RESEARCH
www.commprac.com
ISSN 1462 2815

ANALYSIS OF ADVANCED GLYCATION END PRODUCTS (AGES) AND BODY MASS INDEX WITH LEPTIN SERUM IN TYPE 2 DIABETES MELLITUS

Rahmah Apriyani Rasyid [1]*, Liong Boy Kurniawan [2], Yuyun Widaningsih [3], Burhanuddin Bahar [4], Fabiola MS Adam [5], Nurahmi [6] and Darwati Muhadi [7]

[1] Magister of Biomedical Sciences, Graduate School, Hasanuddin University, Makassar, South Sulawesi, Indonesia. *Corresponding Author Email: rahmahapriyani@gmail.com)
[2,3,6] Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.
[4] Department of Public Health, Hasanuddin University, Makassar, South Sulawesi, Indonesia.
[5] Subdivision of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.
[7] Clinical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital, Makassar, South Sulawesi, Indonesia.

DOI: 10.5281/zenodo.12705480

Abstract

Type 2 diabetes mellitus (T2DM) is a chronic health problem characterized by chronic hyperglycemia, insulin resistance, and is often followed by oxidative-carbonyl stress, hyperlipidemia, resulting in the accumulation of AGEs. This accumulation is a major pathogenic mechanism that induce end-organ damage. In T2DM patients, leptin induces insulin resistance, obesity, and inflammation. A high Body Mass Index (BMI) impacts adipocytokines, increasing the risk of insulin resistance. Hence, this research aims to evaluate the relationship of AGEs and BMI with serum leptin in T2DM subjects. Observational analytic with a cross-sectional design was employed using 69 respondents (26 males and 43 females). AGEs and leptin levels were examined utilizing the sandwich Enzyme-Linked Immunosorbent Assay, while the BMI was measured following WHO guidelines. An insignificant negative correlation was found between AGEs and leptin in subjects with T2DM ($r = -0.102$, $p = 0.404$), and no significant association was shown in either male ($r = -0.323$, $p = 0.107$) or female groups ($r = 0.016$, $p = 0.920$). However, BMI and serum leptin showed a significant correlation ($r=0.492$, $p=<0.001$). Similarly, a significant association was observed between BMI and leptin in both gender groups ($r=0.512$, $p=0.007$ and $r=0.533$, $p=<0.001$). In T2DM subjects, the negative relationship between AGEs and leptin is not significant, but there is significant positive correlation between BMI and leptin. Increased leptin concentration is a plausible risk factor for T2DM individuals with a high BMI.

Keywords: Type 2 Diabetes Mellitus, Ages, BMI, and Serum Leptin.

INTRODUCTION

Diabetes mellitus (DM) was reported to have become an epidemic which increased in the 21st century [1]. The rising occurrence of type 2 DM leads to microvascular and macrovascular complications and serves as a precursor to morbidity and mortality in T2DM patients [2]. Management of type 2 DM requires proper monitoring of blood glucose control, particularly through the measurement of HbA1c to observe the progression of DM in reducing the risk of complications [3]. The major contributing factor in type 2 DM complication is insulin resistance which Lead to hyperglycemia. Uncontrolled hyperglycemia induces the formation of advanced glycation end products (AGEs) and oxidative stress [4]. AGEs formation occurs due to nonenzymatic glycation reactions that are accelerated by particular factor such as hyperlipidemia, hyperglycemia, and oxidative stress. This process leads to AGEs accumulation in tissues and plasma, which, accompanied with RAGE or Receptor for Advanced Glycation End Product, causes various signaling events [5]. Excessive accumulation

of AGEs can impair tissue function by disrupting the activity of macromolecules and glycated enzymes; additionally, by binding the surface receptors for AGEs (RAGE) on leukocytes, this can generate the activation of stress transcription factors, by producing proinflammatory and inflammatory mediators including cytokines and acute-phase proteins [4]; [6]. Subclinical inflammation, which is an pivotal risk factor in the growth of T2DM and associated complications, may be exacerbated by AGE accumulation through activation of inflammatory pathways [6]. The consequences of increased level of AGEs are observed in chronic degenerative diseases such as DM, cardiovascular, neurological disorders, several types of cancer and all pathologies involving oxidative stress mechanisms and the aging process [4]. This implies that AGEs potential in glucose metabolism is the key contributor in the development of T2DM. The resistance of insulin is connected to higher levels of circulating insulin, which may stimulate leptin secretion from adipocytes [7].

Leptin may interact with the glucose regulatory system in the body. Previous study stated that leptin may play a part in regulating hepatic glucose production as well as insulin sensitivity in peripheral tissues like muscle and fat. Leptin may contribute to regulating the balance between gluconeogenesis and glycolysis [8]; [9]. Increased levels of leptin have been correlated to the resistance of insulin and T2DM development. In cases concerning T2DM, studies exhibited correlations between elevated concentration of leptin and high risk of cardiovascular disease, as well as the incidents of cardiac autonomic dysfunction and microvascular complications [10]. When insulin resistance occurs, body cells, including adipose (fat) cells, become less responsive to insulin signals. Consequently, adipose cells tend to increase the production of leptin, leading to an increase of leptin in the blood. This condition may result in a high level of leptin, but since the body is already resistant to the effect of leptin, the response becomes ineffective. Furthermore, leptin has been proven to restrain the expression of insulin precursor genes, ultimately affecting insulin secretion. Schmidt et al 2006 revealed that the rise in leptin levels is related to a higher risk of diabetes due to its role in controlling the sensitivity and secretion of insulin [11]. Chronic elevation of leptin levels can interfere with the responsiveness of the cells receptor system that produce insulin, subsequently lead to insulin resistance [12]. The incompetence to regulate the release of insulin is attributable to a decrease in cellular response. Hyperinsulinemia possesses the potential to exacerbate obesity by increasing the expression of leptin and genes in white fat tissue (the primary source of leptin production). Obesity which reflects a high Body Mass Index (BMI) affects adipocytokines, increasing the risk of insulin resistance in T2DM; this can result from excessive leptin production, consequently increasing the risk of undergoing T2DM [13]. Moreover, obesity, hypertension, metabolic syndrome (MetS), and endothelial dysfunction are frequently observed more often in those experiencing T2DM who exhibit high levels of leptin [14].

