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RESEARCH ARTICLE

CLINICAL INVESTIGATION OF SERUM PROTEIN ELECTROPHORESIS IN TYPE 2 DIABETES MELLITUS

Akeem Olayinka Busari¹, Ibrahim Saheed Saka², Renata Teritin Perdomo³, Malson Neilson de Lucena⁴, Sulaiman Adebayo Nassar⁵, Imam Malik Kabir⁶, Munirudeen Ibrahim⁷ and Shefiat Bashir⁸

1. Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brasil
2. Department of Medical Laboratory Science, Kwara State University, Malete, Nigeria
3. Laboratório de Biologia Molecular e Cultura Celular, Laboratório de Fluidos Biológicos e Citologia Clínica, Farmácia-Facfan, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil
4. Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Brasil
5. Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomoso, Nigeria
6. Department of Medical Laboratory Science, Bayero University, Kano, Nigeria
7. Department of Medical Laboratory Science, Kwara State University, Malete, Nigeria
8. Department of Medical Laboratory Science, Al-Hikmah University, Ilorin, Nigeria

Abstract

Background: Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. Circulating proteins in the pathogenesis of T2DM have been implicated with different degrees of glucose intolerance.

Objective: This study assessed the serum protein pattern in T2DM patients.

Method and Material: A comparative cross-sectional study was conducted on 120 participants comprising 60 T2DM patients and 60 non-diabetic individuals in General Hospital Ilorin, Kwara State, after the ethical approvals were obtained from both the Kwara State Ministry of Health and General Hospital Ilorin with the reference numbers ERC/MOH/2022/04/089 and GHI/IRC/246/VOL1/03, respectively. About 5 ml of venous blood sample was collected from each participant after an overnight fast of 10–12 hours for glycated hemoglobin and serum protein estimation. The glycated hemoglobin was estimated using the fluorescence immunoassay technique, while protein and albumin were estimated spectrophotometrically using biuret and bromocresol green dye-binding methods, respectively. The serum protein pattern was determined by serum protein electrophoresis.

Results: A total of 60 T2DM patients were included in the study; 8 (13.3%) showed hypergammaglobulinemia, 7 (11.7%) demonstrated hyperbetaglobulinemia, and 13 (21.7%) revealed hyperalpha-2-globulinemia serum protein patterns compared to normal serum protein patterns observed in the non-diabetic individuals. Also, a significant increase ($p < 0.05$) in glycated hemoglobin was observed in T2DM patients compared to non-diabetic individuals. However, no significant difference ($p > 0.05$) was observed in serum protein, albumin, globulin, and A/G ratio of T2DM patients compared to non-diabetic individuals.

Conclusions: This study observed that diabetes mellitus significantly affects positive acute phase proteins, such as beta, alpha-2, and gamma globulin, compared to negative acute phase proteins, such as albumin, globulin, and the albumin/globulin ratio.

Keywords: Glycated hemoglobin, serum protein electrophoresis, type 2 diabetes mellitus.

Corresponding Author: Akeem Olayinka Busari, Institute of Biological Sciences, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil, Email: busakeem@yahoo.com

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. In 2021, 366 million people were reported to have diabetes mellitus worldwide, with more than 23 million people living in the United States. This number was projected to reach 552 million by 2030.¹ T2DM is associated with chronic complications affecting many organs, including microvascular, macrovascular, and neuropathic complications. The microvascular complications include retinal, renal, and possibly neuropathic disease; the macrovascular complications include coronary artery and peripheral vascular disease; and diabetic neuropathy affects autonomic and peripheral nerves.² As part of measures to limit the development of its devastating complications, it requires long-term medical attention. It is a disproportionately expensive disease; according to the American Diabetes Association (ADA), in 2022, the annual cost of diabetes in the United States reached \$412.9 billion, with \$306.6 billion representing direct medical costs and \$106.3 billion representing indirect costs. Medical expenditures for people with diagnosed diabetes were 2.6 times greater, on average, than those expected had they not had diabetes.³

