELISA assay. $P<0.05$ was considered statistically significant.

Results: Our results showed that the two groups had a statistically significant difference in length, width, thickness, and volume of the spleen ($P<0.001$, $P<0.017$, $P<0.002$, and $P<0.001$, respectively). However, there was no significant difference in the elasticity of the spleen ($P=0.58$). Plasma levels of IL-6 were significantly higher in subjects with obesity compared to the control group ($P<0.001$). However, the two groups had no significant difference in IL-10 levels ($P=0.92$).

Conclusion: The increase in spleen dimensions and high plasma levels of IL-6 may reflect the development of obesity.

Keywords: Interleukin 6, Interleukin 10, Obesity, Spleen

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Introduction

The increasing rate of overweight and obesity has made obesity one of the most critical health challenges in the world. Obesity is a risk factor for many other chronic diseases, such as cardiovascular diseases, diabetes, stroke, osteoarthritis, and cancer. It is considered the most important health risk factor in populations of all age groups (1). The most critical factors of obesity are bad eating habits and lack of proper physical activity. Obesity is defined as excessive or abnormal fat accumulation in adipose tissue (2). There are various methods to check and measure obesity, including body mass index (BMI), waist circumference, waist-to-hip ratio, and body fat mass percentage (3).

Meanwhile, measuring BMI is considered the first step in determining the degree of obesity. BMI is an easy and reliable measure of obesity and is related to body fat percentage mass. BMI is obtained by dividing the weight by the square of the height (4).

BMI is a statistical index calculated based on the weight and height of individuals to evaluate obesity. Obesity is defined as BMI greater than or equal to 30 kg/m^2 and it is subdivided into three categories: obesity class I (BMI 30 to 34.9 kg/m^2), obesity class II (BMI 35 to 39.9 kg/m^2), obesity class III (BMI greater than or equal to 40 kg/m^2), also referred to as severe, extreme, or massive obesity. Obesity is one of the problems raised by the World Health Organization (WHO). Different solutions have been proposed to solve it. Numerous research studies and investigations have been done on other treatments and the use of traditional and complementary medicine in obesity (5).

According to the growing tendency towards traditional and complementary medicine around the world, the WHO, with a comprehensive review of the current world situation and its analysis, has developed its strategic policies regarding complementary medicine and traditional medicine in the form of four strategies to make the practice of medicine work better (6). The WHO has developed the Traditional and Complementary Medicine Strategy 2014-2023 (7). Complementary medicine in Iran includes methods of health maintenance, etiology, diagnosis, and treatment of diseases based on individual differences, which relies on the scientific support and

experience of several thousand years of Iranians and other nations and focuses on moral and educational aspects and Islamic teaching. Iranian medicine has a history that dates back 3000 years, before Greek medicine (8). The spleen is of great importance in Traditional Persian Medicine due to its relationship with obesity. The spleen, through the secretion of some appetizers such as soda, can stimulate the appetite (9). Additionally, due to its proximity to organs such as the stomach, diaphragm, and intestines, as well as the connection with organs such as the liver and kidneys, the spleen can affect the functioning of these organs (10). In this regard, and according to recent studies, the size of the spleen in tall and obese males is larger than in females and even in thin and short males (11,12).

Moreover, in patients with metabolic syndrome, the spleen is reported to be larger than in healthy cases. Therefore, the spleen can play an important role in obesity. Interleukins (ILs) are cytokines produced by various immune cells that play essential roles in the immune system (13). Among ILs, IL-6 and IL-10 are more related to obesity. IL-6 stimulates acute phase protein synthesis, hematopoiesis, cell differentiation, and inflammation. IL-10 inhibits the production of Th1 cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor (TNF), and IL-2 (14,15). IL-6 was reported to be associated with both obesity and insulin resistance in diabetic patients, whereas IL-10 was not associated with either obesity or insulin resistance (16). In this study, we aim to investigate spleen characterization and plasma levels of IL-6 and IL-10 in obese and control subjects.

Materials and Methods

Study population

According to the results of a previous study (17), the sample size was determined considering three type errors including an alpha level of 5%, a beta level of 10%, and an effect size (d) of 0.95. Accordingly, the sample size was calculated using the G*Power 3.1.9.2 software, with 25 people in each group. Two participants withdrew from the study, leaving 48 individuals included in the research.