Previous studies presented that AGEs and leptin each contribute to T2DM complications. Sabbatinelli et al 2022, stated that a high level of AGEs was linked to an increased risk of cardiovascular complications [15], while Bidulescu et al 2020 stated that insulin resistance intervened the relationship between leptin and the occurrence of T2DM [16]. Furthermore, a study conducted by Kalousová et al 2003, exhibited a significant correlation between AGEs and leptin, as well as the ratio of leptin/body fat in hemodialysis patients with DM [17]. These studies, however, are mainly focused on the individual role of AGEs or leptin, without exploring the potential

relationship between these two variables. Furthermore, the research examining the relationship between these two variables was only conducted on DM subjects with hemodialysis.

So far, there is a limited research that comprehensively study the association between AGEs and BMI with the levels of serum leptin in individuals diagnosed with type 2 DM. Considering the importance of these variables in the pathophysiology of type 2 DM, studies that integrate the analysis of these variables are crucial to deepen the understanding of the mechanisms causing the progression of T2DM. Therefore, the current study aims to fill that gap by exploring the correlation between AGEs and BMI with the level of serum leptin in research subjects suffering from T2DM. We hope that the results of this study will offer significant new insights into the interaction between AGEs and BMI with leptin in the context of type 2 DM and may assist the development of more effective treatment plan.

METHODS

Research Plan and Population

This research was carried out by employing the observational analytic method and a cross-sectional study design. All T2DM patients who visited the Central General Hospital (RSUP) Dr. Wahidin Sudirohusodo, Makassar, for treatment were determined as the research population. The inclusion criteria were men and women with type 2 DM aged >18 years who received information related to this research and were willing to take part by filling out and signing an informed consent form. Meanwhile, the exclusion criteria included pregnant women, patients taking statins, patients with acute infection or malignancy, and those with cardiovascular disease, osteoporosis, diabetic nephropathy, liver cirrhosis, and autoimmunity. A total of 69 samples were involved, comprising 26 males and 43 females. This study was conducted at the laboratory of Hasanuddin University Medical Research Center (HUM-RS) after the Health Research Ethics Commission (KEPK) of Hasanuddin University Hospital (RSPTN UH) granted the ethical approval number 910/UN4.6.4.5.31/PP36/2023.

Laboratory Measurements

This current study was conducted by recording the patient's identity. Prior to blood collection, patients underwent a fasting period and an anthropometric examination. Examination of BMI was performed by dividing body weight (kg) by height (cm^2). Whole blood samples were collected on red cap blood tubes without anticoagulant to examine the levels of serum AGEs and serum leptin. The blood samples were then left to stand in a vacuum tube for 15-30 minutes to let it clot and then they were centrifuged at 3000 rpm for 10 minutes. After that, the serum that had been separated from the blood cells was transferred to sample cups using a disposable pipette, with a volume of 250 µl per cup. They were then stored in a calibrated freezer at -20°C to keep the samples stable. To examine the level of Serum AGEs and serum leptin, the Enzyme-Linked Immunosorbent Assay (ELISA) kit MyBioSource on a Thermo ELISA Reader instrument was utilized.

Data Analysis

To analyze the obtained data, SPSS software version 22 was employed. All variables, including age, gender, BMI, and laboratory examination results, were examined. Two types of tests, Kolmogorov-Smirnov and Shapiro-Wilk, were performed to examine the

normality of distribution of data using a significant value (α=0.05). Additionally, the Spearman correlation test was performed in this study.

RESULTS

Based on the analysis, there were 69 patients suffering from T2DM, consisting of 26 males and 43 females. They had average age of 57.49 ± 11.03 years, average BMI of 25.047 ± 4.14 kg/m2, average GDP of 146.99 ± 45.93 mg/dL, average AGEs levels of 22.27 ± 6.00 ng/mL, and average Leptin levels of 8.30 ± 7.83ng/mL (Table 1).

Table 1: Variable Description of Age, AGEs Levels, and Leptin Levels of Research Subjects

Characteristics	N	Mean±SD	Median	Min-Max	*p*
Age (Year)	69	57.49±11.03	58	22-83	-
BMI (kg/m^2)	69	25.047±4.14	24.1	16.87-36.89	0.009*
FBG (mg/dL)	69	146.99±45.93	141	75-291	0.040*
Level of AGEs (ng/mL)	69	22.27±6.00	22.018	11.02-39.68	0.200*
Level of Leptin (ng/mL)	69	8.30±7.83	5.572	0.03-28.61	<0.001*

Description: BMI = Body Mass Index, FBG = Fasting Blood Glucose, AGEs = Advanced Glicated End Products, N = Total of Subject, Mean= Average value, SD = Standard Deviation, Min = Minimum, Max = Maximum, *the Kolmogorov-Smirnov test

Analysis of the AGEs level revealed p>0.05, indicating normally distributed data, in contrast to other data that is not normally distributed. This study also found that the mean level of AGEs in the male group (22.80 ng/mL) was higher than that of the female group (21.94 ng/mL). Conversely, the mean leptin levels in the male group (4.85 ng/mL) were lower than in the female group (10.38 ng/mL), as can be seen in Table 2.

Table 2: Differences in AGEs and Leptin levels in male and female groups

	Gender	N	Mean	SD	*p*
Level of AGEs (ng/mL)	Male	26	22.80	5. 63	0.568*
	Female	43	21.94	6.25	
Level of Leptin (ng/mL)	Male	26	4.85	5. 58	0.004*
	Female	43	10.38	8.30	

Description: AGEs = Advanced Glicated End Products *Independent Sample T-test (p<0.05)

The Table 3 below displays the correlation between AGEs and Leptin in T2DM research subjects according to their gender group. AGEs and leptin levels were observed to have a negative correlation in the male group and an inverse correlation in the female group. Thus, it can be concluded that no significant association was found between the levels of AGEs and leptin based on gender.

