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To study the levels of cytokines in metabolic syndrome and non-metabolic syndrome individuals

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Abstract: Background: Metabolic syndrome is characterized by abnormalities in blood pressure, blood sugar, waist circumference, and cholesterol or triglyceride levels.
Aim: The unique aspect of this study is its primary focus on determining whether there is a distinction in inflammatory markers between individuals with metabolic syndrome and those without the condition.
Material and Methods: The study included 50 patients with metabolic syndrome and 50 healthy controls. The research was conducted at Indore Index Medical College & Research Centre. After obtaining approval, the study's researchers commenced their work. Each subject provided informed consent prior to the study. Individuals with type 1 diabetes or clinical symptoms for fewer than five years and documented T2DM duration were excluded. Healthy controls (non-metabolic syndrome) were individuals without diabetes, multivitamin use, or comorbidities.
Statistical analysis: Regressions were used to determine the relationship between two variables. Additionally, percentages were calculated. A significance level of $p < 0.05$ was considered significant.
Conclusion: The significance of these results cannot be overstated. The study's findings unequivocally demonstrate that both pro-inflammatory and anti-inflammatory cytokines contribute to the development of secondary illnesses associated with metabolic syndrome.

Keywords: Metabolic syndrome; Tumor necrosis factor; Cytokines; Body mass index; Inflammation.

1. Introduction

Metabolic syndrome is characterized by abnormalities in blood pressure, blood sugar, waist circumference, and cholesterol or triglyceride levels. Cytokines, produced by immune system cells, act as messengers to regulate inflammation and immune response [1–5].

Studies have demonstrated the contribution of cytokines to metabolic syndrome. Inflammatory cytokines such as Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α) have been associated with metabolic syndrome and insulin resistance, a hallmark of the condition [6–8].

Adipose tissue, or fat cells, can also produce inflammatory cytokines like IL-6 and TNF-α, leading to systemic low-grade inflammation [7]. This inflammation can exacerbate insulin resistance and increase the risk of developing metabolic syndrome. Consequently, targeting cytokines and reducing inflammation may prove to be a valuable therapeutic approach for managing metabolic syndrome [8–10]. Treatment options may include medications that target cytokines, as well as lifestyle modifications such as exercise and a healthy diet. Successful identification of risk factors for metabolic syndrome, including inflammatory markers, can aid in the development of early detection and prevention strategies for the disease [11–15]. This study focuses specifically on investigating potential distinctions in inflammatory markers between individuals with and without metabolic syndrome.

2. Material and methods

Two hundred people participated in the current study, 100 in the metabolic syndrome group and 100 in the control group of healthy subjects. The participants at the Index Medical College & Research Centre in Indore were the focus of this study. The researchers behind the study got to work after getting the go-light from the appropriate authorities. Each subject gave their informed consent before the present study began. Patients with type 1 diabetes or with less than five years of pathological symptoms and proven T2DM duration were not included. People who were considered healthy controls did not have diabetes, did not use multivitamins, and did not suffer from any comorbid conditions.

All participants in both groups were examined by a qualified physician from the hospital's medical department, who followed standard procedures and accounted for the study's exclusion and inclusion criteria. The health control group consisted of 100 participants of the same age and gender who did not have metabolic syndrome. Patients being treated for metabolic syndrome numbered 100 in the second group. Metabolic syndrome was identified using criteria established by the ATP-III. Normal glycemic state human volunteers of similar age and sex served as the control group. Each person was examined by a licensed medical professional who followed established medical procedures. Metabolic syndrome was identified using criteria established by the ATP-III. The BMI (Body Mass Index) of each individual was determined by dividing their weight in kilos by their height in square meters. Subjects were divided into groups once their body mass index had been recorded. Within each cohort, subjects were once again categorized into three categories according to the World Health Organization's diagnostic standards for obesity in BMI for Asian populations: obese (30kg/m2), overweight (25-29.9kg/m2), and normal weight (18.5-24.9kg/m2). Each person in both groups had 5ml of their fasting venous blood extracted into flat vials using a disposable syringe and needle in a sterile environment. Following centrifugation at 3000rpm for 20 minutes to separate the serum from the blood, samples were aliquoted and kept at 20°C.

