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ANALYSIS OF ADVANCED GLICATED END PRODUCTS (AGEs) WITH SERUM GHRELIN IN TYPE 2 DIABETES MELLITUS SUBJECTS

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Abstract

Diabetes mellitus (DM) is a metabolic disease characterised by elevated blood glucose levels. High levels of glucose in the blood can be caused by impaired insulin secretion or insufficient production. The World Health Organisation (WHO) reports that, globally, more than 425 million people are living with diabetes. Patients with diabetes mellitus are at high risk of chronic comorbidities and secondary complications, such as neuropathy, retinopathy, nephropathy, and cardiovascular disease. Type 2 diabetes mellitus patients experience hyperglycaemia, which causes the production of AGEs to increase and impacts the development of complications in type 2 diabetes mellitus patients. Ghrelin may play a role in the development of type 2 DM. AGEs and serum Ghrelin levels were examined using sandwich Enzyme-Linked Immunosorbent Assay. This study aimed to determine whether there is a relationship between AGEs and serum Ghrelin in type 2 DM subjects and involved 70 subjects with type 2 DM, consisting of 28 male subjects and 42 female subjects. From the results of the correlation test found between AGEs and serum Ghrelin in type 2 DM subjects (r = 0.142, p = 0.240), it can be concluded that there is no significant correlation between AGEs levels and serum Ghrelin levels. No correlation was found between AGEs levels and serum Ghrelin levels in male and female subjects with type 2 DM (r = 0.107, p = 0.587) and women (r = 0, 163, p = 0.302), the direct relationship between AGEs and serum Ghrelin may not be significant. Further research is needed to understand the complex interactions between these factors and their influence on diabetes progression and related complications.

Keywords: Type 2 Diabetes Mellitus, AGEs and Serum Ghrelin.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by elevated blood glucose levels. Elevated blood glucose levels can result from deficient insulin secretion or inadequate hormone production. According to the World Health Organisation (WHO), there are more than 425 million people worldwide living with diabetes. The incidence of diabetes mellitus has almost doubled and is projected to increase by 50% by 2045.

Individuals with diabetes mellitus face a high risk of chronic comorbidities and secondary complications such as neuropathy, retinopathy, nephropathy, and cardiovascular conditions, which contribute significantly to the substantial economic burden estimated at 327 billion [1].

Comprehensive monitoring of glycaemic control in patients with type 2 DM, through measurement of HbA1c levels to track disease progress to reduce the likelihood of complications [2].

Haemoglobin (Hb) A1c is a glycated haemoglobin used worldwide as a reference test to assess long-term (past 2-3 months) glycaemic monitoring for decades. In addition to glucose monitoring, HbA1c assesses the risk of microvascular and macrovascular complications in DM. Currently, various approaches are commonly used for the assessment of HbA1c levels, such as immunoassay, enzymatic method, boronate affinity method, IEHPLC, and capillary electrophoresis method, which are able to identify haemoglobin or other haemoglobin variants in the chromatogram results. The determination of HbA1c levels is strongly influenced by the lifespan of red blood cells, indicating glucose exposure to HbA1c during the erythrocyte survival period of about three months [3].

Type 2 diabetes mellitus is a metabolic disorder characterised by hyperglycaemia due to abnormalities in insulin secretion, insulin function, or both. Prolonged hyperglycaemia in diabetes mellitus can lead to long-term impairment or damage to various organs such as the eyes, kidneys, nerves, heart and blood vessels, risking complications such as atherosclerosis, neuropathy, kidney failure and retinopathy.

Several studies have investigated the causes of hyperglycaemia, one of which is sugar-derived substances known as Advanced Glycated End Products (AGEs) [4]. AGEs not only produce a state of hyperglycaemia but are also influenced by oxidative reactions that occur within cells. Reactive Oxygen Species (ROS) play a role in the formation of AGEs and mediate the effects of AGEs on target tissues [5].

Unregulated hyperglycaemia is considered a major cause of diabetic complications due to the production of AGEs. AGEs arise as a consequence of the classical Maillard reaction, in which reducing sugars react non-enzymatically with the amino groups of proteins, lipids, and nucleic acids, leading to a series of reactions forming Schiff bases, followed by Amadori rearrangement and subsequent oxidative modification (glioxidation) resulting in the formation of AGEs [6]. A growing body of research demonstrates the potential of AGEs as a risk factor for the development of diabetes and its complications [7].