Table 3: Correlation of AGEs and Leptin with Gender Groups in Type 2 DM Subjects

Variable		Leptin (ng/mL)			Gender Group				
					Males			Females	
	n	p*	r*	n	p*	r*	n	p*	r*
AGEs (ng/mL)	69	0.404	-0.102	26	0.107	-0.323	43	0.920	0.016

Description: * Spearman's rank correlation test

Data exploration can be effectively carried out by using scatterplot graphs to visually analyze the pattern of linear relationships between data on AGEs levels and Leptin levels. The scatterplot image presented in Figure 1 clearly illustrates that the data points form a random pattern, indicating no significant association between the levels of AGEs and Leptin. The random pattern of the scatterplot is an evidence that there is no discernible relationship between the variables. In addition, the significant variability observed in Leptin levels across the AGEs range suggests that factors other than AGEs may influence the level of Leptin. The scatterplot of AGEs and leptin levels in both male (Figure 2) and female groups (Figure 3) exhibit similar results, with scatter points forming a random pattern. This indicates no correlation or substantial relationship between AGEs level and leptin levels by gender.

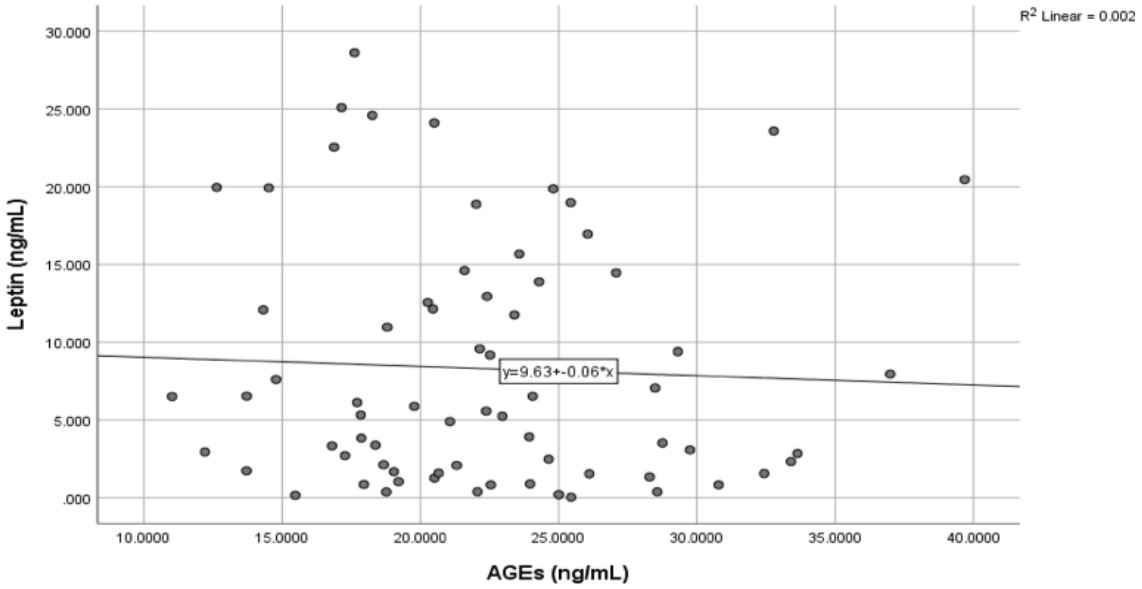

Figure 1: Correlation of AGEs and Leptin Levels in All Subjects with Type 2 DM

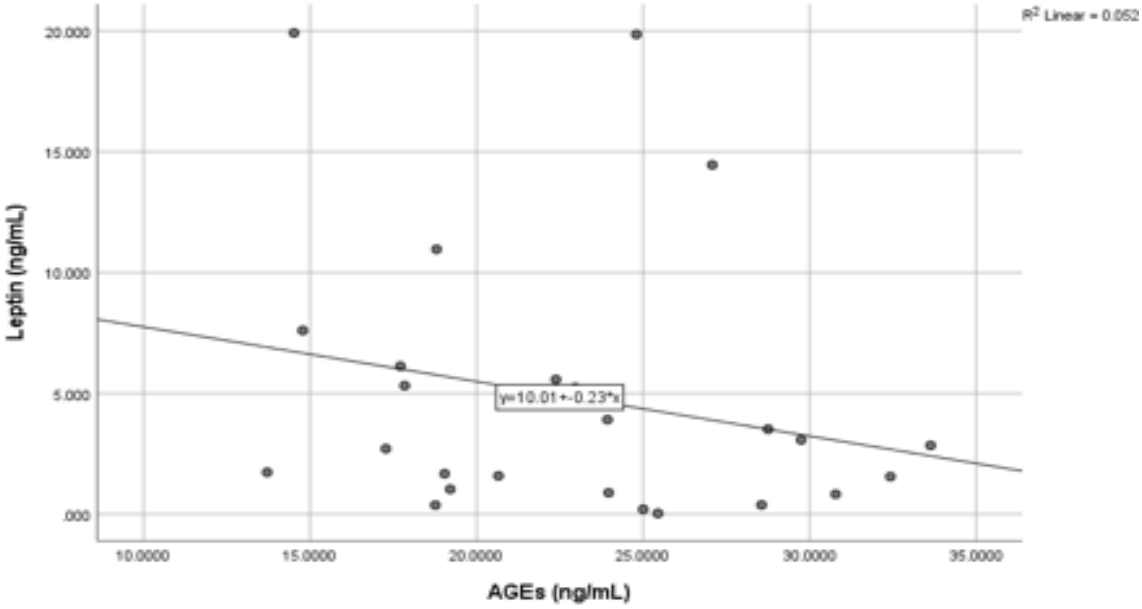

Figure 2: Correlation of AGEs and Leptin Levels in the Male Group

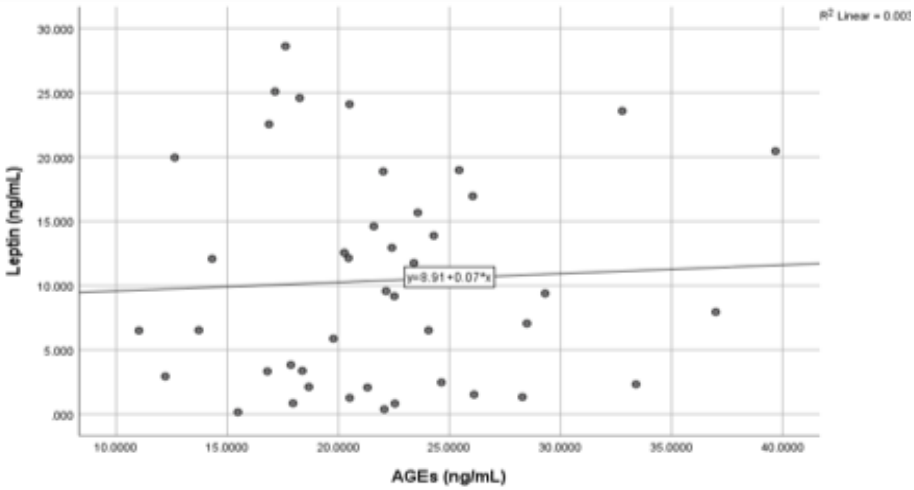

Figure 3: Correlation of AGEs and Leptin Levels in the Female Group

Table 4 displays the association between BMI and serum leptin levels in type 2 DM subjects of both male and female gender groups. In addition, BMI and leptin were also observed to be positively correlated with type 2 DM subjects both in male and female groups. The p-value <0.05 for BMI and leptin as well as the gender groups showed a significant correlation.

Table 4: Correlation between BMI and Serum Leptin Levels in Type 2 DM Patients and Gender Groups

Variable		BMI			Gender Group				
					Males			Females	
	n	p*	r*	n	p*	r*	n	p*	r*
Leptin (ng/mL)	69	<0.001	0.492	26	0.007	0.512	43	<0.001	0.533

Description: *Spearman's rank correlation test

The scatterplot illustration in Figure 4 shows a linear association between BMI and leptin levels. The data distribution in the scatterplot clearly forms a linear pattern. This indicates a noteworthy correlation or relationship between BMI and leptin levels. A similar linear pattern can also be observed in Figures 5 and 6, which represent the data for male and female groups, respectively.

