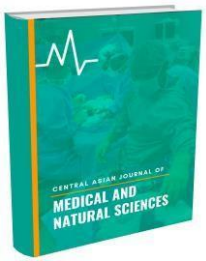

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Article

Estimation the levels of Nuclear Factor Erythroid 2–Related Factor 2 (Nrf-2) in Patients with type II diabetes mellitus

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Abstract: Type II Diabetes Mellitus (T2DM) and its precursor, pre-diabetes, are characterized by chronic oxidative stress and metabolic dysfunction. Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), a master regulator of antioxidant defenses, are crucial in cellular protection. This study aimed to estimate the levels of serum Nrf2 in T2DM patients, pre-diabetic individuals, and healthy controls, and to evaluate their diagnostic potential. A case control was conducted. This study involved 88 individuals, including 46 type II diabetes patients,12 prediabetic and 30 volunteers as a control, with inclusion criteria including normal fasting blood sugar and haemoglobin A1c results. Blood samples were collected from both control and patient groups after 12 hours of fasting. Data were collected via a structured questionnaire covering sociodemographics, medical history, and lifestyle, complemented by clinical evaluations by specialist physicians. Serum lipid panel were measured . Elisa system was used for the detection of Nrf2 level. The study groups were well-differentiated by HbA1c and lipid profiles, with DM and pre-DM groups exhibiting typical glycemic and dyslipidemic patterns. median serum Nrf2 levels were remarkably similar and largely overlapping across all three groups (medians: Healthy 26, Pre-DM 30, DM 29), indicating no significant difference. Nrf2 demonstrated poor diagnostic utility (AUCs < 0.63, p-values > 0.05). Serum Nrf2 levels do not appear to be a reliable prognostic indicator for these conditions. These findings underscore the potential of Nrf2 as a valuable non-invasive screening and monitoring tool in the context of impaired glucose metabolism.

Keywords: Type II Diabetes Mellitus, Nuclear Factor Erythroid 2–Related Factor 2 (Nrf2), Oxidative Stress, Metabolic Dysfunction, Antioxidant Defenses

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1. Introduction

Diabetes Mellitus is a significant metabolic disorder characterized by elevated blood glucose levels, influenced by a complex interplay of genetic and environmental factors [1]. Over 1.4 million Iraqis suffer from diabetes, a condition divided into three types: T1DM, T2DM, and GDM, according to the World Health Organization. [2]. Type II diabetes: The pancreas produces insulin, but in inadequate amounts to meet the body's requirements, or the cells exhibit an improper response to insulin [1].

T2DM is a disease characterized by a nonautoimmune heterogeneously progressive loss of adequate islet β cell insulin secretion frequently in the presence of insulin resistance (IR) and metabolic syndrome (MS) [3]. There are several factors that can be considered

risk factors for type 2 diabetes, including obesity, high blood pressure, lifestyle, age, nutrition, physical activity, In addition to the genetic factor [4]. The nuclear factor erythroid 2-related factor 2 (Nrf2) It is located in the nucleus. A crucial transcription factor plays an essential role in mitigating oxidative stress [5]. Any impairment in the function of this crucial antioxidant significantly contributes to the onset of diabetes and its associated consequences [6-7].

Pre diabetes: The term "prediabetes" denotes persons with glucose levels that are elevated above normoglycemia yet remain below the diagnostic threshold for diabetes, characterized by impaired glucose metabolism. Laboratory markers for identifying prediabetes include fasting blood glucose (FBG), 2-hour post-load blood glucose, or HbA1c. [8]

