Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Ischemia modified albumin levels in patients with insulin resistance

Rabia Seker[a], Elif Ayca Sahin[b], Aziz Sener[a,*]

[a] *Ankara Etlik City Hospital, Department of Medical Biochemistry, Ankara, Türkiye*
[b] *Gazi University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Türkiye*

ARTICLE INFO

Keywords:
Insulin resistance
Oxidative stress
Ischemia-modified albumin
HOMA-IR
Type 2 diabetes mellitus

Received: Aug 27, 2024
Accepted: Jan 09, 2025
Available Online: 26.02.2025

DOI:
10.5455/annalsmedres.2024.08.178

Abstract

Aim: Insulin resistance (IR) is a crucial characteristic of type 2 diabetes mellitus (T2DM) and can also occur independently or in other conditions such as obesity and metabolic syndrome. Oxidative stress significantly contributes to IR pathology. Ischemia-modified albumin (IMA) is a biomarker of oxidative stress and has been studied for its potential link to IR. This study aimed to identify the relationship between IMA levels and IR.

Materials and Methods: We conducted a prospective study involving 101 IR patients without diabetes mellitus and 101 healthy controls. HOMA-IR was used to classify IR and control groups. IMA levels were measured using a colorimetric method. The groups' IMA values were compared, and correlations between IMA and other parameters were determined.

Results: There was no statistically remarkable difference in IMA levels between the IR and control groups ($p=0.27$). In addition, no significant correlation was observed between IMA and HOMA-IR ($p=0.60$). Significant differences were found in glucose, insulin, and HOMA-IR levels between the IR and control groups ($p<0.001$ for insulin and HOMA-IR, $p<0.01$ for glucose).

Conclusion: Despite higher IMA levels in the IR group, the difference was not statistically notable, and no significant correlation with HOMA-IR was found. This may be due to the finite number of patients with high HOMA values, the need for more precise data on body mass index, and longer follow-up. Further prospective studies are necessssary to define the connection between IMA and IR.

Introduction

Insulin resistance (IR) is elucidated as the lack of appropriate response by muscle, fat, and liver cells to insulin. Therefore, increased blood glucose levels are observed because of decreased glucose uptake by tissues [1]. The pancreas tries to compensate by producing more insulin to help absorb the glucose. Over time, this leads to beta-cell problems and can result in type 2 diabetes (T2DM). IR is a hallmark feature of T2DM, but it can also be observed in obesity, hypotyroidism, Gestational diabetes mellitus, and metabolic syndrome [2]. The extant research evidence substantiates a strong and direct relationship between oxidative stress and insulin resistance (IR) [3, 4]. Oxidative stress is characterized as a state where an overabundance of reactive oxygen species (ROS) surpasses the inadequate cellular antioxidant defenses, significantly contributing to the pathophysiology of IR. These ROS interfere with insulin signaling pathways through oxidative modifications, either by tyrosine nitration or serine phosphorylation of key proteins [5, 6]. Consequently, there is an impairment in either the insulin receptor itself or its downstream signaling molecules, which reduces glucose uptake in both adipose tissues and muscle. Moreover, the production of ROS also amplifies the existing inflammatory reaction by triggering nuclear factor kappa B, thereby enhancing the production of pro-inflammatory cytokines [7]. This inflammatory milieu also contributes to the worsening of insulin resistance. This chronic oxidative environment may lead to endoplasmic reticulum stress and mitochondrial dysfunction, thereby promoting cellular damage [8]. Thus, these mechanisms, taken together, disrupt metabolic homeostasis and participate in the progressive nature of insulin resistance as well as its complications.

Ischemia-modified albumin (IMA) is a changed form of serum albumin that arises under an ischemic background owing to oxidative stress. The N-terminal region of albumin undergoes structural changes during ischemia, reducing its metal-binding capacity, particularly for cobalt,

*Corresponding author:
Email address: dr.azizsenerr@gmail.com (Aziz Sener)

making IMA a valuable biomarker for ischemic events and oxidative stress [9]. Various studies have shown that IMA has both diagnostic and prognostic value in diseases characterized by oxidative stress [10]. Studies have also showed a connection between IMA levels and IR [11, 12]. The close relationship between IR and oxidative stress and the fact that IMA indicates oxidative stress explain the possible relationship between IR and IMA.