Ghrelin is a peptide hormone produced primarily by epsilon cells found in the lining of the stomach and is also secreted in small amounts from epsilon cells of the pancreas [8]. Ghrelin was first discovered in 1999 from human and rat stomach isolates. Ghrelin has a role in glucose metabolism, and is involved in insulin secretion. Elevated ghrelin may be associated with hyperglycaemia and IR in populations with type 2 diabetes mellitus, as ghrelin induces insulin resistance [9].

Identification Ghrelin emerged as a novel endocrine pathway involved in regulating feeding behaviour and maintaining energy balance over the last decade [10]. Ghrelin is a hormone that has many physiological functions. One of its main functions involves strong GH releasing activity [11].

Ghrelin levels in patients with type 2 diabetes mellitus, plasma levels are decreased, and compensatory hyperinsulinaemia due to insulin resistance is associated with significantly decreased ghrelin levels, suggesting that total fasting plasma ghrelin levels are lower in patients with type 2 diabetes than those without type 2 diabetes [12].

Elevated ghrelin may be related to hyperglycaemia and Insulin resistance (IR) in our population with type 2 diabetes mellitus, as ghrelin induces insulin resistance or it may be related to body mass index (BMI) as our subjects were within normal BMI, previous studies reported peripheral ghrelin to be inversely correlated with BMI and they had lower Ghrelin levels in type 2 diabetes mellitus patients with higher BMI [13].

Examination of AGEs and ghrelin in individuals with type 2 diabetes mellitus is necessary, given the recognised significance of AGEs in the development and complications of diabetes. While serum Ghrelin plays a role in glucose metabolism and insulin secretion, previous studies have presented conflicting findings regarding changes in serum Ghrelin levels in individuals with type 2 diabetes mellitus, prompting further investigation into the analysis of AGEs alongside serum Ghrelin in this population.

MATERIALS AND METHODS

Study Design and Population

The design of this study was a cross sectional study, the population in this study were type 2 DM patients who came for treatment at Hasanuddin University State College Hospital (RSPTN-UH) with a total sample of 70 samples consisting of 28 male gender and 42 female gender. This study was conducted at the Clinical Pathology Laboratory of Hasanuddin University Hospital (RSUH), Clinical Pathology Laboratory of Labuang Baji Hospital, UPK Laboratory of Makassar City Health Service Centre. This study was conducted after obtaining ethical approval from the Health Research Ethics Committee (KEPK) Faculty of Medicine, Hasanuddin University RSPTN-UH with ethical number 960/UN4.6.4.5.31/PP36/2023.

The research samples used are samples that meet the inclusion criteria and for exclusion criteria are samples that are not used by researchers, the inclusion criteria in this study consist of men and women with type 2 diabetes mellitus and accept the provision of information and agree to participate voluntarily and in writing (informed consent) to undergo blood collection and sample examination in the laboratory while the exclusion criteria in this study consist of pregnant women, icteric, lipemic or hemolysis serum samples, currently experiencing infection / inflammation that has been diagnosed by a doctor and suffering from tumours or cancer.

Level Measurement

This study was conducted by recording the patient's identity. Prior to blood collection, patients underwent a fasting period and anthropometric examination. Whole blood samples were collected on red cap blood tubes without anticoagulant for examination of serum AGEs levels and serum Ghrelin levels.

Then the blood samples were allowed to stand for 60 minutes in a vacuum tube, allowing them to clot. The sample was centrifuged at 3000 rpm for 10 minutes, after centrifuging, transfer the serum that has been separated from the blood cells to the sample cup using a disposable pipette with a volume of 150 µl per serum cup.

Serum was stored in a calibrated freezer at -20°C to keep the sample stable. Serum AGEs and serum Ghrelin levels were analysed using the Enzyme-Linked Immunosorbent Assay (ELISA) kit MyBioSource on a Thermo ELISA Reader instrument.

Data Analysis

The data obtained were then analysed using SPSS software version 22, data analysis was carried out by entering all variables including age, gender, and laboratory examinations. The Kolmogorov-Smirnov test was performed to determine whether the data distribution was normal or not with a significance value (α=0.05). If the data was not normally distributed, a non-parametric test was used, namely the Spearman correlation test.