Nuclear factor erythrocyte-associated factor 2 (Nrf-2) is located in the nucleus and belongs to the Cap n Collar family of transcription factors. It is an essential transcription factor that plays a key role in mitigating oxidative stress and inflammation in cells. Under normal conditions, Nrf-2 is restricted to the cytoplasm by binding to the repressor protein Keap1 (Kelch-like ECH-associated protein 1), which leads to the ubiquitination of Nrf-2 and its subsequent destruction via the proteasome system, preventing its accumulation in the nucleus. Keap1 consists of several cysteine residues that act as sensors of oxidative stress and a negative regulator of Nrf-2 [5]. Modification of cysteine residues in Keap1 triggers and facilitates translocation of Nrf-2 to the nucleus, binds to a DNA sequence where it forms an asymmetric dimer with a small Maf protein, and binds to the antioxidant response element (ARE), stimulating the expression of heme oxygenase 1 (HO-1), with mRNA levels significantly lower in the diabetic group compared to the healthy control group [9]. The small musculoaponeurotic fibrosarcoma (sMaf) proteins form a heterodimer with NRF2 when it is stabilized through its release from Keap1. NRF2 then translocates to the nucleus and binds to target genes at the antioxidant response element (ARE) consensus sequence [10-11]. The transcription of enzymatic antioxidant defense proteins, such as glutathione (GSH; through de novo synthesis of glutamate-cysteine ligase, GCLC), heme oxygenase 1 (HO-1), NAD(P)H dehydrogenase quinone 1 (NQO1), catalase, superoxide dismutase (SOD), thioredoxin, and others, is regulated by NRF2 binding. NRF2 has also been demonstrated to modulate genes associated with metabolic processes, such as malic-enzyme 1 (ME-1), peroxisome proliferator-activated receptor (PPAR), and transaldolase 1 [12].

This study aimed to estimate the levels of serum Nrf2 in T2DM patients, pre-diabetic individuals, and healthy controls, and to evaluate their diagnostic potential.

2. Materials and Methods

A case-control study was conducted from September 2024 to July 2025 in Iraq, at the Imam Hassan Endocrinology and Diabetes Center and Imam Hassan Al-Mujtaba Teaching Hospital in Karbala. This study included 88 individuals, categorized into type 2 diabetes, prediabetes, and controls, with inclusion criteria including normal fasting blood glucose and hemoglobin A1c results. Blood samples were collected after 12 hours of fasting. Data were collected through a structured questionnaire covering sociodemographics, medical history, and lifestyle, and were supplemented by clinical assessments by specialized physicians. Serum lipid panel was measured using a fully automated chemistry analyzer (SMART-120, Geno TEK, USA). An ELISA system was used to detect Nrf2 levels.

Inclusion criteria: Type II Diabetes Mellitus, without insulin therapy, across all age groups and both genders, selected controls exhibiting normal fasting blood sugar and haemoglobin A1c (HbA1c) results.

Exclusion criteria: Diabetes Mellitus I (DM I),Gestational Diabetes Mellitus (GDM), Chronic Heart diseases, Chronic Joints diseases, Cancer, Auto-immunity patient.

Collection of The Blood Samples and running Tests.

A case-control study was conducted , This study included 88 individuals, classified as type 2 diabetes cases, prediabetics, and controls. Inclusion criteria included normal fasting blood glucose, glycated hemoglobin A1c, and serum lipids. Data were collected through a structured questionnaire covering sociodemographics, medical history, and lifestyle, and were supplemented by clinical assessments performed by specialized physicians. Human Nuclear Factor Erythroid 2-Related Factor 2 (also known as NFE2L2) was measured in serum using Sandwich ELISA. Fasting blood glucose was determined by a Clinical chemistry analyzer (Monarch 240, Biorex Diagnostic, United Kingdom). serum lipid concentration ((total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL)and triglycerides (TG)) fully automatic chemistry analyzer (SMART-120, Geno TEK, United States of America).

Ethical Considerations: In order to obtain the necessary ethical permits, the team of the ethical committee, the faculty of medicine, the university of Karbala (The ethical approval letter No.:24-61) , and the Karbala Health Directorates / Karbala-Iraq contributed their support.

Real Statistics Resource Pack for Excel 2016 (Release 7.2) and SPSS version 28.0 (IBM, Chicago, IL, USA) were used for data analysis.. The Shapiro-Wilk test was used to determine normality, and descriptive statistics were employed. The best diagnostic thresholds were found using ROC curve analysis. A p-value of less than 0.05 was deemed significant.