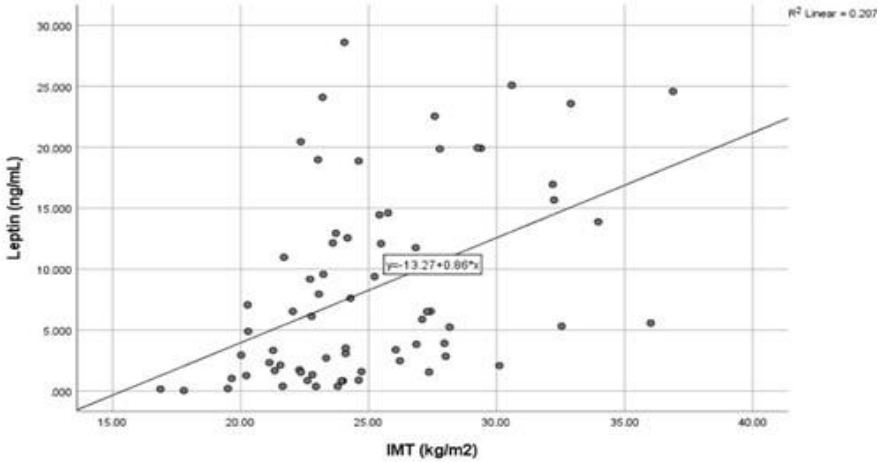

Figure 4: Correlation of BMI values and Leptin levels in T2DM Subjects

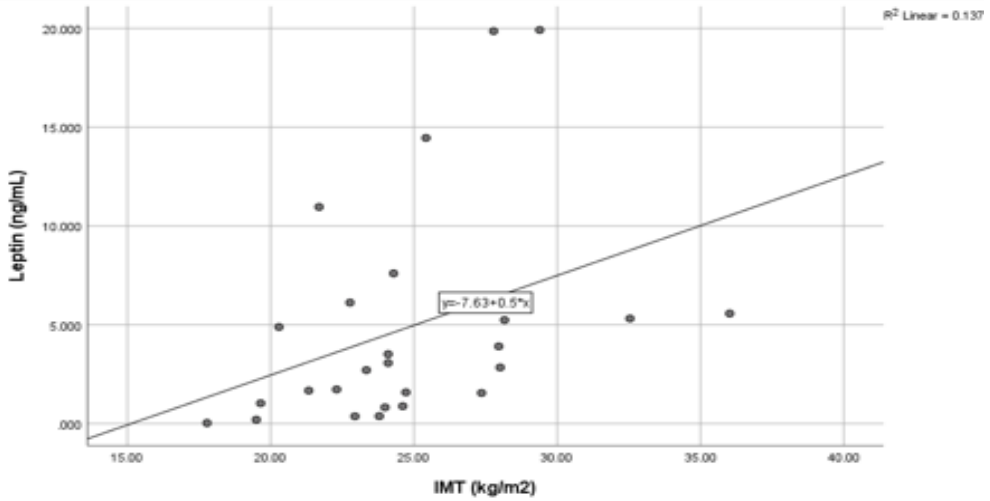

Figure 5: Correlation between BMI and Leptin Levels in Male Type 2 DM Subjects

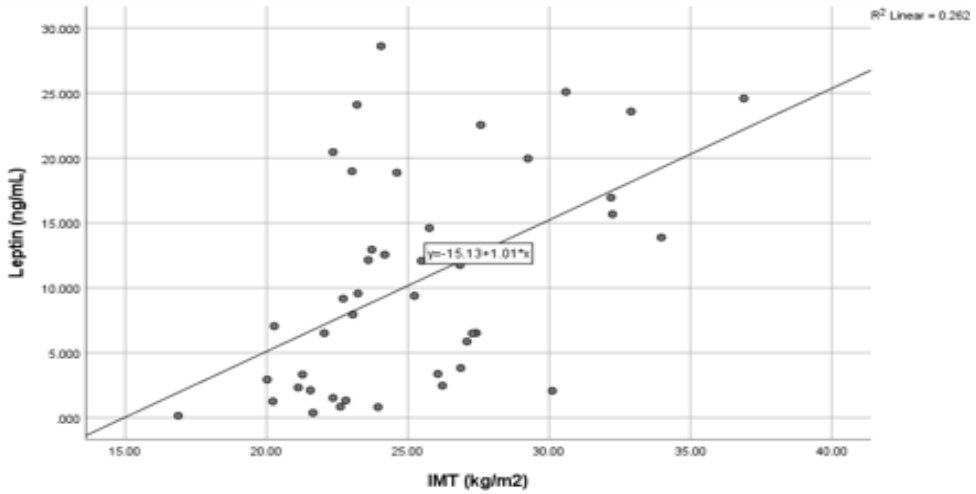

Figure 6: Correlation between BMI and Leptin Levels in Female Type 2 DM subjects

DISCUSSION

Globally, the impact of T2DM is caused by factors such as decreased physical activity levels, urbanization, and higher socioeconomic status. The occurrence rate tends to increase in urban areas compared to rural areas, and in countries with higher level of income than those with lower income levels [18]. This chronic disease may potentially cause various complications, both acute and chronic. These complications will affect various other organs like diabetic kidney disease (DKD), Diabetic retinopathy (DR), and neuropathic and cardiovascular diseases; individuals with T2DM possess higher risk (two to ten times) of suffering from heart disease compared to those without diabetes [19]. Dysfunction in insulin secretion, insulin action, or both can lead to hyperglycemia, which is regarded as a sign of DM. Persistent hyperglycemia triggers the AGEs and oxidative stress formation [4]. The interaction occurred between AGEs with their receptors triggers pathways that can cause elevated oxidative stress and inflammation, which then exacerbates tissue damage in individuals with DM [20]. In subjects with T2DM, the accumulation of AGEs increases, contributing to the development of complications such as nephropathy, vascular complications, and

cardiovascular disease (CVD) [21]; [20]. The intake of chronic AGEs leads to increased oxidative stress and inflammation, resulting in insulin resistance characterized by elevated fasting plasma insulin and leptin levels as well as decreased adiponectin levels [2]. Individuals with type 2 DM experience elevated levels of leptin, which may contribute to insulin resistance. Chronically elevated leptin levels can lead to leptin resistance, where the body becomes less sensitive to the leptin effect. This leptin resistance can hinder the normal signaling pathways involved in insulin action, consequently result in impaired glucose metabolism and insulin resistance [7].

The current study demonstrates an important perspective on the involvement of AGEs and BMI with serum leptin in the onset and progress of T2DM. The study found a non-significant negative correlation between the level of AGEs and serum leptin in T2DM subjects due to a small amount of serum leptin levels being significantly lower in these individuals. This is attributable to poorer pancreatic function, as evidenced by lower B-cell function; it as can be seen from the homeostatic model assessment for B-cell function, and high insulin resistance, as indicated by HOMA-IR values [22]. Similarly, Xing-ping et al 2009, found that patients with hyperglycemia crisis had lower leptin levels and higher levels of oxidative stress markers compared to healthy individuals, suggesting a significant imbalance in their body's stress response and energy regulation mechanisms [23]. In