Type 2 diabetes is a global health problem posing substantial burdens on human health, with reports from studies of increasing prevalence globally.^{4,5} In 2021, the global diabetes prevalence in 20–79-year-olds was estimated to be 10.5% (536.6 million people), rising to 12.2% (783.2 million) in 2045. Diabetes prevalence was similar in men and women and was highest in those aged 75–79 years. The estimated prevalence was higher in urban (12.1%) than rural (8.3%) areas and in high-income (11.1%) compared to low-income countries (5.5%) in 2021. The greatest relative increase in the prevalence of diabetes between 2021 and 2045 is expected to occur in middle-income countries (21.1%) compared to high-income (12.2%) and low-income (11.9%) countries.⁶ In Nigeria, the most populous country in Africa, the prevalence of T2DM has been high and is still increasing, with the country widely reported as having Africa's highest burden of diabetes, with a 2013 IDF global study reporting a prevalence of 5% estimate for Nigeria, accounting for 3.9 million cases among persons aged 20–79 years.⁴

The increasing prevalence of T2DM has called attention to finding causes for deranged glucose homeostasis. Hypotheses implicating circulating proteins in the pathogenesis of T2DM have been tested by measuring levels of these proteins in serum obtained from persons with different degrees of glucose intolerance.⁷ Studies have corroborated the crucial role of inflammation in T2DM pathologies, with low-grade inflammation characterized by elevated inflammatory protein levels linked with T2DM pathogenesis.⁸ This low-grade systemic inflammation releases Tumor Necrosis Factor (TNF), making cells more insulin resistant, leading to diabetes associated with hyperglycemia and glycated end products.⁹

In general, concentrations of these plasma protein changes help detect inflammation and can often be used to monitor the progress of the inflammation or its response to treatment. The increment and decrease of these acute-phase proteins have been depicted in traditional serum protein electrophoresis. Generally, five bands are shown in serum proteins' cellulose acetate sheet electrophoresis. They are albumin, α 1, α 2, β -, and γ -bands starting from the anode to the cathode ends due to variability in the mobility of different serum proteins and quantification by densitometer.⁹ Thus, this study aims to assess the serum protein pattern using cellulose acetate electrophoresis in T2DM patients.

MATERIAL AND METHODS

Study Design

A comparative cross-sectional study was conducted on 120 participants, comprising 60 diagnosed T2DM patients attending the diabetic clinic and 60 apparently healthy individuals diagnosed as non-diabetic individuals within the General Hospital Ilorin.

Ethical Approval

Approval for this research was obtained from both the Research Ethics Committees of Kwara State Ministry of Health and General Hospital Ilorin with reference numbers ERC/MOH/2022/04/089 (MOH/KS/777/905) and GHI/IRC/246/VOL.1/03, respectively. The research was carried out in accordance with the Helsinki Declaration of 1975, as reviewed in 2022. Both verbal and written informed consent were obtained from the respondents before their recruitment into the research study

Inclusion Criteria

- i. Type 2 diabetic patients aged 20 to 60 years and age-matched non-diabetic individuals.
- ii. Participants who gave consent.

Exclusion Criteria

- i. Individuals with infectious and inflammatory diseases such as hepatitis B, C, and HIV/AIDS.
- ii. Individuals with complications such as neuropathy, retinopathy, and nephropathy.
- iii. Those who did not give consent.

Sampling Technique

The study was explained in detail, including the benefits and risks to the prospective participants. A convenient random sampling technique was used to recruit voluntary participants for the study. The volunteers were assured of their confidentiality for participation in the study, and they could withdraw at any point if they wished. Both written and verbal informed consent were obtained from each participant before sample collection. The participants were made to fast overnight for a period of 10 to 12 hours before sample collection. All participants were requested to be seated at the clinic 15 minutes before sample collection, which commenced at 8:00 am. All participants received clear instructions to produce a spot urine sample to screen out overt proteinuria. A semi-structured questionnaire was administered to the qualified participants to obtain their socio-demographic characteristics. The height and weight of each participant were measured and recorded to the nearest 0.1 m (meter) and kg (kilogram), respectively, using a standard stadiometer and well-calibrated weight scale manufactured by ONE-Mi. Their body mass index (BMI) was calculated by dividing the weight measured in the nearest kg by the square of the height in meters of each participant and recorded in kg/m².