A multi-analyte Elisarray kit from Qiagen laboratories was used to quantify cytokines in the serum. The capture antibodies are more able to bind to their target protein after being incubated. After the elimination of free protein, the collected analyte can be bound by biotinylated detection antibodies that have been added to the wells. After a final wash, an avidin-horseradish peroxidase conjugate is used to get rid of any remaining free particles. After another wash, a colorimetric substrate solution is added to the wells, which turns the sample a shade of blue that is directly proportionate to the protein analyte concentration in the original sample. After adding a stop solution, you may measure the absorbance of your samples at 450 nm and make meaningful comparisons between them. There was a 4.9% difference between replicates and a 6.3% difference between assays. A sensitivity of 0.5 pg/mL was established.

2.1. Statistical Analysis

Statistical analysis was performed using IBM SPSS version 20. A two-sample independent t-test was used to compare the means of variables between the two groups. Additionally, percentages were calculated. A significance level of $p < 0.05$ was considered statistically significant. Regression analysis was conducted to determine the relationship between two variables.

3. Results

Table 1 displays the increased mean levels of IL-1, IL-5, IL-10, IL-13, and TNF-α for both groups included in the study. Subjects with metabolic syndrome had higher levels of TNF-α and IL-1, while healthy controls had lower levels. Significant differences in TNF-α and IL-1 serum levels between the two groups were observed. Healthy controls also exhibited higher blood levels of weight, IL-2, and IL-15 compared to individuals with metabolic syndrome. These differences in parameters between patients with metabolic syndrome and healthy controls were unexpectedly large.

Table 2 demonstrates that, on average, individuals with metabolic syndrome had decreased cytokine levels compared to healthy controls. The study revealed statistically significant differences in IL-2, IL-4, and IL-15 blood levels between the metabolic syndrome group and the control group. However, no significant differences were found in IFN-γ and IL-15 levels between the two groups.

We explored the correlation between weight and IL-2 in the blood of healthy controls. We observed a consistent positive relationship ($y = 0.182x + 57.5$) between body mass index and IL-2 concentrations in

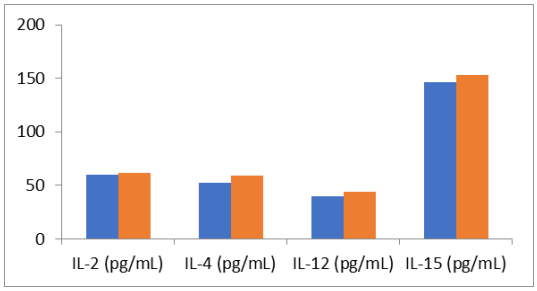

Figure 1. Reductions in cytokine levels among the study population are depicted as bars in the figure below

healthy subjects. Patients with metabolic syndrome showed a negative correlation between blood weight and IL-15 levels ($y = -0.027x + 4.9$). Additionally, patients with metabolic syndrome exhibited a negative relationship ($y = -0.062x + 9.315$) between body mass index and tumor necrosis factor-α.

Table 1. Those with metabolic syndrome had lower mean values of many cytokines compared to the study's control group.

Variable	Metabolic syndrome group (n=100)	Control group (n=100)	P Value
IFN-γ (pg/mL)	60.8 ± 10.6	63.2 ± 10.5	>0.05
IL-2 (pg/mL)	52.9 ± 9.2	59.7 ± 15.5	<0.05
IL-4 (pg/mL)	41.2 ± 11.9	45.4 ± 15.5	<0.05
IL-12 (pg/mL)	147.9 ± 44.9	154.4 ± 13.0	>0.05
IL-15 (pg/mL)	4.6 ± 1.2	7.6 ± 1.8	<0.05

Table 2. Those with metabolic syndrome had higher mean values of many cytokines compared to the study's control group.

Variable	Metabolic syndrome group (n=100)	Control group (n=100)	P Value
IL-1β (pg/mL)	21.6 ± 5	12.6 ± 10.6	<0.0001
IL-5 (pg/mL)	16.9 ± 1.1	13.4 ± 0.6	<0.05
IL-10 (pg/mL)	24.2 ± 2.4	23.1 ± 8.7	>0.05
IL-13 (pg/mL)	79.9 ± 41.9	62.9 ±10.8	<0.001
TNF-α (pg/mL)	7.3 ± 3.2	4.7 ± 1.2	<0.05

4. Discussion

Significant differences were observed in the blood concentrations of IL-4, IL-5, and IL-13 between the control and metabolic syndrome groups. Individuals with metabolic syndrome exhibited distinct serum concentrations of these cytokines compared to healthy controls. The study documented and published this finding in an academic journal [1]. Another study by the same researchers and collaborators reported significantly reduced IL-4 expression in individuals with metabolic syndrome [2]. Conversely, a different study found higher levels of IL-4 gene expression in metabolic syndrome patients compared to healthy controls [3]. However, a study [4] found no correlation between IL-4 presence and the occurrence of metabolic syndrome. The collective evidence supports the conclusion that reduced IL-4 production is associated with metabolic syndrome, particularly when compared to healthy controls with normal IL-4 production levels.