In this study, we aimed to evaluate IMA levels in a cohort with insulin resistance and in a control group. In this way, we aimed to assess the relation between IMA and IR.

Materials and Methods

Patient selection

This prospective study included 101 IR patients without DM (serum glucose levels between 70-100 mg/dL and HbA1c level between 4%-6%) and 101 healthy controls between the ages of 15 and 60. The exclusion criteria for the study included a documented history of DM, the use of medications such as corticosteroids that induce insulin resistance, ongoing pharmacological treatments, a history of malignancy, recent or chronic infections, and the presence of any other underlying diseases.

Study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Ankara Dr. Sami Ulus Training and Research Hospital on April 20, 2022, under protocol number E-2022/04-326. Informed consent was not obtained from patients, as the research involved a retrospective analysis of anonymized blood test results. These results were used solely for disease monitoring and included waste blood samples collected for ischemia-modified albumin (IMA) measurements.

Grouping and cut-off values

HOMA-IR values were calculated using fasting insulin and fasting glucose levels in the patient and control groups for both sexes. Individuals with HOMA-IR values exceeding 2.5 formed the insulin resistance (IR) patient group, while those with HOMA-IR values at 2.5 or lower made up the control group.

Laboratory measurements

Glucose levels were measured using the hexokinase method on an AU 5800 analyzer (Beckman Coulter), while insulin levels were determined through the chemiluminescence method on an Advia Centaur XPT system (Siemens Healthcare).

Blood samples from all subjects were collected by venipuncture in serum separating tube, and instantly centrifuged at 4,000 X g for 10 min at 4 C in terms of their rutine blood analysis. Waste serum samples for IMA measurement were frozen at -20°C up to the working day. On the day of the study, all samples were brought to room temperature, and the study was completed on the same day, and the results were evaluated. A colorimetric determination method based on the cobalt binding property of albumin was used to determine the IMA level, and the colored compound formed by dithiothreitol and cobalt II, which does not bind to albumin, was measured colorimetrically. Measurements were made on a Human Humalyzer 2000 spectrophotometer at 470 nm, and the results were obtained using an absorbance unit (ABSU) [13]. The intra- and inter-study coefficient of variation (CV) determined for this method was lower than 3.6%.

Statistical analysis

All statistical analyses were examined with IBM SPSS Statistics for Windows, Version 24.0. (Armonk, NY: IBM Corp.). The normal distribution was assessed through the Kolmogorov-Smirnov test, kurtosis and skewness of the data distribution. An independent samples t-test was conducted to evaluate statistically notable differences between the groups. Chi-square test was conducted to assess the gender distribution differences along the groups. The relationship between IMA and the parameters was investigated using the Pearson correlation test. Descriptive statistics were expressed as mean ± standard deviation. Statistical significance was set at $p < 0.05$ for all analyses. To determine the appropriate sample size, a priori power analyses were conducted using G*Power software (version 3.1.9.7). For the independent samples t-test, with parameters set at $\alpha = 0.05$, an effect size of $d = 0.5$, and a statistical power of 0.95, the minimum required sample size to compare two groups was calculated as 176. The study included 202 participants, exceeding the required threshold to ensure sufficient power for the analysis. Similarly, for the Pearson correlation analysis, with a significance level of $\alpha = 0.05$, an effect size of $r = 0.30$, and a power of 0.80, the minimum sample size needed was 178. Since the actual sample size was 202, the study had adequate power to detect meaningful correlations.

Results

The mean age was 30.42±11.41 years in the control group and 27.76±11.52 years in the group with insulin resistance. The mean IMA was 0.37±0.10 (ABSU) in the control group and 0.39±0.17 (ABSU) in the group with insulin resistance (Table 1).

Table 1. Comparison of parameters between groups.