RESULT

Characteristics of Research Subjects

This study involved 70 subjects with type 2 diabetes mellitus as a whole consisting of 28 male and 42 female subjects. The variable frequency distribution of the sex of the research subjects is shown in Table 1.

Table 1: Frequency Distribution of Gender Variables of Research Subjects

Characteristics	Category	DM Type 2	
		n	%
Gender	Male	28	40
	Female	42	60

The frequency distribution of age variables in this study with an age range between 22 years and 83 years, with an average BMI of 25.27 kg/m2, an average FBG of 151.23 mg/dL, an average AGEs level of 22.08 ng/mL, and an average serum Ghrelin level of 0.96 ng/mL. Descriptive analysis of age variables, AGEs levels and serum Ghrelin levels of research subjects are shown in Table 2.

Tabel 2: Descriptive Analysis of Age Variables, AGEs Levels and Serum Ghrelin Levels of Study Subjects

Characteristics	Mean±SD	Median	Min-Maks
Age (Years)	57,14±11,93	58	22-83
BMI (kg/m^2)	25,269±3,95	24,51	17,78-36,89
FBG (mg/dL)	151,23±48,46	147	75-291
AGEs Level (ng/mL)	22,086±5,01	22,03	12,20-36,99
Ghrelin Level (ng/mL)	0,958±0,15	0,97	0,61-1,30

Description : BMI : *Body Mass Index*
HbA1c : *Hemoglobin A1c*
FBG : *Fasting Blood Glucose*
AGEs : *Advanced Glicated End Products*

Normality Test

The normality test of the research results of AGEs levels and serum Ghrelin levels in type 2 diabetes mellitus subjects as a whole and based on gender was carried out to determine whether the data distribution was normal or not. The test used is the Kolmogorov-Smirnov test with a significance value (α = 0.05) for samples > 50 and the Shappiro Wilk test for data below < 50. The normality test of AGEs and Ghrelin serum levels of the research subjects is shown in Table 3.

Table 3: Normality Test of AGEs Level and Serum Ghrelin Level of Study Subjects

	Description		
	n	p	
BMI (Overall)	70	0,092*	Normal
AGEs Level (Overall)	70	0,200*	Normal
Ghrelin Levels (Overall)	70	0,200*	Normal
BMI (Male)	28	0,672*	Normal
AGEs Level (Male)	28	0,322*	Normal
Ghrelin Levels (Male)	28	0,228*	Normal
BMI (Female)	42	0,004	Not Normal
AGEs Level (Female)	42	0,155*	Normal
Ghrelin Levels (Female)	42	0,892*	Normal

Description: **Test Kolmogorov-Smirnov, **Test Shappiro Wilk*

BMI : *Body Mass Index*

AGEs : *Advanced glicated end products*

Based on the Kolmogorov-Smirnov test in Table 3 for the whole subject, the p value of BMI value in type 2 diabetes mellitus subjects is 0.09>α (0.05), indicating that the data is normally distributed. For the p value of AGEs levels in type 2 diabetes mellitus subjects is 0.20>α (0.05), indicating that the data is normally distributed, while for the p value of serum Ghrelin levels in all type 2 diabetes mellitus subjects obtained is 0.20>α (0.05), indicating that the data is normally distributed. For male subjects, the p value of BMI value in type 2 diabetes mellitus subjects is 0.67>α (0.05), indicating that the data is normally distributed. For the p value of AGEs levels in type 2 diabetes mellitus subjects is 0.32>α (0.05), indicating that the data is normally distributed, while the p value of serum Ghrelin levels for type 2 diabetes mellitus subjects with male gender is 0.23>α (0.05), indicating that the data is normally distributed. For female subjects, the p value of BMI value in type 2 diabetes mellitus subjects is 0.00 <α (0.05), indicating that the data is not normally distributed. For the p value of AGEs levels in type 2 diabetes mellitus subjects is 0.16>α (0.05), indicating that the data is normally distributed, while the p value of serum Ghrelin levels for type 2 diabetes mellitus subjects with male gender is 0.89>α (0.05), indicating that the data is normally distributed.