3. Results

The study revealed significant differences in medical history between the diabetic, prediabetic, and non-diabetic groups. 23.9% of diabetic patients had a family history of type 2 diabetes. 41.7% of the prediabetic group had a family history of the disease, compared to 13.33% of the healthy control group. 65.2% of diabetic patients had hyperlipidemia. The incidence of hyperlipidemia (in the prediabetic group) was 0.00%, while the non-diabetic group had hyperlipidemia (6.7%). 45.6% of diabetic patients were physically active. 66.6% of the prediabetic group were physically active, compared to 90.0% of the non-diabetic group. The study included 88% of diabetic patients who were treated with blood sugar-lowering medications. The study also found that 12% of diabetic patients were not receiving treatment and were following a diet.

The study examined serum glycated hemoglobin (HbA1c) levels in participants, revealing a pattern of increasing levels across three groups: Healthy Control, Pre-DM, and DM Patients. The median HbA1c values align with standard clinical diagnostic criteria. The wide range in DM Patients indicates significant heterogeneity in glycemic control, with some diabetic patients showing better control and others very poor control. The tighter ranges for Healthy Controls and Pre-DM suggest a more consistent level of glycemic control within non-diabetic and pre-diabetic populations. See the following Table 1 and Figure 1.

Table 1. Serum Levels of glycated Hemoglobin among study groups.

	DM Patients	**Pre DM group**	**Healthy Control**
Hba1c Median (Mini-Max)	7.9 (5.2-13)	5.9 (5.7-6.1)	4.9 (4.7-5.6)

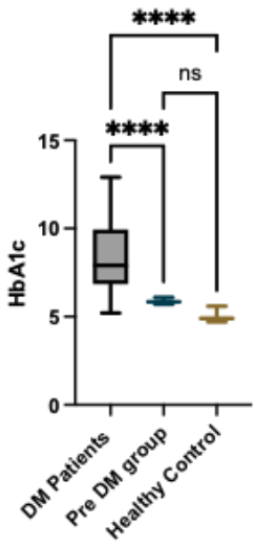
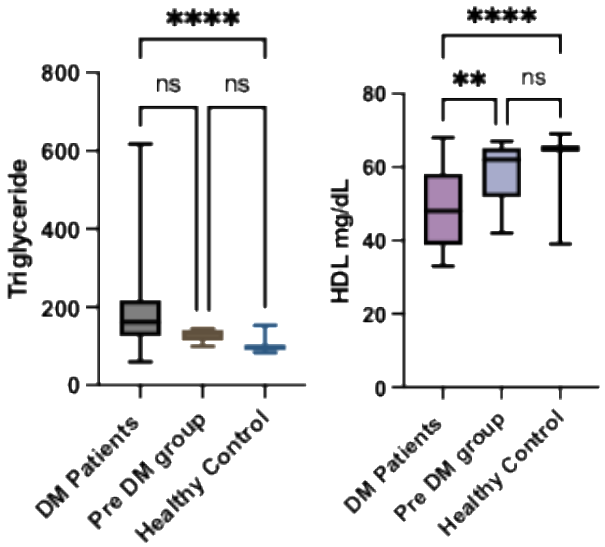


Figure 1. Distribution of Serum Levels of HbA1c among study groups (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$), ****: significant at $p \leq 0.0001$.

The lipid profile data reveals distinct patterns across three groups, indicating progressive metabolic dysregulation from a healthy state to pre-diabetes and overt diabetes. Triglycerides (TG), High-Density Lipoprotein (HDL), and Total Cholesterol are all correlated with the glycemic status of the groups. TG levels show an ascending trend from Healthy Controls to the Pre-DM group, and further to DM Patients, See the following Table 2 and Figure 2.

Table 2. Serum Levels of Lipid profile among study groups.