Parameters	Insulin resistant group (n=101)	Control group (n=101)	p-value
Age (years)	27.76 ±11.52	30.42±11.41	0.10[a]
Gender (Female/Male)	75/26	76/25	0.99[b]
IMA (ABSU)	0.39±0.17	0.37±0.10	0.27[a]
Glucose (mg/dL)	91.94±7.98	88.64±5.94	**<0.01[a]**
Insulin (pmol/L)	21.56±10.06	7.39±2.51	**<0.001[a]**
HOMA-IR	4.89±2.39	1.61±0.55	**<0.001[a]**

Parameters were compared between the two groups. The gender parameter was compared with Chi-square test and other tests were compared with Independent T-Test. P-values less than 0.05 are considered statistically significant for Independent T-Test[a] and Chi-Square test[b] and are shown in bold. Continuous variables are presented as mean ± standard deviation. Abbrevations: IMA, ischemia modified albumin; HOMA-IR, homeostasis model assessment for insulin resistance; ABSU, absorbance unit.

Table 2. Correlation analysis of IMA with other parameters.

Parameters	r-value	p-value
Age	-0.02	0.81
Glucose	-0.11	0.11
Insulin	-0.02	0.74
HOMA-IR	-0.04	0.60

Pearson correlation test was used to determine relation between IMA and parameters. P-values less than 0.05 are considered statistically significant for Pearson correlation test. None of the parameters showed statistically significant correlation with IMA. Abbrevations: IMA, ischemia modified albumin; HOMA-IR, homeostasis model assessment for insulin resistance; r-value. Pearson correlation coefficient.

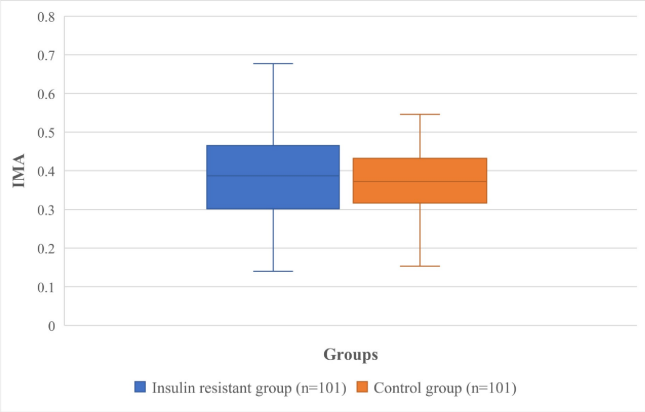

Figure 1. Box plot showing the distribution of IMA values according to groups. Abbrevations: IMA, ischemia modified albümin.

It is found that there was no statistically remarkable difference between the control group and the group with insulin resistance regarding age and IMA (p=0.10 and p=0.27 respectively) (Figure 1).

There was a statistically remarkable disparity between the control group and the group with insulin resistance regarding glucose, insulin, and HOMA-IR parameters ($p<0.01$ for glucose, $p<0.001$ for insulin and HOMA-IR).

It is found that there was no statistically notable correlation between IMA and age, glucose, insulin, and HOMA-IR (p=0.81, p=0.11, p=0.74 and p=0.60 respectively) (Table 2).

Discussion

Although IR is a hallmark of T2DM, IR can be seen in many clinical conditions and on its own. Oxidative stress plays a vital role in the pathology of IR. IMA draws attention as an important parameter to indicate oxidative stress. This study inspected the relation between IMA and IR in a cohort aged 15-60 years. Although the IMA levels were higher in the IR group than in the control group, the disparity was not statistically significant. There was also no notable correlation between IMA and HOMA-IR.

IR alone has been established as a separate risk factor for endothelial dysfunction, cellular damage and cardiovascular events, even in patients without T2DM [12, 14]. Gast KB et al. conducted a meta-analysis of 65 (involving 516,325 participants) published between 2011 and 2012 and found possible relationships between cardiovascular disease (CVD) and fasting glucose, fasting insulin and HOMA-IR values. This study found that cardiovascular disease (CVD) risk was greater when HOMA-IR increased by one standard deviation compared to when fasting glucose or insulin concentration increased by one standard deviation. Additionally, research suggests that including HOMA-IR in a cardiovascular risk prediction model could be advantageous [15]. Mottillo and colleagues also evaluated the relationship between metabolic syndrome and CVD. They conducted a meta-analysis of 87 studies (involving 951,083 participants) published until 2009. They found that all components of CVD and metabolic syndrome were separately related. They reported that the overall risk is higher than the sum of the individual risks of the components [16].

