**EVALUATION OF SERUM AMYLOID A (SAA) AND GLYCATED HAEMOGLOBIN (HBA1C) IN TYPE 2 DIABETIC SUBJECTS ATTENDING NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL DIABETIC CLINICS**

**ABSTRACT**

**Background**: Diabetes mellitus is a chronic disease characterized by hyperglycemia and various complications. Serum amyloid A (SAA) and glycated hemoglobin (HbA1c) are biomarkers that may indicate inflammation and glucose control, respectively.

Objective: To evaluate the levels of SAA and HbA1c and their relationships in type 2 diabetic subjects.

**Methods**: This is a cross-sectional study involving 130 participants which comprises of 65 diabetic subjects and 65 non-diabetic control subjects. Fasting blood samples was collected from each participant following standard procedure and SAA and HbA1c levels were determined using ELISA and Ion-exchange chromatographic methods respectively. Statistical analysis was performed using SPSS version 26 to determine the levels and correlation between SAA and HbA1c.

**Results**: the result showed a significant high levels of SAA and HbA1c in type 2 diabetic group when compared with the non-diabetic group (p<0.05). There was a significant positive correlation between SAA and HbA1c levels in type 2 diabetic subjects (p<0.05). The results further showed elevated SAA levels in subjects with poor glucose control. However, the mean levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), basal mass index (BMI) and age did not differ significantly in type 2 diabetics when compared with non-diabetic group (p>0.05).

**Conclusion**: This study is suggestive of increased inflammation in type 2 diabetics especially in those with poor glycemic control. This may increase diabetic complications in these subjects.

**Keywords**: Type 2 diabetes mellitus, Serum amyloid A (SAA), Glycated hemoglobin (HbA1c), glycaemic control, Inflammation.

**Introduction**

**Background of study**

Type 2 diabetes is a chronic and progressive cardiometabolic disorder that affects more than 10% of adults worldwide and is a major cause of morbidity, mortality, disability, and high costs (Galindo *et al*., 2023). It is one of the most common metabolic disorders worldwide and its development is primarily caused by a combination of two main factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin (Galicia-Garcia *et al*., 2020). Type 2 diabetes mellitus (T2DM) is also known as non-insulin-dependent diabetes mellitus. It is polygenic and multifactorial in its pathogenesis, with genetics, lifestyle habits, and the acquired health status of the organism influencing the development of the disease (Su *et al*., 2023; Okwara *et al*., 2021).

The global prevalence of T2DM in adults was 536.6 million people (10.5%) in 2021, and that there would be 783.2 million people (12.2%) living with diabetes worldwide by 2045 (Sun *et al*., 2022). Incidence and prevalence of T2DM vary according to geographical region, with more than 80% of patients living in low-to-middle-income countries, which pose additional challenges in effective treatment (Galicia-Garcia, 2020). In Nigeria, the prevalence of diabetes is estimated at 4.3% (WHO, 2022), with local studies showing rates between 0.8% and 11%. Type 2 diabetes accounts for over 90% of diabetes cases in Nigeria, with an estimated 4.7 million Nigerians affected (Dahiru *et al*., 2016; Ajikobi, 2018).

Type 2 diabetes is associated with many complications such as retinopathy, nephropathy, neuropathy and other macrovascular diseases like myocardial infarction and stroke (Ihim *et al*., 2019; Weinberg *et al.*, 2024). It has also been shown that type 2 diabetes is associated with decreased essential trace elements like zinc and selenium (Onah *et al*., 2013a) and disorder in hormonal profile especially the testosterone which is usually low (Onah *et al*., 2013b). Most of these complications are consequences of interplay between type 2 diabetes and inflammation. Elevated glucose levels generate free radicals which in turn initiates chronic inflammation that heightens oxidative stress which is also implicated in the development of insulin resistance (Ehiaghe *et al*., 2025a; Ehiaghe *et al*., 2025b), subsequently fostering hyperglycemia (Ogbodo *et al*., 2019; Weinberg *et al*., 2024). This interplay between inflammation, oxidative stress, insulin resistance, and hyperglycemia fosters a detrimental cycle by increasing the risk of diabetes-related complications (Papachristoforou *et al*., 2020; Weinberg *et al*., 2024).

Serum amyloid A (SAA) is a class of 104 amino acid conservative acute-phase proteins, which is essential in immune-mediated inflammatory processes (Chen *et al*., 2023). SSA has been found to be more expressed in vivo under conditions of reduced insulin sensitivity (Poitou *et al*., 2005). Also, persistent insulin resistance elevates glycosylation end products (Analike *et al*., 2019) which stimulate macrophages and moncytes to release more inflammatory factors, inducing a chronic low-grade inflammatory state in the body (Engin, 2017; and Permana *et al*., 2006; Ehiaghe *et al*., 2025a; Ehiaghe *et al*., 2025b) and this accelerates the progression of type 2 diabetes mellitus and its complications (Liu *et al*., 2023).