		DM	*Pre DM*	*Non DM*
TG	**Median (Mini-Max)**	162 (60-617	131 (100-145)	99 (84-153)
HDL	**Median (Mini-Max)**	48 (33-68	62 (42-67)	65 (39-69)
LDL	**Median (Mini-Max)**	93 (21-150	90 (86-94)	68 (50-104)
CHOLESTROL	**Median(Mini-Max)**	188 (90-308	185 (168-193)	164 (124-199)



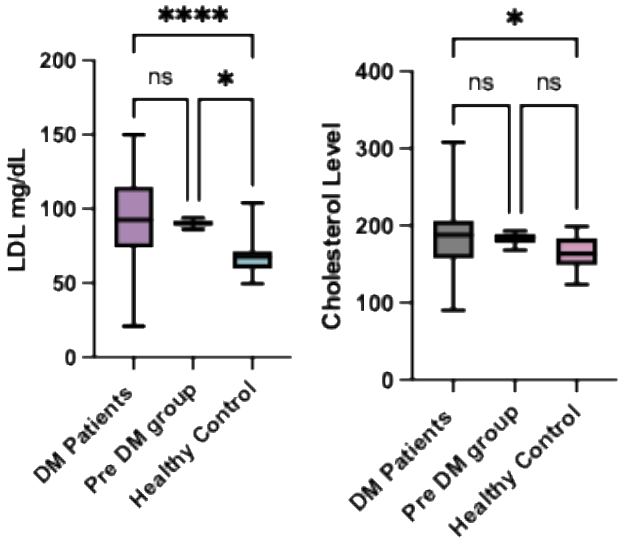


Figure 2. Distribution of Serum Levels of Lipid profile among study groups (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$), ****: significant at $p \leq 0.0001$.

Table 3 & Figure 3 presented the median and range for Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in diabetic patients (DM), pre-DM group, and healthy controls. The median ALT levels showed a descending trend from healthy controls to DM patients, with the highest median ALT in healthy controls, followed by the Pre-DM group, and the lowest in DM Patients. AST showed a descending trend across the groups, with Healthy Controls having the highest median, followed by the Pre-DM group, and DM patients. The Healthy Control group had the widest overall range for AST, possibly due to outliers or a broader spectrum of physiological conditions.

Table 3. Serum Levels of Liver function enzymes among study groups.

		DM Patients	*Pre DM group*	*Healthy Control*
ALT	**Median (Mini-Max)**	20 (9.5-61	29 (10-36	31 (29-32
AST	**Median (Mini-Max)**	23 (15-42	26 (16-33	28 (16-83

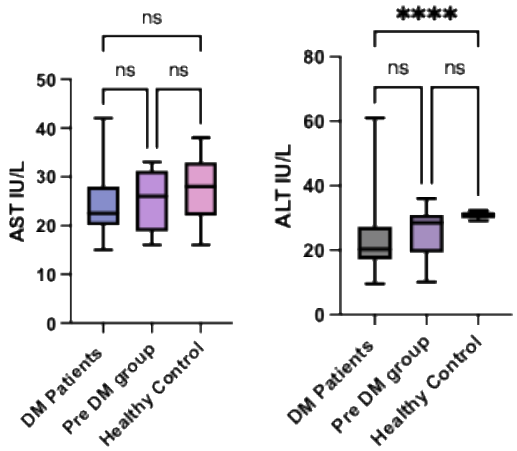


Figure 3. Distribution of Serum Levels of Liver function enzymes among study groups (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$), ****: significant at $p \leq 0.0001$.

While the median values for both ALT and AST show a slightly decreasing trend from healthy controls to DM patients, the broad ranges indicate heterogeneity within each group, suggesting differences in liver health or metabolic compensatory mechanisms among these specific cohorts or a need for more nuanced statistical analysis beyond medians and ranges.