In a study conducted by Yigitbası et al., they examined IMA and oxidative stress parameters in obese and IR, obese and non-IR, and control groups. They found that obesity or IR had no effect on IMA and no notable disparity between the groups regarding the oxidative stress index [17]. In a study conducted by Ates et al. in obese women with and without IR, it was found that the IMA value was lower in the group with IR. They also found no significant correlation between IMA and IR [18]. In a study by Kazanis et al. on postmenopausal women, IMA was positively correlated with body mass index, insulin and HOMA-IR. They also found that obesity was the strongest determinant of IMA levels in regression analysis [19].

There are varying results in studies in the literature. In our study, we found no significant difference among the groups regarding IMA and no correlation between IMA and HOMA-IR. This may be attributed to the insufficient number of patients with high HOMA values. The mean HOMA in our patient group is 4.89 (±2.39). Only 5 of our patients have an HOMA value above 10. In addition, we could have obtained more precise results if we had included the body mass indexes of our sample group in the study. Moreover, we could have obtained more precise results if we could have followed the patients for a while.

Previous studies have reported elevated IMA levels in various conditions associated with insulin resistance and diabetes mellitus (DM). Reddy et al. found high IMA levels in diabetic patients, particularly in those with retinopathy [20]. D'Souza et al. also reported remarkably increased IMA levels in T2DM patients, both with and without complications [21]. Similarly, Gulpamuk B et al. observed higher IMA values in diabetic patients with proliferative retinopathy, suggesting its utility as an indicator for monitoring tissue ischemia in DM [22].

Recent studies have highlighted the relationship between IMA and inflammation, as evidenced by high levels of high-sensitivity C-reactive protein (hs-CRP). In patients with T2DM, both IMA and hs-CRP levels were observed to be significantly elevated in relation to healthy controls, suggesting that hyperglycemia and inflammation may contribute to the observed increase in IMA levels. The positive correlation between hs-CRP and IMA levels indicates that inflammation, alongside oxidative stress, plays a significant act in the pathophysiology of ischemia and related complications in diabetic patients. This association underscores the complex interplay between oxidative stress, in-

flammation, and the progression of insulin resistance [23]. Considering that the IR pattern is well established in DM and that conditions other than IR, such as accumulation of advanced glycation end products and chronic hyperglycemia-induced activation of the polyol pathway, contribute to oxidative stress, IMA is expected to be elevated in DM patients. Most studies on this topic analyze the connection between IR and IMA in patients with DM. The novelty and strong aspect of our study is that it applied the diagnosis of DM as an exclusion criterion and inspected the connection between IR and IMA only in the presence of IR. It is the first study in this category. In addition, applying strict exclusion criteria is another strong aspect of our study. We think that our study will also shed light on future prospective studies.

Conclusion

In conclusion, IR is a serious condition. IR can lead to complications even when seen independently rather than as part of T2DM or other clinical conditions. More comprehensive and prospective studies are required.

Ethical approval

Ethical approval was granted by the Ethics Committee of Ankara Dr. Sami Ulus Training and Research Hospital on April 20, 2022, under protocol number E-2022/04-326.