Inclusion and exclusion criteria

In this study, inclusion criteria included age between 18 and 65 years, BMI ≥ 30 for the experimental group and normal BMI for the control group, not having minor thalassemia, liver diseases such as viral hepatitis, acute or chronic liver failure, cholestasis, and liver transplant, weight loss more than 3 kg in the last 6 months, not having a history of taking any slimming drugs in the previous 6 months, not having a history of surgery for weight loss, not having spleen diseases such as splenomegaly, hypersplenism, or not having a spleen for any reason, not having a history of damage to the spleen such as accidents and surgery, not suffering from pancreatic diseases such as pancreatitis, doing sports regularly or being a professional athlete, using certain drugs such as corticosteroids, amiodarone, valproate, prednisone, tamoxifen, perhexiline, and methotrexate, sulfa drugs, chlorothiazide, ursodeoxycholic acid in the last 6 months, pregnancy, breastfeeding, AIDS, malignancy, not following a special diet, and not using particular herbal and chemical medicines. Individuals who failed to follow up during the study, were absent, or did not comply with the inclusion criteria were excluded from the study.

Data collection and measurement

The judgmental sampling method was used in this study. The health of the patients was assessed based on medical history, examination by a researcher and a doctor, tests, and sonography. A checklist (health assessment questionnaire) with confirmed validity and reliability using similar studies was used to record information. If they met the inclusion and exclusion criteria, they were included in the study by obtaining consent. However, they were allowed to withdraw from the study whenever they wished.

Primary data included age, gender, height, and weight (height and weight of patients were measured using standard anthropometric technique). Height was measured in cm, and weight was measured in kg. BMI was calculated as the body weight (kg) divided by the square of height (m^2). The length, thickness, and width of the spleen were measured for each subject. For the accuracy of the result, each parameter was measured three times, and their average was obtained. Spleen volume was calculated for each subject using the standard prolate ellipsoid formula (length x thickness x width x 0.523); this formula is usually used to estimate the volume of irregularly shaped organs. Fasting blood samples were taken from the subjects to measure IL-6 and IL-10. After the blood sample was clotted, blood serum was separated by centrifugation at 1500 rpm and stored in a freezer at -70 °C for measurement.

The plasma levels of IL-6 and IL-10 were measured using commercially available kits from Qmedx, Iran. The details are as follows:

- IL-6
- Accession Number: hIL-696
- Dynamic Range: 8-500 pg/mL
- Sensitivity: 6.1 pg/mL
- Protocol: Sandwich-based and colorimetric
- IL-10
- Accession Number: hIL-10
- Dynamic Range: 16-1000 pg/mL
- Sensitivity: 9.4 pg/mL
- Protocol: Sandwich-based and colorimetric

Statistical analysis

Data were analyzed using SPSS version 19.0. We used the independent samples *t* test to compare the means of two independent groups. Categorical data were compared using Fisher's exact test. One-way analysis of variance (ANOVA) was used to compare the means of different

age groups. A post hoc test (Hochberg GT2) was used to determine the significance of the difference between age groups. Pearson's correlation coefficient was used to measure the strength of correlation between two numerical variables. P value < 0.05 was considered to show a statistically significant difference.

Results

Demographic data and the relationship of the studied variables with the degree of obesity

Twenty-two males and 26 females were included in this study. Twelve males and 12 females had normal BMI, and 10 men and 14 women had BMI ≥ 30. The demographic data of the study population were shown in Table 1. The comparison of the studied variables in the two groups (BMI ≥ 30 and normal BMI) is shown in Table 2. According to the results, the variables of length, width, thickness, and volume of the spleen showed significant differences between the two groups. Moreover, there was a significant difference in the levels of IL-6 between the two groups. However, this difference was not significant in the levels of IL-10.

Comparison of spleen characteristics in the studied groups

Comparing the difference in spleen length between the two groups of study

The mean value of spleen length in the control group was 11.10 ± 0.85 and it was 11.93 ± 0.75 in the group with BMI ≥ 30. The comparison of the two groups showed a statistically significant difference (P < 0.001), and individuals in the obese group had a higher mean value of spleen length than the control group. Figure 1 shows the mean values of the spleen length in the study groups.