Blood Sample Collection and Processing

About 5 ml of venous blood sample was collected from the antecubital fossa of the study participants after an overnight fast (10–12 hours). Two milliliters (2 mL) of the blood sample were dispensed into an ethylene diamine tetraacetic acid (EDTA) tube, and the other 3 mL into plain tubes. The samples collected into the plain bottles were allowed to clot and spun at 3,000 revolu-

tions per minute (RPM) for 5 minutes, and the serum was separated from the clotted whole blood sample and labeled and stored at -20°C before sample analysis.

Measurement of glycated hemoglobin

HbA1c was assayed using the fluorescence immunoassay method by Finecaré™.¹⁰

Principle

The quantitative determination of HbA1c is based on fluorescence immunoassay technology. It uses the sandwich immunodetection method to measure the percentage of HbA1c in human blood. After mixing with the sample and buffer, the sample mixture is added to the sample well of the test cartridge, and the fluorescence-labeled detector HbA1c antibody binds to HbA1c in the blood specimen. As the sample mixture migrates on the nitrocellulose matrix of the test strip by capillary action, the complexes of the detector antibody and HbA1c are captured by the HbA1c antibody that has been immobilized on the test strip. The fluorescence-labeled detector Hb antibody binds to Hb in the blood specimen; the complexes are captured by the Hb antibody immobilized on the test strip. Signal fluorescence intensity is proportional to the concentrations of HbA1c and Hb in blood specimens. The ratio between the inflorescent signals of HbA1c and Hb is the ratio between HbA1c and Hb.

Quantitative Estimation of Serum Protein

The serum protein was estimated using the Biuret method modified by Cheesbrough.¹¹

Principle

Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum samples forms a blue-colored complex when treated with cupric ions in an alkaline solution. The intensity of the blue color is proportional to the protein concentration.

Albumin Determination Method

The serum albumin was estimated using the Bromocresol Green (BCG) dye binding method modified by Cheesbrough.¹¹

Principle

Albumin (pI 4.9) at pH 4.2 is sufficiently cationic to bind the anionic dye bromocresol green (BCG) to form a blue-green-colored complex.

PH 4.2

Albumin + BCG -----> BCG complex

The intensity of the blue-green color is directly proportional to albumin concentration in the specimen. It is determined by measuring the increase in absorbance at 630 nm.

Determination of Serum Protein Electrophoresis

The serum protein fractions were separated using the cellulose acetate electrophoresis method modified by Varley.¹²

Principle

Proteins are large molecules composed of covalently linked amino acids. Depending on electron distributions resulting from covalent or ionic bonding of structural subgroups, proteins have different electrical charges at a given pH. In the Helena Serum Protein procedure, the proteins are separated according to their respective electrical charges at pH 8.8 on a cellulose acetate plate using both the electrophoretic and electroendosmotic forces present in the system. After the proteins were separated, the plate was placed in a solution of sulfosalicylic acid and Ponceau S (to stain the protein bands). The staining intensity is related to protein concentration. After dehydration in methanol, the plate background is then rendered transparent by treatment with a clearing solution.

Data Analysis

Data obtained were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 23 statistical software. All measured data were presented as mean \pm SD. An independent t-test was used to compare the means of serum protein, albumin, and glycated hemoglobin between T2DM patients and non-diabetic individuals. Chi-square (X^2) was used to compare the percentage of serum electrophoretic patterns between T2DM and non-diabetic individuals, as well as body mass index (BMI) among the T2DM patients. The level of significance was considered at $p < 0.05$.

RESULTS

Socio-Demographic Characteristics of the Study Participants

The socio-demographic characteristics of enrolled participants are presented in Table 1. A total of sixty (60) participants with type 2 DM (T2DM) were recruited, and 60 age-sex-matched non-diabetic individuals were recruited for this study. The mean

age for the non-DM individuals and T2DM patients was approximately 49.15 and 51.60 years, respectively. The male-to-female ratios were similar and comparable for the non-DM individuals and T2DM patients (0.7 and 1.3), respectively. Also, the body mass index (BMI) of non-DM individuals and cases was computed and was comparable. All participants were of Black ethnicity.

Comparison of SPE Pattern Between T2DM and Non-DM Individuals

The number and percentage of protein band patterns were visually enumerated in T2DM and non-DM individuals; the result is shown in Table 2. It was observed that, out of the 60 T2DM samples examined, 8 (13.3%) revealed a hypergammaglobulinemia pattern, 7 (11.7%) manifested hyperbetaglobulinemia, and 13 (21.7%) hyperalpha-2-globulinemia. The outcome of the chi-square analysis showed that the pattern of only hyperalpha-2-globulinemia was significantly elevated in T2DM.