References

1. Lee SH, Park SY, Choi CS. Insulin Resistance: From Mechanisms to Therapeutic Strategies. *Diabetes Metab J.* 2022 Jan;46(1):15-37.
2. Szablewski L. Insulin Resistance: The Increased Risk of Cancers. Curr Oncol. 2024 Feb 13;31(2):998-1027.
3. Onyango AN. Cellular Stresses and Stress Responses in the Pathogenesis of Insulin Resistance. *Oxid Med Cell Longev.* 2018(7):1-27. doi: 10.1155/2018/4321714.
4. Sędzikowska A, Szablewski L. Insulin and Insulin Resistance in Alzheimer's Disease. Int J Mol Sci. 2021 Sep 15;22(18):9987.
5. Li H, Ren J, Li Y, Wu Q, Wei J. Oxidative stress: The nexus of obesity and cognitive dysfunction in diabetes. *Front Endocrinol (Lausanne).* 2023 Apr 3;14:1134025. doi: 10.3389/fendo.2023.1134025.
6. Erdemli Z, Erdemli ME, Gul M, Altinoz E, Gul S, Kocaman G, Kustepe EK, Gozukara Bag H. Ameliorative effects of crocin on the inflammation and oxidative stress-induced kidney damages by experimental periodontitis in rat. *Iran J Basic Med Sci.* 2021 Jun;24(6):825-832.
7. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev.* 2016 Sep 22;2016:7432797. doi: 10.1155/2016/7432797.
8. Kasai S, Kokubu D, Mizukami H, Itoh K. Mitochondrial Reactive Oxygen Species, Insulin Resistance, and Nrf2-Mediated Oxidative Stress Response-Toward an Actionable Strategy for Anti-Aging. *Biomolecules.* 2023 Oct 19;13(10):1544.
9. Coverdale JPC, Katundu KGH, Sobczak AIS, Arya S, Blindauer CA, Stewart AJ. Ischemia-modified albumin: Crosstalk between fatty acid and cobalt binding. *Prostaglandins Leukot Essent Fatty Acids.* 2018 Aug;135:147-157.
10. Shevtsova A, Gordiienko I, Tkachenko V, Ushakova G. Ischemia-Modified Albumin: Origins and Clinical Implications. *Dis Markers.* 2021 Jul 19;2021:9945424. doi: 10.1155/2021/9945424.
11. Kaefer M, Piva SJ, De Carvalho JAM, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem.* 2010;43(4-5):450-454.
12. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C, Zuñiga FA. Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol.* 2018 Aug 31;17(1):122.
13. Zoroddu S, Zinellu A, Carru C, Sotgia S. Analytical Insights into Methods for Measuring Ischemia-Modified Albumin. *Molecules.* 2024;29(19):4636.
14. Laakso M, Kuusisto J. Insulin resistance and hyperglycaemia in cardiovascular disease development. *Nat Rev Endocrinol.* 2014 Mar;10(5):293–302. doi: 10.1038/nrendo.2014.29
15. Gast KB, Tjeerdema N, Stijnen T, Smit JW, Dekkers OM. Insulin resistance and risk of incident cardiovascular events in adults without diabetes: meta-analysis. *PLoS One.* 2012;7(12):e52036.
16. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol.* 2010 Sep 28;56(14):1113-32.
17. Yigitbasi T, Baskin Y, Akgol E, Calibasi Kocal G, Ellidokuz H. Association of ischemia-modified albumin with oxidative stress status and insulin resistance in obese patients. *Rev Rom Med Lab.* 2017;25(3).
18. Ates E, Set T, Karahan SC, Bicer C, Erel O. Thiol/Disulphide Homeostasis, Ischemia Modified Albumin, and Ferroxidase as Oxidative Stress Markers in Women with Obesity with Insulin Resistance. *J Med Biochem.* 2019 Mar 26;38(4):445-451.
19. Kazanis K, Dalamaga M, Kassi E, Nounopoulos C, Manolis AS, Merantzi G, et al. Serum levels of ischemia modified albumin in overweight/obese postmenopausal women: a potential biomarker of atherosclerotic burden associated with oxidative stress. *Maturitas.* 2011 Oct;70(2):182-7.
20. Reddy VS, Agrawal P, Sethi S, Gupta N, Garg R, Madaan H, et al. Associations of FPG, A1C and disease duration with protein markers of oxidative damage and antioxidative defense in type 2 diabetes and diabetic retinopathy. *Eye (Lond).* 2015 Dec;29(12):1585-93.
21. D'Souza JM, D'Souza RP, Vijin VF, Shetty A, Arunachalam C, Pai VR, et al. High predictive ability of glycated hemoglobin on comparison with oxidative stress markers in assessment of chronic vascular complications in type 2 diabetes mellitus. *Scand J Clin Lab Invest.* 2016;76(1):51-7.
22. Gulpamuk B, Tekin K, Sonmez K, Inanc M, Neselioglu S, Erel O, et al. The significance of thiol/disulfide homeostasis and ischemia-modified albumin levels to assess the oxidative stress in patients with different stages of diabetes mellitus. *Scand J Clin Lab Invest.* 2018 Feb-Apr;78(1-2):136-142.
23. Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, et al. Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem.* 2010 Mar;43(4-5):450-4.