Comparing the difference in spleen width between the two groups of study

The mean width of the spleen in the control group was 7.27 ± 0.61 and it was 7.82 ± 0.87 in the case group. The comparison of the two groups showed a statistically significant difference (P < 0.017), and individuals in the obese group had a higher mean value of spleen width than the control group (Figure 2).

Comparing the difference in spleen thickness between the two groups of study

The mean thickness of the spleen in the control group was 4 ± 0.49 and it was 4.64 ± 0.79 in the case group. The comparison of the two groups showed a statistically significant difference (P < 0.002), and individuals in the obese group had a higher mean value of spleen thickness than the control group (Figure 3).

Comparing the difference in spleen elasticity between the two groups of study

The mean value of the spleen elasticity in the control group was 17.02 ± 0.73 and it was 16.83 ± 0.54 in the case group. The comparison of the two groups showed no statistically significant difference (P = 0.58) between them. Figure 4 shows the mean values of spleen elasticity in the study groups.

Comparing the difference in spleen volume between the two groups of study

The mean value of spleen volume in the control group was 170.76 ± 36 and it was 227.64 ± 54 in the case group. The comparison of the two groups showed a statistically significant difference (P < 0.001) between the two groups. Figure 5 shows the mean values of spleen volume in the study groups.

Comparing the plasma levels of IL-6 and IL-10 between the two groups of study

The mean value of IL-6 in the control group was 38.88 ± 7.1, and it was 133.2 ± 59.4 in the case group. IL-6 was significantly different between the two groups (P < 0.001), and individuals in the case group had higher IL-6 levels than the control group. However, there was no significant difference in IL-10 levels between the two groups (P = 0.92) (Figure 6).

Table 1. Demographic data of the study population

Group	Variables	Mean ± standard deviation	*P* value
Normal BMI (n = 24)	Age	32.45 ± 15.53	0.78
BMI ≥ 30 (n = 24)		31.79 ± 21.33	
Normal BMI (n = 24)	Height	167.79 ± 75.64	0.42
BMI ≥ 30 (n = 24)		165.54 ± 83.03	
Normal BMI (n = 24)	Weight	64.33 ± 43.26	< 0.001
BMI ≥ 30 (n = 24)		93.12 ± 54.89	
Normal BMI (n = 24)	Female	12 people	0.45
BMI ≥ 30 (n = 24)		14 people	
Normal BMI (n = 24)	Male	12 people	
BMI ≥ 30 (n = 24)		10 people	

Table 2. Comparison of characteristics of spleen and IL-6 and IL-10

Group	Variables	Mean ± standard deviation	*P* value
BMI normal	Spleen length	11.10 ± 0.85	< 0.001
BMI ≥ 30		11.93 ± 0.75	
BMI normal	Spleen width	7.27 ± 0.61	0.01
BMI ≥ 30		7.81 ± 0.87	
BMI normal	Spleen thickness	4.01 ± 0.48	0.001
BMI ≥ 30		4.63 ± 0.78	
BMI normal	Spleen volume	170.76 ± 36	< 0.001
BMI ≥ 30		227.64 ± 54	
BMI normal	Spleen elasticity	17.02 ± 0.73	0.3
BMI ≥ 30		16.83 ± 0.54	
BMI normal	IL-6 plasma level	38.87 ± 7	< 0.001
BMI ≥ 30		133.2 ± 59	
BMI normal	IL-10 plasma level	4.8 ± 0.44	0.3
BMI ≥ 30		4.79 ± 0.45	