However, this study seeks to determine the levels of HbA1c, the best indicator of glycaemic control in patients with diabetes, and SSA, a marker of inflammation in type 2 diabetic subjects.

**METHODS**

**Method**

**Study site**

This study was carried out at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state.

**Study Design and population**

This was a cross-sectional study designed to evaluate the levels of SAA and HbA1C in type 2 diabetic subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria. A total of 130 subjects were recruited for the study which includes sixty five (65) type 2 diabetic subjects within the range of 30-70 years and 65 age-matched non-diabetic control subjects. They were randomly selected after obtaining their informed consent.

**Sample Size**

The sample size was calculated using the method described by Charan and Biswas (2013);

N = (Z2pq)

D2

Where;

N= Desired number of sample when population of facility is limited

Z= Z value, where Z is the standard normal variance where confidence level is 1.96 at 95%

P= Prevalence rate of type 2 diabetes in Nigeria 4.3% (WHO, 2022)

Q= 1-P

d= 5% i.e. degree of precision as desired by the researcher

Applying the method,

N = Z2 x P x (1-P)

D2

N= 1.962 X 0.043 (1-0.043)

0.052

N= 63.

Sixty five individuals were recruited in each group to give room for attrition.

**Inclusion Criteria**

This includes individuals who have been diagnosed with type 2 diabetes and are under treatment; and between the aged range of 18-70 years that gave their written informed consent.

The control subjects were apparently healthy individuals that gave their informed consent.

**Exclusion Criteria**

This includes individuals who have obvious diabetic complications; and those that were outside the age range; and those who declined interest in participating in the study.

**Ethical Approval**

This study adhered to the ethical guidelines for human research. Ethical clearance was sought from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria prior to data collection. Also, informed consent was obtained from all participants.

**Sample Collection**

Blood samples were collected from the anticubital vein after a maximum of 12 hours of overnight fast. Rubber tourniquet was applied for less than a minute and site to be punctured was cleaned with 70% methylated spirit. Part of 5ml of blood sample that was collected was dispensed into EDTA containers for the measurement of glycated heamoglobin (HBA1c). The samples were stored at 2 ºC until analysis within 3 days of collection. The remaining 3 ml of whole blood was dispensed into plain container and allowed to clot and retracted. The serum was separated into a new plain container and stored at -20 ºC until analysis of serum amyloid A (SAA) within one month.

**Assay Method:**

SAA was analyzed by ELISA method using commercial kit from Nanjing MornMed Medical Equipment Co., Ltd, China. HbA1c was analyzed by Ion-exchange chromatographic method using commercial kit from TECO diagnostics, USA.

**RESULTS**

There was no significant difference in the mean age, BMI, Systolic blood pressure and diastolic blood pressure in type 2 diabetic group when compared with the control group (p > 0.05) (table 1).

The mean level of SAA was significantly higher in type 2 diabetic group when compared with non-diabetic group (p<0.05). Likewise, the mean level of HbA1c was significantly higher in type 2 diabetic group when compared with non-diabetic group (p<0.05) (table 2).

There was a significant positive correlation between SAA and HBA1C in type 2 diabetic group (p<0.001). Likewise, a significant positive correlation was observed between SAA and Age in type 2 diabetic group (p<0.05). However, no correlation was observed between SAA and other variables (BMI, SBP, and DBP) in type 2 diabetic group (p>0.05). Similarly, no correlation was observed between HBA1c and other variables (age, BMI, SBP, and DBP) in type 2 diabetic group (p>0.05) (table 3).

There was no significant correlation between SAA and other variables (age, BMI, SBP, and DBP) in control group (p>0.05). Similarly, no correlation was observed between HBA1c and other variables (age, BMI, SBP, and DBP) in control group (p>0.05) (table 4).

**Table 1:** **Age, BMI and BP in Type 2 diabetes mellitus patients and in the control group**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Control | Test | *t*-value | *p*-value |
| **Age** | 46.77 ± 11.07 | 46.14±10.81 | 0.331 | 0.741 |
| **BMI** | 26.41 ± 2.63 | 24.42±2.50 | 4.448 | <0.001 |
| **SBP** | 124.86±12.81  | 121.03±16.02 | 1.504 | 0.135 |
| **DBP** | 73.66±9.50 | 71.97±10.70 | 0.956 | 0.341 |

**Table 2 Determination of** **Serum Amyloid A and glycated heamogloin in the test and control groups.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Type 2 diabetic group | Control group | *t*-value | *p*-value |
| **Serum Amyloid A** | 272.16±111.58 | 94.08± 47.59 | 11.913 | <0.001\* |
| **Glycated Haemoglobin** | 8.11±1.79 | 5.40± 1.12 | 10.386 | <0.001\* |