On the other hand, patients with metabolic syndrome exhibited higher levels of IL-5 and IL-13 compared to healthy controls. These values were unexpectedly elevated, as shown in Table 2. Due to limited research on IL-5 and metabolic syndrome, our conclusions are drawn from previously conducted studies.

Furthermore, the study compared IL-2, IL-15, IL-1, TNF-α, and IFN- serum levels between the two study groups. Statistically significant differences were observed in TNF-α, IL-2, IL-15, and IL-1 levels in the blood of individuals with metabolic syndrome compared to healthy individuals. Notably, a stable and positive

correlation between weight and IL-2 levels was found in healthy controls, suggesting an increasing association over time. IL-2, an anti-inflammatory cytokine that regulates white blood cells, specifically T-lymphocytes, has been investigated in the context of metabolic syndrome [5–9]. A recent study on IL-2 function revealed significantly lower levels of IL-2 in individuals with newly diagnosed metabolic syndrome compared to the control group of non-diabetic volunteers [10]. Another study reported higher IL-2 concentrations in metabolic syndrome patients compared to the control group, which was published in Diabetes Care [11–16]. These findings significantly differ from individuals without metabolic syndrome. The study specifically included participants with metabolic syndrome for less than five years, considering the average duration of the condition. The study identified decreased body mass index as one of the primary contributors to lower IL-2 levels in the metabolic syndrome group, as observed through the analysis of weight and IL-2 concentrations. The higher levels of weight and IL-2 in the control group, along with the statistically significant differences between the control group and metabolic syndrome patients, can be attributed to the control group's normoglycemia. The comparison was made between individuals with metabolic syndrome and those without it, considering the established correlation between increased weight and decreased metabolic syndrome incidence. Overall, the study findings indicate lower levels of anti-inflammatory cytokines in individuals with metabolic syndrome [11–13].

There was an inverse relationship between weight and IL-15 levels in individuals with metabolic conditions, as well as a similar relationship between TNF-α and weight levels. This finding, which was our third discovery during the investigation, can be attributed to the fact that individuals with metabolic syndrome tend to have lower body weight compared to healthy controls. The increase in IL-15 and TNF-α production in individuals with metabolic syndrome is therefore significant and can lead to complications due to their underweight condition [16]. The association observed in individuals with metabolic syndrome is subject to various alternative interpretations due to the complexity of pathogenesis. In a recent study, Manohar M. found that IL-15, a widely distributed cytokine present in various organs including the heart, liver, and kidneys, plays a crucial role in the pathophysiology of the inflammatory response [17]. The involvement of IL-15 in the onset and progression of inflammation associated with cardiovascular disorders has been convincingly demonstrated [17]. Elevated levels of IL-15 have been observed in human and animal atherosclerotic lesions according to multiple investigations [11–14,16]. Diabetes and hyperglycemia are also considered risk factors for the development of atherosclerotic lesions [11,16].

Several studies [18–20] have shown a link between metabolic syndrome, obesity, nephropathy, and elevated TNF-α levels, corroborating the findings of our investigation [15,16]. Peripheral neuropathy patients were found to have significantly higher serum TNF-α levels, and this discovery was associated with reported nerve conduction velocity in metabolic syndrome [18–20]. Interestingly, our research revealed no significant correlation between weight and TNF-α in the healthy control group, which is an intriguing finding. However, a significant association between these variables was observed in the metabolic syndrome group. The pro-inflammatory cytokines TNF-α and IL-15 play a significant role in the development of secondary conditions such as cardiovascular disorders and atherosclerotic lesions, contributing to systemic inflammation [18–20]. The elevated levels of IL-15 and TNF-α observed in our study participants with metabolic syndrome further support this interpretation. Therefore, metabolic syndrome, characterized by progressive and increasing inflammation, is associated with the secretion of cytokines such as IL-15 and TNF-α [18–20].

5. Conclusion

The results of the current study establish a link between metabolic syndrome and the inflammatory cytokines IL-4, IL-5, and IL-13, emphasizing their significance in the context of metabolic syndrome. Both pro-inflammatory and anti-inflammatory cytokines play a role in the development of secondary conditions associated with metabolic syndrome. Moreover, these analyzed cytokines may serve as biomarkers for early detection and diagnosis of secondary complications in individuals with metabolic syndrome.

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