The Role of Serum CC-Chemokine Ligand 2 (CCL2) in Obesity: A Comparative Study with Normal Subjects

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Abstract. Excess fat in the visceral area in the form of obesity is associated with metabolic syndrome. Various factors have been identified that contribute to the pathophysiology of obesity-related metabolic diseases, including chronic inflammatory factors and immune system activation. One of the components of the immune and inflammatory system is CC-chemokine ligand 2 (CCL2), which is secreted by adipose cells. This study aimed to analyze the role of CCL2 by comparing the serum CCL2 levels between obese and non-obese subjects at Warmadewa University. Employing a cross-sectional design, sixty participants were selected through consecutive sampling and assessed for body weight, height, and serum CCL2 levels using the ELISA method. Data were analysed using the independent t-test. The p-value <0.05 was significant. This study indicated that serum CCL2 levels were significantly lower in obese individuals, measuring 111.57 ± 33.51 pg/mL, compared to 133.44 ± 44.91 pg/mL in individuals with normal weight. This difference was statistically significant, with a p-value of 0.0370. CCL2 levels in normal subjects were found to be higher than in obese subjects, suggesting a possible alteration in chemokine regulation associated with obesity. Further studies are needed to explore the underlying mechanisms.
Keywords: obesity, CCL2, serum

1 Introduction

Excess fat in the visceral area in the form of obesity is associated with a metabolic syndrome consisting of insulin resistance, hyperglycemia, dyslipidemia and hypertension. These metabolic disorders increase the risk of developing type 2 diabetes mellitus and cardiovascular disease which in turn increases morbidity and mortality [1]. The progression from obesity-related insulin resistance to type 2 diabetes mellitus is still unclear. Still, it is known that there is an effect of pancreatic beta cells failure to compensate for insulin resistance leading to chronic hyperglycemia. Low-grade chronic inflammation and immune system activation have been observed in abdominal obesity and play a role in the pathogenesis of obesity-related metabolic diseases [2]. The mechanism underlying the regulation of cytokines in adipose cells is unclear. Current research is exploring how cytokines and chemokines contribute to metabolic diseases and their associated

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complications. During inflammation in adipose tissue, there is an upregulation of several cytokines and chemokines, including TNF-α, TGF-β1, interleukins 1, 6, and 18, tissue factor (TF), adhesion molecules, Smads, monocyte chemoattractant protein-1 (MCP-1), and adipokines [3].

Adipocytes, also known as fat cells, can secrete one of the chemokines, specifically CC-chemokine ligand 2 (CCL2), also referred to as MCP-1, which functions to attract monocytes. Several studies have demonstrated that CCL2 expression is elevated in adipose tissue from both animals and humans. [4, 5]. It was also found that plasma MCP-1 levels were higher in obese than non-obese mice. [5]. In human preadipocytes and mature adipocytes, it was found that the expression of MCP-1 genes and protein was higher in visceral fat tissue than in subcutaneous [6]. However, the correlation between MCP-1 in both circulating serum and plasma and its secretion in adipose tissue still yields different results. [7].

The urgency of this study is to obtain baseline data on serum CCL2, which will complement data collection on inflammatory cytokines and chemokines that play a role in obesity and diabetes mellitus. This will form the basis of serum CCL2 as a marker and predictor of the obesity continuum to metabolic syndrome, which can then develop into diabetes mellitus. Therefore, the specific purpose of this study was to compare serum CCL2 levels between obese and normal subjects in the Warmadewa University environment.

2 Method

This study employed a cross-sectional analytical design to compare obese and normal subjects within the Warmadewa University environment. Data on obese and normal subjects were obtained from registers at the Warmadewa Clinic. The inclusion criteria were healthy subjects, without signs of systemic infection and inflammation, and willing to participate in the study. Samples were selected by consecutive sampling until the targeted number was reached. The total sample consisted of 60 participants, divided into 30 obese and 30 normal individuals. Obesity is determined by calculating the BMI, with a BMI greater than 25 being considered obese and a BMI of 18.5-22.9 being considered normal according to the Asia-Pacific Classification. The Institutional Review Board of Udayana University had granted ethical clearance, as indicated by number 2469/UN14.2.2.VII.14/LP/2019.

The blood of the subjects enrolled in the study was drawn, then centrifuged to obtain the serum, which was later checked for CCL2 levels. The serum collected will be stored in the refrigerator at a temperature of -80 °C before performing the ELISA.

Serum CCL2 level was assessed using the ELISA technique with the CCL2/MCP-1 kit from R&D Systems (Cedarlane, Oakville, Ontario). The procedure begins with preparing the reagent, and then progressed to reading the result at a wavelength of 500 nm. All standards, controls, and samples were duplicated.

Following data collection, the results were organized into tables and analyzed using both descriptive and analytical methods. The independent sample t-test was used if the data were normally distributed, and the Mann-Whitney test was used if the data were not normally distributed. The effect size was assessed using Cohen's d test if an independent-sample t-test was selected as the statistical analysis. The p-value <0.05 was significant.

3 Results and Discussion

A total of 60 participants were enrolled by the end of the study period, comprising 30 individuals with obesity and 30 without. As shown in Table 1, the sample characteristics

include both male and female participants with nearly equal distribution between the sexes. There were no significant differences in blood pressure and random blood glucose levels between the two groups.