Correlation Test

From the results of the Pearson correlation test between AGEs levels and serum Ghrelin levels in patients with type 2 diabetes mellitus, the value of p=0.240 was obtained. Because 0.240>α (0.05), it can be concluded that there is no significant correlation between AGEs levels and serum Ghrelin levels in patients with type 2 diabetes mellitus. Correlation test of AGEs levels and serum Ghrelin levels of all research subjects is shown in Table 4.

Table 4: Correlation Test of AGEs Level Variables and Serum Ghrelin Levels of All Study Subjects

Variable	Serum AGEs Level	
Serum Ghrelin Level	r =	0,142
	p =	0,240
	n =	70

Description: *p: Spearman correlation test

Based on the scatterplot image above, it can be seen that the distribution of data on the scatterplot forms a random pattern. This indicates that there is no significant correlation or relationship between the variables of AGEs levels and Ghrelin levels, because the distribution points on the scatterplot form a random pattern Figure 1.

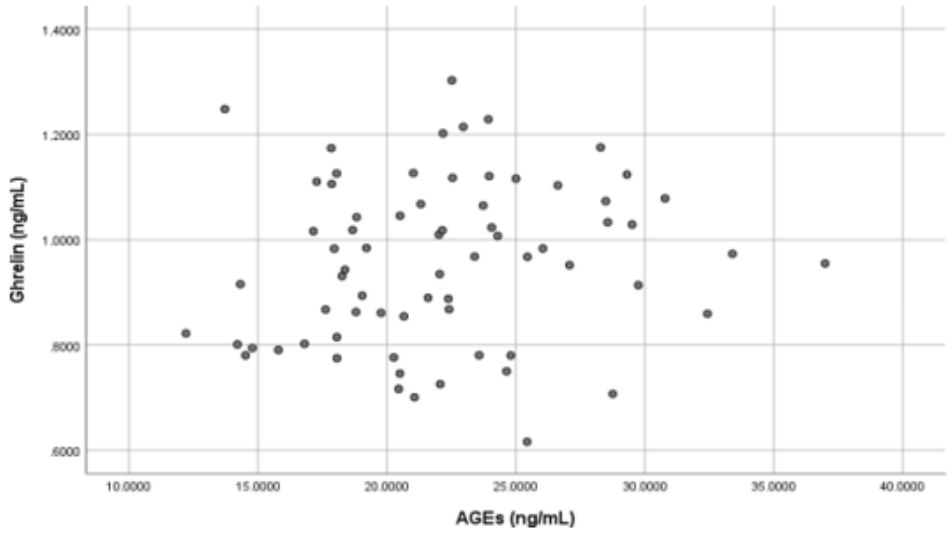

Figure 1: Scatterplot of AGEs Levels and Serum Ghrelin Levels for the Whole Study Subject

Correlation Analysis of AGEs Levels and Serum Ghrelin Levels in Male Type 2 Diabetes Mellitus Subjects

Pearson correlation between AGEs levels and serum Ghrelin levels in male patients with type 2 diabetes mellitus, obtained p=0.59. Since 0.59>α (0.05), it can be concluded that there is no significant correlation between AGEs levels and serum Ghrelin levels in male patients with type 2 diabetes mellitus. The variable test of AGEs and Ghrelin serum levels in male research subjects is shown in Table 5.

Table 5: Correlation Test of AGEs Level Variables and Serum Ghrelin Levels of Male Research Subjects

Variable	Serum AGEs Level	
Serum Ghrelin Level	r =	0,107
	p =	0,587
	n =	28

Description: *p: Pearson Correlation Test

Based on the scatterplot image above, it can be seen that the data distribution on the scatterplot forms a random pattern. This indicates that there is no significant correlation or relationship between the variables of AGEs levels and serum Ghrelin levels in male type 2 diabetes mellitus subjects, because the distribution points on the scatterplot form a random pattern. A flat scatterplot of AGEs levels and serum Ghrelin levels of male research subjects is shown in Figure 2.