Comparisons of Biochemical Parameters Between T2DM and Non-DM Individuals

The comparison of glycated hemoglobin and serum protein parameters between T2DM and non-DM participants was presented in Table 3. There was a statistically significant ($p < 0.01$) increase in glycated hemoglobin in T2DM patients compared to non-diabetic individuals; however, there were no significant differences in the level of proteins in T2DM participants compared to non-DM controls ($p > 0.05$).

Comparison of SPE Pattern In T2DM Based on BMI

The number and percentage of protein band patterns were visually enumerated among the T2DM participants concerning their BMI, and the result is presented in Table 4. It was observed that out of 8 T2DM participants with hypergammaglobulinemia, 7 (87.5%) had a BMI greater than 25 kg/m². Also, out of 7 with hyperbetaglobulinemia, 4 (57.2%) and 3 (42.8%) were in the optimal BMI and overweight categories, respectively. Furthermore, out of 13 T2DM participants with hyperalpha-2-globulinemia, 11 (84.6%) were overweight. The outcome of the chi-square analysis showed that the patterns of hypergammaglobulinemia and hyperalpha-2-globulinemia were significantly elevated with BMI in T2DM patients.

DISCUSSION

The comparison of socio-demographic data of the study participants was based on age, sex, BMI, and blood pressure. There was no statistically significant difference ($p > 0.05$) between the ages of non-DM individuals compared to T2DM patients, contrary to an earlier study by Sattar et al.¹³, who reported that the age of onset of T2DM varies from one locality to another. Environmental and lifestyle factors are part of the condition that determines the age of onset. The body mass index (BMI) was compared between T2DM patients and non-diabetic individuals, although the pre-morbid weights of the study subjects were not known. T2DM participants were categorized into two groups based on their BMI: <25 and $25-30$ kg/m². It was observed that the T2DM patients had a significantly higher BMI compared to the non-DM participants ($p = 0.001$). This finding is in agreement with a previous study by Schofield¹⁴, who reported that DM is usually associated with dyslipidemia, which is mostly responsible for the tendency to become overweight.

We observed that the serum protein pattern was significantly altered in T2DM patients. The alteration pattern in serum proteins observed included a significant increase in gamma bands (hypergammaglobulinemia), beta bands (hyperbetaglobulinemia), and alpha-2 bands (hyper-alpha2-globulinemia). This agrees with the study of Zhang et al.¹⁵, who reported that hepatobiliary diseases such as necrosis, inflammation, or non-alcoholic hepatic steatosis could be induced by diabetes mellitus, and the mortality rate caused by advanced-stage hepatic diseases in diabetic patients is higher than cardiovascular causes; therefore, hepatic injuries can develop in diabetes mellitus, and these hepatocellular injuries probably play a striking role in serum protein electrophoretic pattern alteration in diabetes mellitus. Some mechanisms, such as oxidative stress, chronic hyperglycemia, chronic inflammation, lipotoxicity, and other mechanisms, can induce pancreatic β -cell dysfunction in diabetes mellitus.¹⁶ An investigation has demonstrated that oxidative stress and hepatocellular fat accumulation (hepatic steatosis) can play a significant role in hepatic disorders caused by diabetes mellitus.¹⁷ It has also been investigated that endoplasmic reticulum stress (ERS) is another important factor in pancreatic β -cell dysfunction in diabetes mellitus.¹⁸ Pro-inflammatory cytokines such as TNF- α and interleukin-1 β (IL-1 β) can activate

a signaling cascade of cell death, including ASK-1 and p38 MAPK in the diabetic liver, and this could lead to hepatocellular damage in diabetes mellitus.¹⁹ These hepatic damages induced by diabetes mellitus could lead to many alterations in serum protein concentration, especially the serum protein electrophoretic pattern.

In addition, this study observed no significant change in total protein, albumin, globulin, and A/G ratio in T2DM patients and non-diabetic individuals. This contradicts the findings of Hasan et al.²⁰ and Su et al.²¹, who found a significant decrease in these acute-phase proteins in diabetes patients.