Table 4 and Figure 4 shown the mean and range (minimum-maximum) of serum Nrf2 in diabetic patients, prediabetic patients, and healthy controls. Mean Nrf2 levels are remarkably similar and overlap considerably across all three groups: healthy (26), diabetic patients (29), and prediabetic patients (32). The ranges for all groups are very wide and overlap, indicating a high degree of variability within each group, making it difficult to discern a clear pattern or significant difference based on these mean values alone. Nrf2 does not appear to be a strong discriminator between healthy individuals, prediabetics, and diabetic patients based on mean serum levels. This may indicate that systemic Nrf2 levels in the circulation do not consistently reflect its activity or cellular response within specific tissues relevant to diabetes (such as the pancreas, liver, and muscle), or that its dysregulation manifests differently in diabetes compared to thyroid disease. Serum Nrf2 levels do not show any significant differences between groups associated with diabetes status.

Table 4. Serum Levels of Nuclear respiratory factor 2 (Nrf2) among study groups

		DM Patients	Pre DM	non-DM
Nrf2	Median (Mini-Max)	29 (12-69	30 (12-49	26 (13- 70)

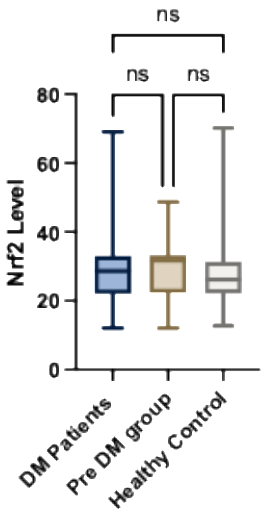


Figure 4. Distribution of Serum Levels of Nrf2 among study groups (Post Hoc ANOVA test was *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$, ***: significant at $p \leq 0.001$), ****: significant at $p \leq 0.0001$.

Table 5 displays the Pearson correlation coefficient (r) and its corresponding p-value for different pairs of biomarkers in the diabetic group. A p-value less than 0.05 is considered statistically significant. Nrf2 levels tended to be lower, suggesting a potential interaction or parallel regulation between these two antioxidant-related molecules. For all other biomarkers (cholesterol, TG, HDL, LDL, HbA1c, ALT, and AST): Nrf2 did not show any statistically significant associations with any of these parameters in the diabetic group (all p-values > 0.05). This key factor in cellular defense against oxidative stress may be

regulated in a somewhat coordinated manner, or antioxidant status may influence cardiovascular health.

Table 5. The correlation coefficient (r) between Serum Levels of Nuclear respiratory factor 2 (Nrf2 among DM group.

Biomarkers	*NRf2/p*	
	®	*P value*
NRf2/p	1[a]	-
Chol	0.097	0.504[NS]
TG	-0.054	0.707[NS]
HDL	-0.053	0.714[NS]
LDL	**0.204**	0.155[NS]
HbA1c	-0.003	0.986[NS]
ALT	0.038	0.793[NS]
AST	-0.061	0.677[NS]

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ®:Correlation Coefficient

Table 6 displays the Pearson correlation coefficient (r) and its corresponding p-value for different biomarker pairs in the prediabetic group. According to the table's definition, p-values less than 0.05 are considered statistically significant.

Table 6. The correlation coefficient (r) between Serum Levels of Nuclear respiratory factor 2 (Nrf2), among Pre-DM group.

Biomarkers	*NRf2/p*	
	®	*P value*
NRf2/p	1[a]	-
Chol	-0.163	0.653[NS]
TG	-0.218	0.545[NS]
HDL	0.094	0.796[NS]
LDL	-0.028	0.939[NS]
HbA1c	-0.032	0.931[NS]
ALT	-0.128	0.027[NS]
AST	-0.527	**0.003[S]**

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant ®:Correlation Coefficient

Correlations with liver enzymes suggest a protective association with Nrf2, but the absence of significant correlations with conventional lipid parameters contrasts with the diabetic group, suggesting dynamic changes in biomarker interactions during the progression of metabolic disease

Table 7 evaluates the ability of Nrf2 to predict the likelihood of an individual falling into the diabetic or prediabetic group, as opposed to the healthy control group. An odds ratio (OR) of 1 indicates no association, an OR greater than 1 indicates increased odds, and an OR less than 1 indicates decreased odds.

Table 7. The binary logistic regression of Serum Levels of Nuclear respiratory factor 2 (Nrf2) among study groups.