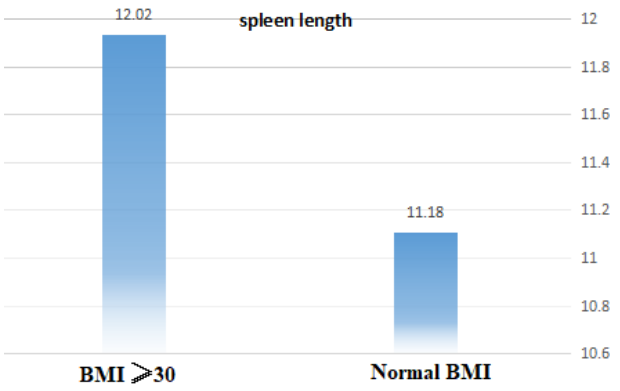

Figure 1. Mean values of spleen length in the study groups

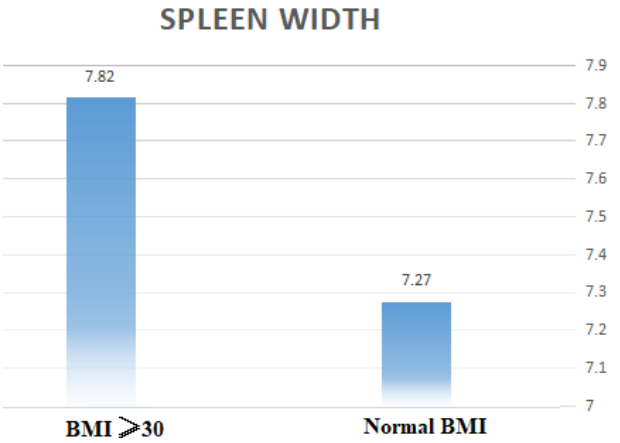

Figure 2. Mean values of spleen width in the study groups

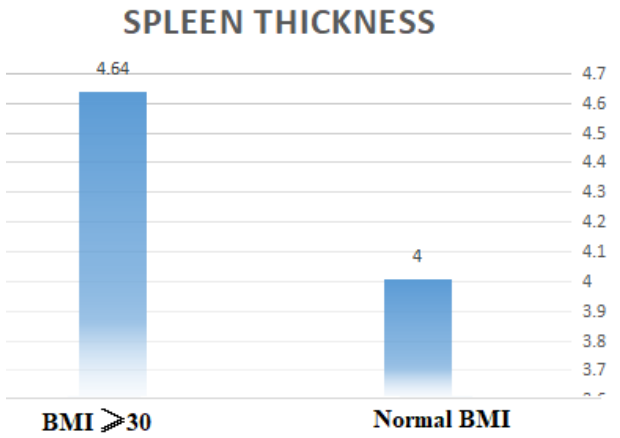

Figure 3. Mean values of spleen thickness in the study groups

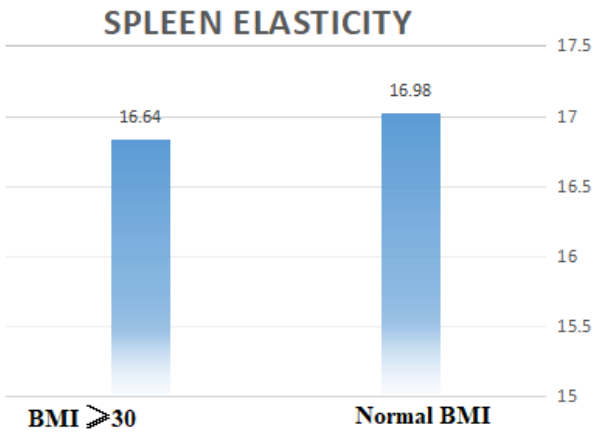

Figure 4. Mean values of spleen elasticity in the study groups

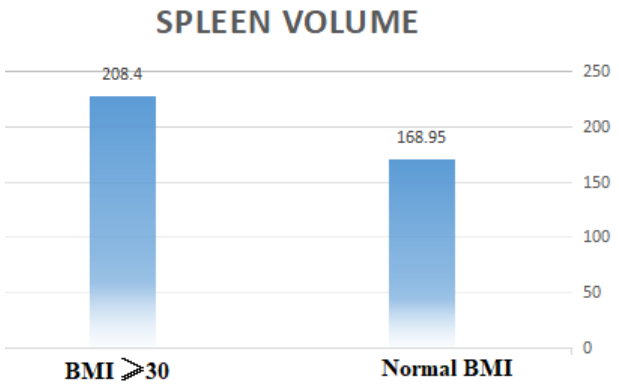

Figure 5. Mean values of spleen volume in the study groups

Discussion

This study investigated the relationship between obesity and spleen length, width, thickness, volume, and elasticity, as well as IL-6 and IL-10 levels. Our results showed that the spleen volume in the study group was significantly different from that in the control group, and the individuals in the BMI ≥ 30 group had larger spleen volume than the control group. Additionally, previous studies have examined spleen volume in different individuals, which aligns with our research. In this regard, Fateh et al reported that spleen volume was associated with height, weight, and BMI, which is consistent with a recent study (18). Moreover, a lower spleen volume was reported by Harris et al in the Japanese population in comparison with the Kurdish population, which might be due to their smaller body size compared to Kurds (19). Mustapha et al also reported a smaller mean spleen volume for African adult population, which can be due to regional and national differences and the mean size of individuals in the same region that affect the spleen volume (20). This finding was also reported by Hosey et al, who concluded that African American athletes had smaller spleens despite being taller and heavier than White American athletes (21).