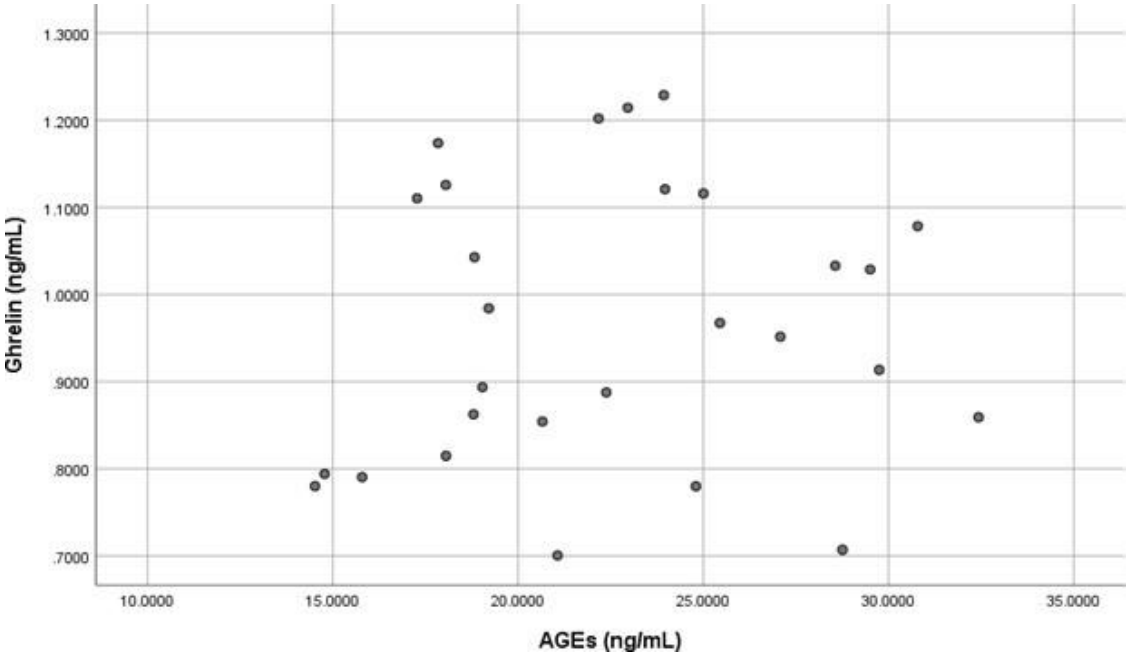

Figure 2: Scatterplot of data on AGEs levels and Serum Ghrelin levels of Male Research Subjects

Correlation Analysis of AGEs Levels and Serum Ghrelin levels in Female Type 2 Diabetes Mellitus Subjects

From the results of the Pearson correlation test between AGEs levels and serum Ghrelin levels in female patients with type 2 diabetes mellitus, a p value of 0.30 was obtained. Since 0.30>α (0.05), it can be concluded that there is no highly significant correlation between AGEs levels and serum Ghrelin levels in female patients with type 2 diabetes mellitus. Correlation test of AGEs levels and serum Ghrelin levels of female research subjects is shown in Table 6.

Table 6: Correlation Test of AGEs Levels and Ghrelin Levels of Female Research Subjects

Variable	Serum AGEs Level	
Serum Ghrelin Level	r =	0,163
	p =	0,302
	n =	42

Description: *p: Pearson Correlation Test

Based on the scatterplot image above, it can be seen that the data distribution on the scatterplot forms a random pattern. This indicates that there is no significant correlation or relationship between the variables of AGEs levels and serum Ghrelin levels in female type 2 diabetes mellitus subjects, because the distribution points on the scatterplot form a random pattern. A flat scatterplot of AGEs levels and serum Ghrelin levels of female research subjects is shown in Figure 3.

