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Original Research Article

Assessment of fasting plasma glucose, insulin, insulin resistance and glycated haemoglobin as markers of glycemetic control in apparently healthy older adults in Nnewi

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ABSTRACT

Background and Aim: Biochemical changes in glycemetic indices have been linked to aging, and many individuals have asymptomatic hyperglycemia as they get older. This leads to metabolic dysregulation, which heightens vulnerability to a number of age-related chronic conditions, such as diabetes mellitus.

Materials and Methods: This cross-sectional study assessed the levels of fasting plasma glucose, insulin, insulin resistance and glycated haemoglobin as markers of glycemetic control in apparently healthy older adults in Nnewi. A total of 144 participants were involved in this study: 72 older persons and 72 control subjects. The older adults were aged 45 to 75; while the control group was composed of individuals aged 18 to 30. Six milliliters (6 ml) of fasting venous blood samples were collected from each participant for the determination of insulin (INS), glycated haemoglobin (HbA1c), and plasma glucose (FPG) levels. Enzyme-linked immunosorbent assay was used to evaluate INS, resin ion-exchange was used to estimate HbA1c, and glucose oxidase peroxidase was used to determine FPG. The Homeostasis Model Assessment Index (HOMA-IR) was used to calculate insulin resistance.

Results: The mean FPG, HbA1c, INS and HOMA-IR were significantly higher in the older adults compared to control subjects respectively ($p < 0.05$). Also, the mean FPG, HbA1c, INS and HOMA-IR were significantly higher in the older adult males and females compared to the control male and female subjects respectively ($p < 0.05$). There was significant moderate positive correlation between the level of FBS Vs HbA1c ($r = 0.484$, P -value = 0.000) and strong positive correlation between the level of Insulin Vs HOMA-IR ($r = 0.980$, P -value = 0.000) in the control group. Also, strong significant positive correlations were observed between FPG and HbA1c ($r = 0.704$, P -value = 0.000), FPG and HOMA-IR ($r = 0.778$, P -value = 0.000), Insulin and HOMA-IR ($r = 0.778$, P -value = 0.000) with a moderate significant positive correlation found between HbA1c Vs HOMA-IR ($r = 0.557$, P -value = 0.000) in the older adults.

Conclusion: This study showed that glycemetic indices tend to become altered with advancing in age.

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1. Introduction

Glucose is an important metabolic fuel of the human body, the level of which is tightly regulated within the body by several hormones especially insulin and glucagon. Alterations in plasma glucose level give rise to hyperglycemia which predisposes to diabetes mellitus in older adult population.

Insulin is a polypeptide hormone mainly secreted by cells in the islets of Langerhans of the pancreas which functions to regulate glucose levels in the bloodstream and induces glucose storage in the liver, muscles, and adipose tissue, resulting in overall weight gain.¹ The modulation of a wide range of physiological processes by insulin makes its synthesis and levels critical in the onset and progression of several chronic diseases such as type 2 diabetes mellitus.¹

Insulin resistance (IR) is a prevalent medical condition that accompanies type 2 diabetes, obesity, hypertension, metabolic syndrome and polycystic ovarian disease.² It is a state in which higher than normal concentrations of insulin are needed for normal responses, leading directly to hyperinsulinaemia and impairment in some of its action.² Insulin resistance affects several organs, including adipose tissue, muscle, and the liver, and impairs insulin signaling pathways.³ The disruption of many molecular mechanisms by which insulin acts in target tissues may be the cause of IR.⁴ Insulin-resistant cells do not respond to insulin as they should.⁵ Increased insulin production and circulating insulin levels typically follow an increase in plasma glucose levels. This prevents hepatic gluconeogenesis and results in the transport of plasma glucose into peripheral tissues. An individual is referred to as having Insulin resistance when this is defective.⁴ In this kind of person, elevated insulin secretion does not prevent glucose from entering peripheral tissue or impair hepatic gluconeogenesis.

Haemoglobin is the protein in red blood cells responsible for carrying oxygen throughout the body. Glucose attaches to it to form glycated haemoglobin (HbA1c) and when blood glucose levels are high, the body produces more glycated haemoglobin by chemically attaching plasma glucose to haemoglobin.⁶ The HbA1c test measures the average blood glucose level over the lifespan of red blood cells (approximately 120 days) and is seen as a gold standard for glycemic control or management. It has been shown to have potentials in predicting dyslipidemia and cardiovascular disease.^{7,8} HbA1c readings are the primary factor in determining the risk of diabetes-related morbidity and mortality,^{9–11} in addition to reflecting glycemic control. As compared to controls, HbA1c levels have been found to be higher in type 2 diabetes mellitus.^{12,13}

Aging is characterized with declines in tissue and cell functions which culminate in significant increases in the risk of a number of aging-related disorders, such as immune