Another parameter investigated in this study was the elasticity of the spleen. The elasticity of the spleen was not significantly different between the two groups. According to our research, previous studies did not report a significant difference in spleen elasticity between normal and obese individuals (22, 23).

In this study, we delved into the characteristics of the spleen and IL-6 and IL-10 in both healthy and obese individuals. The most striking finding was the significant difference in IL-6 levels between the two groups, with obese individuals showing a notably higher level. This confirms that IL-6 is elevated in obese individuals, an important discovery that underscores the role of adipose tissue as a primary source of cytokines and the pro-inflammatory process. This dynamic endocrine role of adipose tissue, which involves the secretion of various

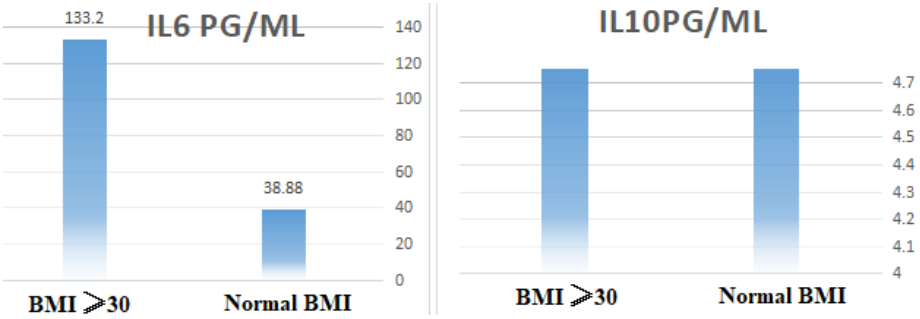

Figure 6. Mean plasma levels of IL-6 and IL-10 in the study groups

factors, including cytokines, could explain the higher IL-6 levels in obese individuals (24). A study by El-Mikkawy et al on overweight and obese Egyptian adults also found significantly higher circulating levels of IL-6 in these subjects (25). However, our study did not find a significant difference in IL-10 plasma levels between the groups. This is consistent with a study conducted by Charles et al, which also found no association between IL-10 and obesity measures or HOMA-IR (16). Other studies have reported a protective effect of IL-10 on obesity, with a reduction in IL-10 levels in obese individuals (26,27). These discrepancies could be attributed to differences in sample size, ethnicity, gender, and the method used to measure IL-10.

It is important to note the limitations of this study. First, due to its cross-sectional nature, we could not establish causality. Second, the small sample size may have limited the generalizability of our findings. These factors should be taken into consideration when interpreting the results.

Conclusion

The increase in BMI was significantly related to the increase in the thickness, length, width, and volume of the spleen, as well as the increase in the amount of IL-6 in the blood of obese individuals compared to non-obese individuals, but there was no significant relationship between the elasticity of the spleen and IL-10 in the two groups of study.

Authors' Contribution

Conceptualization: Mohammad Yousofpour, Amirsaeed Hosseini.
Data curation: Mahdis Tanzifi.
Formal Analysis: Jamshid Yazdani Chrati.
Funding acquisition: Mohammad Yousofpour.
Investigation: Mahdis Tanzifi.
Methodology: Mahdis Tanzifi, Amirsaeed Hosseini.
Project administration: Mohammad Yousofpour.
Resources: Mahdis Tanzifi.
Software: Mahdis Tanzifi, Tahereh Amirian.
Supervision: Mohammad Yousofpour.
Validation: Jamshid Yazdani Chrati.
Visualization: Mahdis Tanzifi.
Writing – original draft: Mohammad Yousofpour, Mahdis Tanzifi, Tahereh Amirian.
Writing – review & editing: All authors.

Competing Interests

None declared.

Ethical Approval

The study process was explained to all the participants before it started. If they were satisfied, an informed consent form was received. During the study, any question asked by the study participants was answered. In addition, the study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. The present study was also approved by the Ethics Committee of Sari University of Medical Sciences (IR.MAZUMS.REC.1401.293).

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