contrast, Kalousová et al 2003, found significantly higher levels of AGEs in patients with type 2 DM undergoing hemodialysis than in those without T2DM undergoing regular hemodialysis [17]. The association between AGEs and leptin in hemodialysis patients with DM displayed a strong positive relationship between AGEs and leptin variables in hemodialysis patients suffering from T2DM. Individuals with Type 2 DM receiving hemodialysis showed increased levels of AGEs which is attributed to nutritional influences in conjunction with changes in carbohydrate metabolism associated with AGEs [17]. In contrast, individuals with renal dysfunction usually exhibit hyperleptinemia, anorexia, and a tendency towards decreased body fat. The increased leptin levels observed in hemodialysis patients are influenced by reduced renal clearance and the potency of increased action of proinflammatory cytokines [24]. Moreover, serum leptin is closely linked to atherosclerotic index in male and female individuals receiving hemodialysis, while AGEs are recognized for their significant contribution to atherosclerosis as a complication of certain chronic medical conditions [25]. The current study has different findings because the type 2 DM subject being studied did not have complications related to kidney disease while the previous study used type 2 DM subjects with hemodialysis. Another study carried out by Han et al 2013, on test animals revealed that a total of 51.9% of B cells expressed AGEs/RAGE on the cell surface with high levels of leptin [26]. This study revealed that glycolipotoxicity characterized by increased FFA and AGE formation due to prolonged hyperglycemia can lead the failure of pancreatic B cell through upregulation of RAGE expression on B cells as a result of insufficient leptin action in T2DM. Prevention of leptin resistance or provision of adequate leptin action may help protect against glycolipotoxicity in B cells of individuals with type 2 DM. Adipose tissue is not only regarded as an inert storage site for surplus energy. It is considered an active endocrine organ with biologically active adipokine networks such as leptin, adiponectin, and free fatty acids (FFAs).

The correlation of AGEs and leptin levels in male subjects experiencing T2DM revealed a non-significant negative relationship. This finding is in line with the study by Vinitha et al 2015, which examined a cohort of Asian Indian men whom glucose

tolerance suffer from impairment; this study observed no significant association between the level of leptin and glycated haemoglobin [27]. This lack of correlation is attributed to the leptin levels in both the control group and case group being approximately the same. The correlation of AGEs and leptin levels in female subjects with T2DM also exhibited an insignificant positive relationship. Similarly, a study conducted by Saeed & Binjawhar 2023, on Saudi Arabian women revealed no significant correlation between leptin and glycated hemoglobin due to the substantial association of elevated level of leptin with insulin resistance and other risk factors, including triglycerides, fasting glucose, and BMI [28].