***\* Statistically significant mean difference at P<0.05.***

**Table 3- Correlation of Serum Amyloid A, Glycated haemoglobin, BMI, SBP, DBP and Age in the test group.**

|  |  |  |
| --- | --- | --- |
|  | r | p-value |
| **SAA Vs HBA1C** | 0.758 | <0.001\* |
| **SAA Vs. Age** | 0.271 | 0.029\* |
| **SAA Vs. BMI** | -0.090 | 0.474 |
| **SAA Vs. SBP** | -0.202 | 0.107 |
| **SAA Vs. DBP** | -0.042 | 0.742 |
| **HBA1C Vs. Age** | 0.096 | 0.448 |
| **HBA1C Vs. BMI** | 0.001 | 0.994 |
| **HBA1C Vs. SBP** | -0.085 | 0.502 |
| **HBA1C vs. DBP** | 0.022 | 0.863 |

***\* Statistically significant mean difference at P<0.05.***

**Table 4:** **Correlation of Serum Amyloid A, Glycated haemoglobin, BMI, SBP, DBP and Age in the control group.**

|  |  |  |
| --- | --- | --- |
|  | r | p-value |
| **SAA Vs HBA1C** | -0.024 | 0.850 |
| **SAA Vs. Age** | -0.101 | 0.419 |
| **SAA Vs. BMI** | 0.103 | 0.410 |
| **SAA Vs. SBP** | 0.034 | 0.789 |
| **SAA Vs. DBP** | -0.084 | 0.503 |
| **HBA1C Vs. Age** | 0.154 | 0.217 |
| **HBA1C Vs. BMI** | 0.112 | 0.369 |
| **HBA1C Vs. SBP** | -0.128 | 0.306 |
| **HBA1C vs. DBP** | -0.202 | 0.107 |

***\* Statistically significant mean difference at P<0.05.***

**DISCUSSION**

This study revealed elevated levels of SAA in type 2 diabetic individuals which is in agreement with the studies of Liu *et al*. (2023), Ebtehaj *et al*. (2017), and Yang *et al*. (2017). This observation also aligns with recent study which demonstrates that SAA is an acute-phase protein associated with inflammation, and its altered levels in T2DM patients suggest changes in the inflammatory response characteristic of the disease (Marzi *et al*., 2013; Diete *et al*., 2016; Liu *et al*., 2022). This study also showed a strong positive correlation between SAA and HbA1c which is suggestive of increased inflammatory reaction in individuals with poor glycemic control. SSA has been found to be more expressed in vivo under conditions of reduced insulin sensitivity (Poitou *et al*., 2005). This chronic inflammatory reaction can accelerate the progression of type 2 diabetes mellitus and its complications (Liu *et al*., 2023).

We also observed that the mean HbA1c level was raised in diabetic individuals even though they were receiving treatment from their clinics. Glycated hemoglobin is a marker for long-term blood glucose levels, and higher levels are indicative of poorer glycemic control. Therefore, the increase in HbA1c in the test group suggests that the intervention or condition might be associated with impaired glucose metabolism or higher blood sugar levels over a prolonged period. This is in line with American Diabetes Association, (2023), which states that elevated HbA1c levels in T2DM patients reflect poor long-term glycemic control, as HbA1c is a measure of average blood glucose levels over the previous two to three months.

This work showed that SAA levels in type 2 diabetic subjects correlated positively with their age but did not show any significant relationship with BMI, SBP or DBP in type 2 diabetic group. This finding is in line with the work of Abdelmotaleb *et al*. (2025) who reported a significant positive correlation between SAA and age. Barbosa *et al*. (2023) also reported that in multivariate analysis, age was found to be independently associated with increased SAA levels. However, our findings did not agree with the work (Carbone *et al*., 2021) who reported no significant correlation between SAA levels and age in adults.

**Conclusion**

This work showed elevated SAA and HbA1c with a significantly strong positive association between these two markers in type 2 diabetic subjects This is suggestive of increased inflammation in type 2 diabetics especially in those with poor glycemic control which may increase diabetic complications in these subjects.

**Recommendation**

Firstly, it is crucial to adopt a dual-focused approach that simultaneously targets inflammation and glycemic control. Healthcare providers should consider incorporating anti-inflammatory treatments alongside standard diabetes therapies to balance these dual aspects of diabetes care.

Additionally, personalized treatment strategies should be considered, given the observed correlation between SAA levels and age. Older patients, who tend to have higher SAA levels, might benefit from tailored interventions that specifically address their unique inflammatory and glycemic profiles.

Finally, further research is needed to explore the underlying mechanisms driving the observed interactions between inflammation and glycemic control in type 2 diabetes. This research should aim to identify specific pathways and potential therapeutic targets that can simultaneously optimize both inflammatory and metabolic parameters.

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