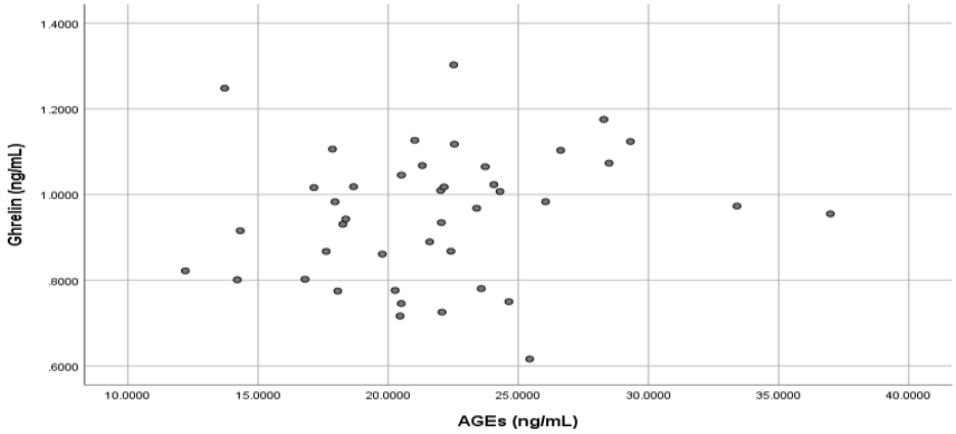

Figure 3: Scatterplot of data on AGEs levels and Serum Ghrelin levels of Female Research Subjects

DISCUSSION

Type 2 diabetes mellitus (DM type 2) is a chronic metabolic disease that can cause long-term complications of type 2 diabetes mellitus. Poorly managed diabetes will result in hyperglycaemia which over time can lead to complications in the form of damage to various body systems, especially the nervous system and blood vessels. Diabetes mellitus is one of the important risk factors for heart disease, stroke, neuropathy, retinopathy, and kidney failure.

A person with diabetes mellitus has a risk of dying twice as fast as a person without diabetes mellitus [14] . When blood glucose levels are high, a process called non-enzymatic glycation occurs where sugar molecules react with the body's proteins. The result of this reaction is the formation of AGEs.

Advanced Glycated End Products (AGEs) are compounds formed when sugar molecules combine with proteins, lipids, or nucleotides without enzymatic regulation. This process is known as glycation and can occur both in the human body and during food processing. In patients with type 2 diabetes, chronic hyperglycaemia leads to the accumulation of AGEs in various body tissues. Elevated levels of AGEs in type 2 diabetes are believed to contribute to the development of chronic complications.

AGEs can damage the structure and function of body tissues through various mechanisms, including the formation of abnormal cross-linking between proteins, activation of the receptor for AGEs (RAGE) that can trigger inflammatory responses and oxidative stress, as well as disruption in the regulation of certain genes. The process begins with the glycation reaction, in which sugar molecules combine non-enzymatically with proteins, lipids or nucleotides to form AGEs.

AGEs can trigger pro-inflammatory pathways by activating RAGE which can involve the activation of Nuclear factor Kappa B (NF-B), a transcription factor that plays an important role in the regulation of pro-inflammatory genes. Activation of NF-B can trigger the expression and release of pro-inflammatory cytokines such as TNF-α and IL-6 [15].

From the results of research conducted by Kilhovd et al, the average levels of AGEs in the blood serum of patients with cardiovascular disease and diabetes were significantly higher than the control group. From the results of research conducted by [16] said Advanced glycation end products (AGEs) are formed through non-enzymatic glycation processes, where reducing sugars react with free amino groups from proteins, lipids, and nucleic acids.

This spontaneous reaction occurs under physiological conditions, but the rate increases sharply under hyperglycaemic conditions, such as diabetes. As a result, diabetics tend to experience increased levels of AGEs, thus contributing to the development of diabetic complications, including cardiovascular disease. In a previous research journal [17] said that in individuals with diabetes, serum and tissue AGEs levels are much higher than non-diabetic controls, AGEs are formed faster than usual in the arteries and circulation of diabetics [17].

Ghrelin is an orexigenic peptide, which is secreted from the stomach and duodenum. In normal weight and fasting individuals, Ghrelin levels increase causing hunger, and preventing hypoglycaemia through increased glucagon secretion and decreased glucose-stimulated insulin secretion. Ghrelin can stimulate the release of growth hormone, which is responsible for metabolism, growth, and tissue repair. In the central nervous system Ghrelin functions to regulate the body's fat storage and energy expenditure. Some studies suggest that Ghrelin may affect insulin sensitivity and blood glucose regulation. Ghrelin may also affect neuronal activity in the brain, including areas responsible for appetite, thermoregulation, and hormonal function. Ghrelin may also increase gastric and intestinal motility, which are responsible for the digestion and absorption of nutrients.