Table 1. Characteristics of the subjects

	Obese (n=30)	**Non-obese (n=30)**	**p**
Age (year)	30.43±10.1	32.77±15.05	0.454
Gender			
Male	16 (53.3%)	13 (43.3%)	0.438
Female	14 (46.7%)	17 (56.7%)	
Systolic blood pressure (mmHg)	118.33±10.85	113.67±8.09	0.064
Diastolic blood pressure (mmHg)	74.67±7.76	74.00±7.70	0.740
BMI (kg/m^2)	30.45±2.79	22.76±2.22	0.000
Random Blood Glucose (mg/dL)	106.87±21.55	108.40±14.94	0.750

Obesity is the result of an increase in the number and size of adipose tissue cells, commonly referred to as adipocytes. CCL2 signaling affects the formation and development of obesity [8]. CCL2 can induce adipogenesis via MCP-1-induced protein (MCPIP) in cultured cells without the aid of PPAR gamma activation and stimulate adipocyte remodeling and expansion through angiogenic effects on endothelial cells[9].

Obese people tend to be at risk for degenerative diseases and metabolic syndromes such as diabetes mellitus, hypertension, gout, cardiovascular disease, and cancer. More than 70% of patient with type 2 diabetes mellitus are obese. In general, people with diabetes have abnormal levels of fat in their blood. According to research results, at the age of 20 - 39 years, obese people have a two times greater risk of developing hypertension than those who posseses a normal weight. The results of the study also stated that around 12% an increased risk of coronary heart disease when obese 20 subjects. The findings support the results of this study, which found that obese subjects had higher systolic blood pressure than normal subjects, although not significantly. [10].

The serum CCL2 levels obtained from the ELISA study were also analyzed using a t-test, which revealed that the mean serum CCL2 levels in obese subjects were significantly lower than those in normal subjects ($p < 0.05$).

Table 2. Comparative analysis of serum CCL2 levels in obese and normal subjects

	Obese (n=30)	**Normal (n= 30)**	**p**
Level of CCL2 in serum (pg/ml)	111.57±33.51	133.44±44.91	0.037*

*P<0.05: signifikan

The findings revealed that serum CCL2 levels were significantly elevated in individuals with normal weight compared to those who were obese. These results contrast with earlier studies, which reported higher serum CCL2 levels in obese patients with diabetes mellitus compared to normal-weight individuals [11, 12]. CCL2 can also be found in fat cells (adipocytes) after being induced through oxidative stress, cytokines or growth factors. These chemokines can then stimulate insulin resistance in adipocytes and through the recruitment of macrophages, oxidative stress and inflammatory processes [5]. In addition to its role in the macrophage chemotaxis process, CCL2 also plays a role in angiogenesis, inhibiting cell expression of metalloproteinases, and activating the transcription factor MCPIP. This transcription factor can trigger cell death by inducing oxidative stress and endoplasmic reticulum stress [8].

Obesity is often associated with elevated CCL2 (MCP-1) expression within tissues, particularly in adipose tissue; however, this rise is not consistently observed in serum levels. In some cases, individuals with obesity who are metabolically healthy may even have lower circulating CCL2 levels than those with normal BMI. This paradoxical finding reflects the complexity of CCL2's involvement in obesity and metabolic conditions. [13]. A study identified a direct relationship between BMI and various chemokine levels, including CCL2, where individuals with lower or normal BMI exhibited greater plasma CCL2 concentrations than those with elevated body weight. [14].

Obesity often comes with increased inflammation in the body, and one key player in this process is a molecule called CCL2 (also known as MCP-1). CCL2 helps draw immune cells—especially macrophages—into fat tissue. When these immune cells accumulate, they induce the type of long-term, low-level inflammation commonly observed in individuals with obesity. That's why individuals with obesity usually show higher levels of CCL2 activity in their fat tissue [4]. However, this increase in tissue CCL2 doesn't always translate to elevated serum CCL2 levels. In some obese individuals, particularly those who are considered "metabolically healthy" (meaning they don't exhibit the typical metabolic complications associated with obesity), serum CCL2 levels may be lower than in lean, healthy individuals. [13]

The lower serum CCL2 levels observed in metabolically healthy obese (MHO) individuals are not yet fully understood, but several possible explanations have been proposed. One possibility is that MHO individuals may experience a milder inflammatory response in their adipose tissue compared to metabolically unhealthy obese (MUO) individuals, resulting in reduced CCL2 being released into the bloodstream[13]. Another theory suggests that CCL2 may be retained or used locally within the fat tissue itself, rather than entering circulation[4]. Additionally, genetic predispositions or environmental influences may play a role in regulating the production and release of CCL2 in these individuals [11]. These factors together may help explain why some obese individuals maintain lower systemic levels of inflammation-related markers like CCL2.The elevated levels of CCL2 observed in this study may provide a basis for considering CCL2 as a potential predictor in metabolically healthy obese individuals.

4 Conclusion

The lower serum CCL2 levels in obese subjects suggest that systemic inflammation does not always mirror adipose tissue inflammation, indicating a more complex regulation of inflammatory markers in obesity. The elevated levels of CCL2 observed in this study may provide a basis for considering CCL2 as a potential predictor in metabolically healthy obese individuals. Further research is needed to fully understand the complex interplay between CCL2, obesity, and metabolic health.

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