The findings revealed an interesting link between BMI and serum leptin levels in individuals with T2DM across both male and female groups. This implies a potential hypothesis of the effect of nutrition and changes in carbohydrate metabolism in patients with T2DM. The significant positive association in this finding is similar to those of previous studies [29]; [30], which exhibited that serum leptin levels were higher in obese groups with T2DM and positively correlated with BMI. Furthermore, the significant positive relationship observed in this finding is in line with researach by Moonishaa et al 2017, and Zulfania et al 2020, which exhibited that serum leptin levels were higher in obese groups with T2DM and positively correlated with BMI [29]; [30]. This also aligns with the research by Saeed & Binjawhar 2023, revealing that among women with type 2 diabetes, high insulin resistance, plasma atherogenic index, and BMI were associated with increased leptin levels [28]. This finding suggests that elevated leptin levels in women are due to high waist circumference and BMI, which are linked to the resistance of insulin. Moreover, women with T2DM who are obese and have high leptin levels and low level of adiponectin should be regularly monitored to prevent or reduce complications such as cardiovascular disease. Subcutaneous adipocytes in the abdominal region of women exhibit leptin secretion levels that are threefold higher than those of intra-abdominal fat accumulation. Subcutaneous adipocytes crucially contribute as the main source of circulating leptin in the bloodstream. Obesity causes excessive fat accumulation, which leads to increased levels of free fatty acids in adipose tissue, consequently resulting in increased triglycerides. Leptin, synthesized by adipose tissue, serves a pivotal part in regulating balance of energy and body weight by inhibiting the production of lipids, fatty acids, and acetyl CoA carboxylase, thereby promoting lipid deposition. HDL-c serves to control the synthesis of triglycerides and cholesterol in the arteries, ultimately result in difficult prevention of atherosclerosis.

Prior studies suggest that increased leptin levels are associated with the occurrence of insulin resistance and the progression of T2DM [31]; [29]; [28]; [30]. Conversely, Al-Harithy & Alomari 2021, found decreased leptin levels in their study examining the expression of leptin mRNA as a non-invasive biomarker in T2DM [32]. They found that Leptin mRNA levels were significantly diminished in T2DM subjects compared to the non-diabetic control group. Furthermore, several other studies [33]; [34], also revealed low leptin levels in patients with T2DM. This occurred due to various factors including the distribution of fat, the increase in waist circumference, reduction in BMI, gender, insulin deficiency, and insulin resistance. Although fluctuations in leptin levels in patients with T2DM have been regarded as an issue that is quite controversial, these inconsistent results cannot be associated with differences in gender, ethnicity, or the research methodology.

Individuals with a longer duration of type 2 diabetes may experience an exacerbation of chronic inflammation due to prolonged exposure to diabetes-related risk factors. As blood glucose levels remain elevated and inflammation persists over time, more tissue damage occurs, leading to a more complex inflammatory response.

Sustained chronic inflammation can lead to coronary heart disease and other complications in individuals with diabetes. A study conducted by Bhattacharya 2008, found that patients with T2DM for 5 to 10 years exhibited higher levels of leptin and inflammatory cytokines (IL-2, IL-6, and TNF-alpha), which are regarded as the markers of inflammation [35]. Additionally, it was found that individuals with T2DM who were of normal weight had lower leptin level but higher levels of IL-6 and TNF-alpha inflammatory markers.

Therefore, the duration of T2DM affects AGEs and leptin levels, influencing inflammation, cardiovascular risk factors, and glucose tolerance. Furthermore, a closer examination of the progression of type 2 DM found that more often than not, insulin therapy is needed by the patient as the disease progresses, either alone or combining it with oral hypoglycemic agents. Alfaqih et al 2023, revealed that T2DM patients who received metformin with uncontrolled glycemia exhibited lower serum leptin levels after adjusting the age, BMI, duration of treatment, and HOMA-IR [36].

Another study stated that treatment by using insulin, oral hypoglycemic drugs, and other drugs utilized in the management of T2DM can affect leptin levels in patients [37]. In addition, Abdulrahman et al 2023, who conducted a study on troglitazone, a thiazolidinedione (TZD) drug, found that it lowered serum leptin levels and prevented bone loss in patients suffering from T2DM [38].

Generally, this study's findings align with those of previous research, which has demonstrated a relationship of AGEs, BMI, and serum leptin in the development of T2DM. This research, however, is the first to investigate this association within the Indonesian population and the result suggests that the interaction between AGEs, BMI, and serum leptin may significantly impact insulin resistance and beta cell dysfunction.

This research has several limitations related to variables which influence various factors, including the protracted duration of suffering Type 2 DM, regulation of glycemic levels, and the particular type of medication used. In addition, it is important to note that another limitation is the scarcity of valuable data regarding the duration of patient undergoing regular check-ups, as well as their compliance with the treatment received.

CONCLUSION

In subjects with type 2 DM, the negative relationship between AGEs and leptin is not significant. However, BMI shows a significant positive correlation with serum leptin. The elevated leptin concentrations may be regarded as a potential risk factor in people with T2DM and a high BMI.

Acknowledgement

We would like to extend our gratitude to those who contributed to this research through their financial support, consultation, and assistance in carrying out data collection.

References

1) Glovaci, D., Fan, W., & Wong, N. D. (2019). Epidemiology of Diabetes Mellitus and Cardiovascular Disease. In *Current Cardiology Reports* (Vol. 21, Issue 4). Current Medicine Group LLC 1. https://doi.org/10.1007/s11886-019-1107-y.

2) Nowotny, K., Jung, T., Höhn, A., Weber, D., & Grune, T. (2015). Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. In *Biomolecules* (Vol. 5, Issue 1, pp. 194–222). MDPI AG. https://doi.org/10.3390/biom5010194.

3) Kurniawan, L. B. (2024). HbA1c As Diabetes Mellitus Biomarker and Its Methods Evolution. *Indonesian Journal Of Clinical Pathology And Medical Laboratory*, *30*(2), 198–203. https://doi.org/10.24293/ijcpml.v30i2.2191.

4) Perrone, A., Giovino, A., Benny, J., & Martinelli, F. (2020). Advanced Glycation End Products (AGEs): Biochemistry, Signaling, Analytical Methods, and Epigenetic Effects. In *Oxidative Medicine and Cellular Longevity* (Vol. 2020). Hindawi Limited. https://doi.org/10.1155/2020/3818196.