Biomarkers	*Groups*	*OR (Lower-Upper)*	*P-Value*
NRf2/p	**DM Patient**	0.994 (0.985-1.004)	**0.016[S]**
	Pre DM	0.990 (0.959-1.021)	**<0.03[S]**

Control	1[a]	-	

p<0.05 considered significantly different, [S]= Significant, [NS]= Non significant

1[a]: reference category is Control

The OR for Nrf2 in diabetic patients was 0.994 (95% CI: 0.985–1.004), with a p-value of 0.016, indicating statistical significance. An OR of 0.994 means that for every one-unit increase in serum Nrf2 levels, the odds of being diabetic (compared to the healthy control group) are 0.994 times higher, or slightly less than 1. This indicates a very weak, almost negligible, inverse association. Despite its statistical significance, the odds ratio is very close to 1, and the confidence interval is slightly above 1, suggesting that Nrf2's ability to distinguish diabetics from healthy controls based on linear increase is very limited in practice.

The odds ratio (OR) for Nrf2 in the prediabetic group was 0.990 (95% CI: 0.959–1.021), with a p-value of less than 0.03, indicating statistical significance. Similarly, an OR of 0.990 implies a very weak inverse association; for every one-unit increase in Nrf2, the probability of being in the prediabetic group (compared to the healthy control group) is 0.990 times greater. Despite its statistical significance, the odds ratio is very close to 1, and the confidence interval includes 1, suggesting that Nrf2 is not a strong discriminator of prediabetes either.

Based on logistic regression analysis as described, Nrf2 does not appear to strongly discriminate between diabetes or pre-diabetes compared to the healthy control group, despite achieving statistical significance with odds ratios very close to 1

Table 8 & Figure 5 evaluates the diagnostic performance of serum Nrf2 levels in identifying diabetic and prediabetic individuals. For the diabetic group, the area under the curve is 0.5257. This indicates that Nrf2's diagnostic accuracy is very poor in distinguishing between diabetic and healthy individuals. Using the cutoff, Nrf2 could correctly identify only 51.02% of individuals with actual diabetes.

Table 8. AUC, optimal threshold, Sensitivity, and specificity of Nrf2 levels among patient groups

Variable	AUP	Sensitivity %	Specificity %	Cut-off points	P
DM	0.5257	51.02	65.52	> 28.58	0.7059
Pre-DM	0.6276	68.97	70.00	< 30.26	0.2

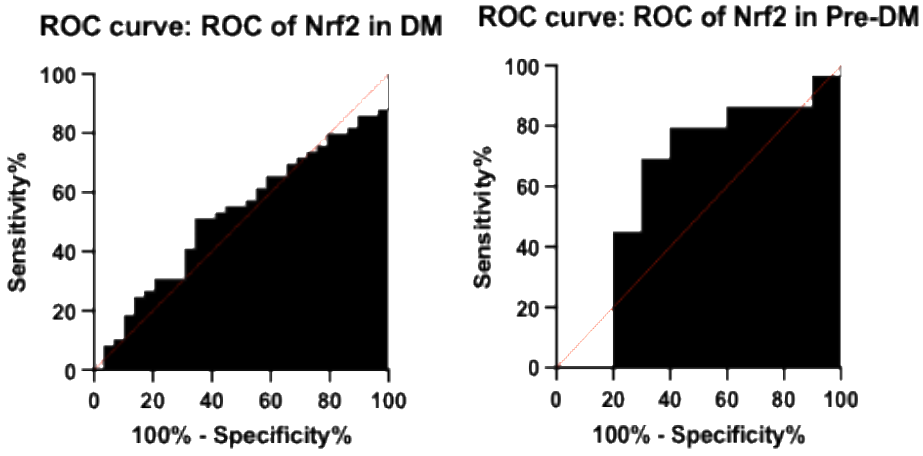


Figure 5. Receiver operating characteristic curve of NRf2/p levels among patients groups.