Decreased ghrelin levels in patients with type 2 diabetes mellitus are associated with increased abdominal adiposity and insulin resistance, ghrelin mentioned previously has demonstrated roles in fat metabolism and glucose homeostasis and the cross talk between lipid and glucose metabolism may result in the physiological role of ghrelin including insulin resistance. Cellular lipid accumulation observed upon ghrelin administration will impact, excess visceral adiposity triggers the release of free fatty acids (FFA) into the circulation. Increased hepatic FFA oxidation triggers insulin resistance and increased glucose expenditure from the liver, Increased circulating FFA may result in decreased insulin sensitivity.

Enlargement of fat-charged adipots is associated with FFA release, physical stress and production of reactive oxygen species. (ROS). The prolonged increase in ghrelin increases visceral adiposity in mice and weakens the transcription of liver x receptor (LXR) and the ATP G1 binding cassette (ABCG1) which increases the volume of adipose due to a decrease in lipid exports, LXR isoform has been to have anti-inflammatory properties, the activation of LXr ligans leading to inhibition of the induction of NFkB-dependent inflammatory genes, increased ROS and the release of destructive inflammation agents as tumor necrosis factors (TNF-α).

These indirect immunomodulator responses can cause insulin resistance and type 2 diabetes mellitus (TNF-α) inducing phosphorylation inhibition of insulin receptor substrate (IRS) -1, leading to systemic insulin resistance [18].

Several studies that that Ghrelin decreased in patients with type 2 diabetes as done by [19] found serum levels of ghrelin in subjects with Type 2 diabetes lower than those without Type 2 Diabetes.

Along with previous studies, this study also showed that obesity is an important determinant of serum ghrelin levels. In the study [20] results showed patients with type 2 diabetes have lower levels of circulating serum Ghrelin compared to healthy people. Serum ggrelin levels in men are lower than in women, although this difference does not reach statistical significance. This study showed lower serum Ghrelin levels in patients with type 2 diabetes mellitus.

The results of the Pearson correlation test between BMI and serum Ghrelin levels in type 2 diabetes patients showed no significant relationship between the BMI value and the serum ghrelin level in subjects with Type 2 diabetes and were carried out by Pearson.

From the results of Pearson's correlations test between the body mass mass mass and the levels of ghreline in male type 2 diabetics, p=0.40 was obtained, the p=0.046 was found, and it was concluded that there was no significant correlated relationship between body mass and Ghrelins serum levels in males with type 2 Diabetes. From Spearman's test results, the correlating value between the Body mass and glycemic levels in female genetic diabetes type 2 patients was found to be P=0.94.

Based on the results of research that obtained the possible factors that influenced so obtaining results that did not correlate are genetic factors, several studies have shown that genetical factors can influence the secretion of Ghrelin and are associated with the risk of diabetes.

Certain genetic polymorphisms have been associated with higher levels of Ghrelin. Some medicines can also affect the levels of Ghrelin in the body, the effects of these medicines on Ghreline may vary depending on the mechanism of the drug's action, the use of SGLT-2 Inhibitor (Co-Transporter Sodium-Glucose Type 2 inhibitor).

Some studies have also shown that smoking can affect the levels of the hormone Ghrelin in the body that triggers hunger and stimulates appetite. The effect of nicotine and other cigarette ingredients on the hormonal system is one of many mechanisms that may be involved in the relationship between smoking and Ghrelin. Research suggests that smoking can affect the neurotransmitter and hormonal pathways that are responsible for regulating appetite.

A history of disease can also increase Ghrelin levels in the body such as traumatic brain injury, stroke, or Parkinson's disease can affect the functions of the autonomous nervous system involved in the regulation of hormone secretion, anorexia nervosa, patients with chronic kidney disease often experience metabolic and hormonal dysfunction. Several studies have shown that Ghrelin levels tend to be higher in patients with chronic kidney disease, and other diseases.

To improve further research, it may be necessary to consider some limitations or limitations in this study, namely the presence of a history of illnesses that the researchers may not know and may affect the levels of serum Ghrelin, variables such as smoking and long-term diabetes.

CONCLUSION

Based on the results of the above study, it can be concluded that no correlation was found between AGEs levels and serum Ghrelin levels in subjects with type 2 diabetes mellitus; No correlations were found between the levels of AGEs and the serum ghrelin in males and females with Type 2 diabetes.

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