5) Sergi, D., Boulestin, H., Campbell, F. M., & Williams, L. M. (2021). The Role of Dietary Advanced Glycation End Products in Metabolic Dysfunction. In *Molecular Nutrition and Food Research* (Vol. 65, Issue 1). Wiley-VCH Verlag. https://doi.org/10.1002/mnfr.201900934.

6) Teichert, T., Hellwig, A., Peßler, A., Hellwig, M., Vossoughi, M., Sugiri, D., Vierkötter, A., Schulte, T., Freund, J., Roden, M., Hoffmann, B., Schikowski, T., Luckhaus, C., Krämer, U., Henle, T., & Herder, C. (2015). Association between Advanced Glycation End Products and Impaired Fasting Glucose: Results from the SALIA Study. *PLOS ONE*, *10*(5), e0128293. https://doi.org/10.1371/journal.pone.0128293.

7) Devi, B. J., Naidu, B. C., Lalitha, D. L., & Kiranmai, C. (2022). Association of leptin with insulin resistance in type 2 diabetes mellitus. *International Journal of Health Sciences*, 11405– 11412. https://doi.org/10.53730/ijhs.v6ns2.8057.

8) Havel, P. J., Uriu-Hare, J. Y., Liu, T., Stanhope, K. L., Stern, J. S., Keen, C. L., & Ahrén, B. (1998). Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: Reversal by insulin. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *274*(5 43-5). https://doi.org/10.1152/ajpregu.1998.274.5.r1482.

9) Morton, G. J., & Schwartz, M. W. (2011). Leptin and the central nervous system control of glucose metabolism. *Physiological Reviews*, *91*(2), 389–411. https://doi.org/10.1152/physrev.00007.2010.

10) Kurajoh, M., Koyama, H., Kadoya, M., Naka, M., Miyoshi, A., Kanzaki, A., Kakutani-Hatayama, M., Okazaki, H., Shoji, T., Moriwaki, Y., Yamamoto, T., Emoto, M., Inaba, M., & Namba, M. (2015). Plasma leptin level is associated with cardiac autonomic dysfunction in patients with type 2 diabetes: HSCAA study. Cardiovascular Diabetology, 14(1). https://doi.org/10.1186/s12933-015-0280-6.

11) Schmidt, M. I., Duncan, B. B., Vigo, A., Pankow, J. S., Couper, D., Ballantyne, C. M., Hoogeveen, R. C., & Heiss, G. (2006). Leptin and incident type 2 diabetes: Risk or protection? *Diabetologia*, *49*(9), 2086–2096. https://doi.org/10.1007/s00125-006-0351-z

12) Thorand, B., Zierer, A., Baumert, J., Meisinger, C., Herder, C., & Koenig, W. (2010). Associations between leptin and the leptin/adiponectin ratio and incident Type 2 diabetes in middle-aged men and women: Results from the MONICA/KORA Augsburg Study 1984-2002. *Diabetic Medicine*, *27*(9), 1004–1011. https://doi.org/10.1111/j.1464- 5491.2010.03043.x

13) Li, Y., Chen, X., Gong, X., Yao, J., He, D., & Du, W. (2022). Effect of Gender on Serum Leptin in Type 2 Diabetes Mellitus: A System Review and Meta-Analysis. *Computational and Mathematical Methods in Medicine*, *2022*. https://doi.org/10.1155/2022/4875799.

14) Katsiki, N., Mikhailidis, D. P., & Banach, M. (2018). Leptin, cardiovascular diseases and type 2 diabetes mellitus. In *Acta Pharmacologica Sinica* (Vol. 39). www.chinaphar.com

15) Sabbatinelli, J., Castiglione, S., Macrì, F., Giuliani, A., Ramini, D., Vinci, M. C., Tortato, E., Bonfigli, A. R., Olivieri, F., & Raucci, A. (2022). Circulating levels of AGEs and soluble RAGE isoforms are associated with all-cause mortality and development of cardiovascular complications in type 2 diabetes: a retrospective cohort study. *Cardiovascular Diabetology*, *21*(1). https://doi.org/10.1186/s12933-022-01535-3.

16) Bidulescu, A., Dinh, P. C., Sarwary, S., Forsyth, E., Luetke, M. C., King, D. B., Liu, J., Davis, S. K., & Correa, A. (2020). Associations of leptin and adiponectin with incident type 2 diabetes and interactions among African Americans: The Jackson heart study. BMC Endocrine Disorders, 20(1). https://doi.org/10.1186/s12902-020-0511-z

17) Kalousová, M., Sulková, S., Zima, T., Deppisch, R., Beck, W., Bednářová, V., Fořtová, M., & Tesař, V. (2003). Advanced glycation end products in hemodialyzed patients with diabetes mellitus correlate with leptin and leptin/body fat ratio. *Renal Failure*, *25*(2), 277–286. https://doi.org/10.1081/JDI-120018728.

18) Wang, H., Li, N., Chivese, T., Werfalli, M., Sun, H., Yuen, L., Hoegfeldt, C. A., Elise Powe, C., Immanuel, J., Karuranga, S., Divakar, H., Levitt, Na. A., Li, C., Simmons, D., & Yang, X. (2022). IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. *Diabetes Research and Clinical Practice*, *183*. https://doi.org/10.1016/j.diabres.2021.109050.

19) Cole, J. B., & Florez, J. C. (2020). Genetics of diabetes mellitus and diabetes complications. In *Nature Reviews Nephrology* (Vol. 16, Issue 7, pp. 377–390). Nature Research. https://doi.org/10.1038/s41581-020-0278-5.

20) Planas, A., Simó-Servat, O., Hernández, C., & Simó, R. (2022). Advanced Glycations End Products in the Skin as Biomarkers of Cardiovascular Risk in Type 2 Diabetes. *International Journal of Molecular Sciences*, *23*(11), 6234. https://doi.org/10.3390/ijms23116234.

21) Lee, J., Yun, J.-S., & Ko, S.-H. (2022). Advanced Glycation End Products and Their Effect on Vascular Complications in Type 2 Diabetes Mellitus. *Nutrients*, *14*(15), 3086. https://doi.org/10.3390/nu14153086.