Nrf2 could correctly identify 65.52% of non-diabetic individuals. This indicates a high rate of false positives. An individual with a serum Nrf2 level above 28.58 is classified as diabetic. This is consistent with the descriptive statistics (Table 3.4), which showed that diabetic patients had a slightly higher mean Nrf2 level (29) compared to healthy controls (26). On the other hand, the diagnostic performance in pre-diabetes (pre-DM) showed an area under the curve (AUC) of 0.6276, but this still indicates poor to moderate diagnostic accuracy for Nrf2 in distinguishing between individuals with pre-diabetes and healthy controls. It is generally considered not clinically useful for differentiation. Nrf2 levels correctly identified 68.97% of individuals with pre-diabetes. Nrf2 levels correctly identified 70.00% of individuals without pre-diabetes. An individual with a serum Nrf2 level below 30.26 is optimally classified as pre-diabetic. Serum Nrf2 levels demonstrate poor diagnostic utility for both diabetes and pre-diabetes. In both cases, the area under the curve values are close to 0.5, and importantly, the p-values are well above 0.05, indicating that Nrf2's ability to discriminate between these patient groups and healthy controls is not statistically significant. Sensitivity and specificity values are relatively low for both classifications. The discrepancy in the cutoff direction for the prediabetic group highlights the lack of clear discriminatory patterns for Nrf2 in this context.

4. Discussion

The study found a clear pattern of HbA1c levels across healthy controls, pre-diabetes, and Type II Diabetes Mellitus patients, indicating a deterioration in glycemic control. HbA1c measures average blood glucose levels over 2-3 months and is a widely accepted diagnostic criterion for diabetes and pre-diabetes. The median values align with major health organizations' diagnostic thresholds, such as <5.7% for non-diabetic, 5.7%-6.4% for pre-diabetes, and ≥6.5% for diabetes [13].

The difference in median HbA1c values between groups highlights the importance of this biomarker in identifying individuals at different glucose metabolism stages [14]. Early detection of pre-diabetes is crucial as individuals in this stage are at a higher risk of developing type 2 diabetes and cardiovascular complications, highlighting the natural continuum of the disease [15]. The clinically relevant HbA1c range in DM patients (5.2%-13%) is influenced by disease duration, treatment efficacy, medication adherence, lifestyle interventions, and diabetes-related complications [16].

The variability in diabetes care necessitates individualized management strategies. Healthy Controls (4.7-5.6%) and Pre-DM group (5.7-6.1%) show a more consistent metabolic state, with stable HbA1c levels in healthy individuals. Pre-diabetes, despite impaired glucose regulation, has not deteriorated as severely as overt diabetes, resulting in a narrower range of elevated but not severely high HbA1c values [13]. In conclusion, the HbA1c data from this study effectively differentiates the three glycemic states, providing clear biochemical evidence for the classification of groups [17].

The lipid profile data reveals patterns of metabolic dysregulation in pre-diabetes and overt diabetes, primarily driven by insulin resistance, a key factor in the pathophysiology of both conditions [18,19]. The study groups showed an ascending trend in Triglyceride (TG) levelsfrom healthy controls (median 99 mg/dL) to the Pre-DM group (131 mg/dL) and significantly higher in DM Patients (162 mg/dL) is a classical feature. Elevated triglycerides in diabetic and pre-diabetic states result from increased hepatic very-low-density, which is a classical feature of diabetic dyslipidemia [20-21]. High-Density Lipoprotein (HDL) showed a descending trend across the groups, with the highest median in healthy controls (65 mg/dL), moderately lower in the Pre-DM group (62 mg/dL), and significantly reduced in DM patients (48 mg/dL). This low HDL, particularly below the clinically desirable threshold (typically >50-60 mg/dL), is another characteristic of diabetic dyslipidemia [13]. Insulin resistance leads to increased catabolism of HDL particles and reduced synthesis of apolipoprotein A-I (apoA-I), a major component of HDL, thereby diminishing its anti-atherogenic functions, such as reverse cholesterol transport [20,22]. The increasing trend

in Low-Density Lipoprotein (LDL) levels from healthy controls (median 68 mg/dL) to Pre-DM (90 mg/dL) and DM Patients (93 mg/dL) is another critical observation. The wide range in LDL among DM patients highlights the diverse metabolic profiles within the diabetic population [23-24].