CONCLUSION

The observed serum protein patterns significantly raised in T2DM patients were hypergammaglobulinemia, hyperbetaglobulinemia, and hyperalpha2-globulinemia. There was no significant alteration in total protein, albumin, globulin, or A/G ratio. This indicates diabetes mellitus affects more positive acute phase proteins, such as beta, alpha-2, and gamma, than negative acute phase proteins, such as albumin, globulin, and the A/G ratio.

Recommendation

This study suggests that serum protein electrophoresis could be evaluated as part of diagnostic and prognostic tests for monitoring type 2 diabetic patients.

Limitations of this Study

The limitations of this study that warrant consideration are as highlighted as follows:

- i. The relatively small sample size and single-center design may limit the generalizability of the results to broader and more diverse populations.
- ii. The cross-sectional nature of the study precludes any causal inference between the observed alterations in serum protein electrophoretic patterns and the pathogenesis or progression of type 2 diabetes mellitus (T2DM).

Future Research

Future studies should therefore employ larger, multicenter cohorts with prospective longitudinal designs to confirm and expand upon these findings.

DECLARATIONS**Acknowledgment**

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Conflict of Interest

No conflict of interest.

Contribution of Authors

All authors contributed to this manuscript.

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ANNEX

TABLE 1. Socio-Demographic Characteristics of Study Group.

Characteristic		Non-DM n (60)	Type 2 DM n (60)	P value
Age (years)	20-60	16 (26.7)	17 (28.3)	1.000
	>60	44 (73.3)	43 (71.7)	1.000
Sex	M	28 (46.7)	29 (48.3)	1.000
	F	32 (53.3)	31 (41.7)	1.000
BMI (Kg/m ²)	18-25.9	49 (81.7)	28 (46.7)	0.029*
	>25.9	11 (18.3)	32 (53.3)	0.542
Systolic		128.44±20.4	145.64±28.3	0.002*
Diastolic		78.24±13.8	82.62±15.3	0.432

TABLE 2. Comparison of SPE pattern between T2DM and non-DM control.

Pattern	Non-DM	T2DM (%)	χ^2 value	P value
Hyper-gammaglobulinemia	1 (1.7)	8 (13.3)	1.27*	<0.05
Hypo-gammaglobulinemia	0	0	NA	NA
Hypo-albuminemia	0	0	NA	NA
Hyper-betaglobulinemia	0	7 (11.7)	2.03*	<0.05
Hypo-alphaglobulinemia	0	0	NA	NA
Hyper-alpha2-globulinemia	0	13 (21.7)		<0.05

Note. Values are in (%). The calculated Chi-square values were compared with the critical χ^2 statistic value for $p = 0.05$ (95% confidence level) with a degree of freedom of 1 (3.8). NA = Not applicable.

*significant difference

TABLE 3. Comparison of serum protein parameters between T2DM and Non-DM Individuals.

Parameters	Non-DM	T2DM	P value
Glycated hemoglobin	4.05 ± 1.21	6.61±2.01	0.001*
Total Protein (g/L)	63.00 ± 14.17	74.98 ± 7.9	0.128
Albumin (g/L)	39.31 ± 7.68	44.65 ± 8.09	0.112
Globulin (g/L)	23.82 ± 3.37	32.63 ± 8.18	0.326
A/G	1.69 ± 2.56	1.49 ± 1.79	0.106

Note. The values are Mean ± SD; p-values were determined by the Student's t-test as appropriate; $p < 0.05$ was considered significantly different, *when there is an intergroup significant difference.

TABLE 4. Comparison of SPE Pattern among T2DM Based on BMI.

Pattern	Optimal BMI	Over weigh	χ^2 value	P value
Hyper-gammaglobulinemia	1 (12.5)	7 (87.5)	2.311*	<0.05
Hypo-gammaglobulinemia	0	0	NA	NA
Hypo-albuminemia	0	0	NA	NA
Hyper-betaglobulinemia	4 (57.2)	3 (42.8)	8.652	>0.05
Hypo-alphaglobulinemia	0	0	NA	NA
Hyper-alpha2-globulinemia	2 (15.4)	11 (84.6)	1.092*	<0.05

Note. Values in (%). The calculated Chi-square values were compared with the critical χ^2 statistic value for $p = 0.05$ (95% confidence level) with degree of freedom 1 (3.8). NA = Not applicable. *significant difference.