22) Mohammadzadeh, G., & Zarghami, N. (2013). Serum leptin level is reduced in non-obese subjects with type 2 diabetes. *International Journal of Endocrinology and Metabolism*, *11*(1), 3–10. https://doi.org/10.5812/ijem.6535.

23) Xing-ping, S., Sen-biao, Z., Hao-jie, W., & Yan, Z. (2009). The relationship between serum level of leptin and oxidative stress in patients with hyperglycemia crisis. *Chinese Critical Care Medicine*, *21*(6), 353-356.

24) Sharma, K., Considine, R. V., Michael, B., Dunn, S. R., Weisberg, L. S., Kurnik, B. R. C., Kurnik, P. B., O'Connor, J., Sinha, M., & Caro, J. F. (1997). Plasma leptin is partly cleared by the kidney and is elevated in hemodialysis patients. *Kidney International*, *51*(6), 1980– 1985. https://doi.org/10.1038/ki.1997.269.

25) Kato, A., Odamaki, M., Maruyama, Y., & Hishida, A. (2001). Association between circulating leptin and soluble receptors for tumor necrosis factor-alpha in hemodialysis patients. *Clinical Nephrology*, *56*(5), 370–377.

26) Han, D., Yamamoto, Y., Munesue, S., Motoyoshi, S., Saito, H., Win, M. T. T., Watanabe, T., Tsuneyama, K., & Yamamoto, H. (2013). Induction of receptor for advanced glycation end products by insufficient leptin action triggers pancreatic β-cell failure in type 2 diabetes. *Genes to Cells*, *18*(4), 302–314. https://doi.org/10.1111/gtc.12036.

27) Vinitha, R., Ram, J., Snehalatha, C., Nanditha, A., Shetty, A. S., Arun, R., Godsland, I. F., Johnston, D. G., & Ramachandran, A. (2015). Adiponectin, leptin, interleukin-6 and HbA1c in the prediction of incident type 2 diabetes: A nested case-control study in Asian Indian men with impaired glucose tolerance. *Diabetes Research and Clinical Practice*, *109*(2), 340–346. https://doi.org/10.1016/j.diabres.2015.05.035.

28) Saeed, W. M., & Binjawhar, D. N. (2023). Association of Serum Leptin and Adiponectin Concentrations with Type 2 Diabetes Biomarkers and Complications Among Saudi Women. *Diabetes, Metabolic Syndrome and Obesity*, *16*, 2129–2140. https://doi.org/10.2147/DMSO.S405476.

29) Moonishaa, T., Nanda, S., Shamraj, M., Sivaa, R., Sivakumar, P., & Ravichandran, K. (2017). Evaluation of leptin as a marker of insulin resistance in type 2 diabetes mellitus. *International Journal of Applied and Basic Medical Research*, *7*(3), 176. https://doi.org/10.4103/ijabmr.ijabmr_278_16.

30) Zulfania, Khan, A., Ghaffar, T., Kainat, A., Arabdin, M., & Ur Rehman Orakzai, S. (2020). Correlation between serum leptin level and Body mass index (BMI) in patients with type 2 diabetes Mellitus. *Journal of the Pakistan Medical Association*, *70*(1), 3–6. https://doi.org/10.5455/JPMA.301135.

31) Paiva, E. S., Andretta, A., Batista, E. D., Lobo, M. M. M. T., de Miranda, R. C., Nisihara, R., Schieferdecker, M. E. M., & Boguszewski, C. L. (2017). Serum levels of leptin and adiponectin and clinical parameters in women with fibromyalgia and overweight/obesity. *Archives of Endocrinology and Metabolism*, *61*(3), 249–256. https://doi.org/10.1590/2359-3997000000248.

32) Al-Harithy, R. N., & Alomari, A. S. (2021). Expression of leptin mRNA as non-invasive biomarker in type 2 diabetes mellitus. *International Journal of Clinical Practice*, *75*(12). https://doi.org/10.1111/ijcp.14844.

33) Abdelgadir. M, ., Elbagir. M, Eltom C. M, & Berne B. Ahr[eacute]n. (2002). Reduced leptin concentrations in subjects with type 2 diabetes mellitus in Sudan. *Metabolism Clinical and Experimental*, *51*(3), 304–305.

34) Uzoamaka Onyemelukwe, O., Ogoina, D., & Onyemelukwe, G. C. (2020). Leptin concentrations in type 2 diabetes and non-diabetes Nigerian-Africans. In *Am J Cardiovasc Dis* (Vol. 10, Issue 4). www.AJCD.us/

35) Bhattacharya SK. (2008). *Relationshi P Between Plasma Leptin And Plasma Insulin Levels In Type-2 Diabetic Patients Before And After Treatment With Glibenclamide And Glimepiride*. https://doi.org/https://doi.org/10.15407/fz60.04.056

36) Alfaqih, M. A., Aljanabi, M., Ababneh, E., Khanfar, M., Alqudah, M., & Sater, M. (2023). Leptin and the rs2167270 Polymorphism Are Associated with Glycemic Control in Type Two Diabetes Mellitus Patients on Metformin Therapy. *Medicina (Lithuania)*, *59*(5). https://doi.org/10.3390/medicina59050997

37) Katsiki, N., Mikhailidis, D. P., Gotzamani-Psarrakou, A., Yovos, J. G., & Karamitsos, D. (2011). Effect of various treatments on leptin, adiponectin, ghrelin and neuropeptide y in patients with type 2 diabetes mellitus. In *Expert Opinion on Therapeutic Targets* (Vol. 15, Issue 4, pp. 401–420). https://doi.org/10.1517/14728222.2011.553609

38) Abdulrahman, Z. S., Alatrakji, M. Q., Al-Maliky, A. A., Hussein, K. I., & Hussain, S. A. (2023). The association of metformin dose up-titration and treatment duration with adiposity, lipid profile indicators, and serum leptin levels in T2DM Iraqi patients. *Journal of Health Sciences*, *13*(1), 20–27. https://doi.org/10.17532/jhsci.2023.1845.