lipoprotein (VLDL) production and impaired catabolism of triglyceride-rich lipoproteins due to reduced lipoprotein lipase (LPL) activity, both consequences of insulin resistance [25,26]. Total cholesterol levels also showed an ascending trend, mirroring the changes in LDL and reflecting the overall lipid burden. The close medians for total cholesterol between Pre-DM and DM groups further reinforce that significant lipid disturbances are present even before a full diagnosis of diabetes, placing these individuals at heightened cardiovascular risk [13].

The study reveals intriguing patterns in serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels, particularly in diabetic patients. These enzymes are biomarkers for liver function and cellular integrity, with elevated levels indicating hepatocellular injury. [27].

The study reveals a significant subset of diabetic patients with type 2 diabetes (DM) exhibit elevated ALT levels, possibly due to NAFLD/NASH, which affects 60-70% of patients [27]. This variability suggests that despite a low median, a significant proportion of individuals are experiencing liver pathology. While ALT and AST are useful screening tools, their median values alone should not rule out liver pathology in high-risk populations. The liver enzyme data, particularly lower median ALT and AST, may reflect NAFLD progression, metabolic adaptations, and individual variability [28].

Serum Nrf2 levels reveal an individual's antioxidant status at different blood glucose levels. Nrf2 is a crucial regulator of cellular defense against oxidative stress and electrophilic effects. Upon activation, it binds to antioxidant response elements in promoters of genes encoding antioxidant enzymes and detoxification proteins [29-30]. Given the significant role of oxidative stress in diabetes pathogenesis, significant changes in Nrf2 levels or activity may occur.

However, the results of this study indicate that mean serum Nrf2 levels are remarkably similar and largely overlap between healthy controls [26], diabetic patients [29], and the prediabetic group [32]. Furthermore, the wide ranges and significant overlap between all groups emphasize the presence of significant individual variation, making it difficult to discern a clear pattern or statistically significant difference based on these mean values alone. This suggests that circulating Nrf2 levels, as measured in this study, do not appear to be a strong discriminator between healthy, prediabetic, and diabetic individuals.

This lack of a clear pattern of serum Nrf2 expression may be attributed to several factors, including complex regulation and tissue specificity. Nrf2 activity is tightly regulated at multiple levels (transcription, translation, posttranslational modifications, and nuclear translocation). Serum levels may not accurately reflect specific Nrf2 activity within relevant target tissues (e.g., pancreatic beta cells, liver, muscle, and adipose tissue), where its dysregulation in diabetes is more pronounced [31,32]. Nrf2 activation is often an acute response to stress. In chronic conditions such as diabetes, there may be an initial compensatory upregulation followed by chronic suppression or functional impairment that is not reflected in total serum levels [33].

The binary logistic regression analysis reveals that serum Nrf2 levels are independent predictors of DM patients and pre-DM groups compared to healthy controls. Nrf2 is a statistically significant predictor for both DM patients (OR = 0.994, $p = 0.016$) and the Pre-DM group (OR = 0.990, $p < 0.03$), confirming earlier descriptive statistics. This finding supports earlier descriptive statistics.

The study found that routine measurement of Nrf2 levels in serum may not be an effective biomarker for diabetes or pre-diabetes due to its complex and compartmentalized nature. The results suggest that Nrf2 dysfunction, a crucial intracellular regulator of antioxidant defenses, is implicated in diabetes pathophysiology [31- 34]. The ROC curve

analysis showed poor diagnostic accuracy for Nrf2, with AUCs close to 0.5 and non-significant p-values [35].

5. Conclusion

Serum nuclear erythroid factor 2-related factor 2 (Nrf2) showed no significant differences between groups and showed little diagnostic utility. This suggests that systemic Nrf2 levels, as measured in this study, do not consistently reflect its intracellular bioactivity or its dynamic tissue-specific responses to oxidative stress in diabetes.

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