system disorders, musculoskeletal disorders, metabolic disorders, cardiovascular disorders, and neurodegenerative disorders.¹⁴ As society ages, a range of chronic illnesses have progressively taken over as the leading causes of disability and mortality among the elderly, despite the fact that the advancement of modern medicine has improved human health and significantly increased life expectancy.¹⁴ There is increasing prevalence of diabetes mellitus among the elderly population in Nigeria.^{15,16} Diabetes mellitus (DM) is a known cause of accelerated aging, and evidence suggests that aging and diabetes mellitus share pathophysiological mechanisms.^{17,18} Diabetes mellitus is a chronic metabolic disorder with impaired insulin production and increased insulin resistance as its key pathophysiological characteristics^{18,19} and its burden is exacerbated by the rising incidence of physical inactivity, dietary changes, increased alcohol consumption and obesity among the older adults.^{20–23} The incidence of type 2 diabetes mellitus (T2DM) is found to increase with age²⁴ with those most at risk aged between 45–64 years.^{25,26} Aging is linked to a decline in physiological function, which can lead to chronic diseases like diabetes and cardiovascular disease. Changes in body composition and insulin resistance are linked to probable deregulation of physiological pathways leading to obesity and diabetes mellitus. Several people develop asymptomatic hyperglycemia as they age, which causes metabolic dysregulation that increases susceptibility to many age-related chronic illnesses like diabetes mellitus and cardiovascular disease mortality over time.¹⁷ Dysglycaemia predisposes the older persons to development of chronic diseases like diabetes and cardiovascular diseases which has a negative impact on their functional abilities and quality of life, resulting in severe morbidity and mortality.²⁷ Despite these, most studies on glycemic indices are focused on diabetic patients while the apparently healthy older adult population seems to be neglected and as such there is paucity of published research work done within and outside Nigeria in this respect. Therefore, this study is focused on the assessment of fasting plasma glucose, insulin, insulin resistance and glycated haemoglobin as markers of glycemic control in apparently healthy older adults in Nnewi.

2. Materials and Methods

2.1. Study design

This cross-sectional study was carried out to assess the levels of fasting plasma glucose, insulin, insulin resistance and glycated haemoglobin as markers of glycemic control in apparently healthy older adults in Nnewi. A total of one hundred and forty four (144) participants were included in this study. They were divided into two equal groups: the test group (older adults) and the control group (younger adults). Seventy two (72) older persons between the ages

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of 45 and 75 who provided written informed consent made up the test group, with thirty six male and female participants each. Furthermore, 72 individuals residing in the same vicinity with the test group between the ages of 18 and 30 comprised the control group, with thirty-six male and female participants each. The objectives and purpose of the study were explained to the volunteers before collecting signed informed consents. The participants age was intentionally unmatched in order to show the possible biochemical alterations that may occur in glycemic control with advancing in age.

2.2. Study area

The study was carried out within Nnewi Metropolis, Anambra State.

2.3. Sample size

The G-Power software, version 3.1.9.2.25, was used to calculate the sample size and power of this study. The sample size of 128 participants was assessed to have 80% power using t-tests for two tail statistics and an error probability of 0.05 to detect differences in replies as low as 0.5 (effect size). The total sample size was established at 144 subjects, with a 10% attrition rate being used as the baseline.

2.4. Inclusion criteria

Apparently healthy older individuals (45-75 years) and control subjects (between 18-30 years) were recruited for this study.

2.5. Exclusion criteria

Individuals with known conditions such as Diabetes mellitus, kidney disease, cardiovascular diseases (heart disease), pregnant women, lactating mothers and children as well as alcohol abusers and smokers were excluded from the present study.

3. Ethical approval

Ethical clearance for the study was sought and obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTH/CS/66/VOL. 16/VER. 3/07/2023/07).

3.1. Participants' informed consent

Prior to the start of the study, the participants' written informed consent was sought and obtained.

3.2. Collection of samples and analysis

Six milliliters (6 ml) of venous fasting blood samples were collected aseptically after 10-12 hours of fast by

venipuncture from each subject via the antecubital vein using a plastic syringe with minimum stasis into fluoride oxalate, EDTA and plain containers in appropriate volumes. 2 ml of venous blood was each dispensed into fluoride oxalate and EDTA bottles respectively for determination of fasting plasma glucose (FPG) and HbA1c levels which were assayed immediately without storage. The remaining 4 ml of the venous blood sample was dispensed into plain container and allowed to clot and retracted. It was then centrifuged at 4000 rpm for 5 minutes (Centrifuge 80-2, Techmel and Technel USA). The serum was separated and used for analysis of biochemical markers at Onamec-Lab, Medical and Diagnostic Services Limited, Nnewi. Serum samples that were not analyzed immediately were stored frozen at minus twenty degree Celsius (-20°C).

3.3. Laboratory methods

3.3.1. Determination of fasting plasma glucose

Fasting plasma glucose was determined using the glucose oxidase peroxidase method described by.²⁸

3.3.2. Determination of glycosylated haemoglobin (HbA1c)

Glycosylated haemoglobin (HbA1c) level was determined using the ion exchange resin method described by.²⁹

3.3.3. Estimation of serum human insulin

Serum Insulin level was determined by Enzyme-linked immunosorbent assay (ELISA) method as described by.³⁰

3.3.4. Determination of insulin resistance

Insulin resistance was calculated by Homeostasis Model assessment insulin resistance index (HOMA-IR) with formula $HOMA-IR = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mmol/l)} / 22.5$.³¹

3.4. Statistical analysis

The SPSS statistical tool, version 26.0, was used to analyze the data collected in this study. Independent t-test was used to compare the results obtained in the test and control groups. The correlation between the various parameters in the test group and control group was examined using Pearson's correlation coefficient. At $p < 0.05$, statistical significance was assumed.

4. Results

The mean age of the older adults was significantly higher compared to the control individuals (59.96 ± 8.47 Vs 23.21 ± 3.41 ; P -value= 0.000). There was significantly higher mean plasma glucose level in the older adults compared to the control group (P -value= 0.000). The mean glycated haemoglobin level was also significantly higher in

the older adults than in the control group (P-value= 0.000). Also, the mean levels of serum Insulin and Homeostasis Model assessment insulin resistance (HOMA-IR) were significantly higher in the older adults when compared to the observed values in the control individuals (P-value= 0.000) respectively (Table 1).

Furthermore, the mean age of the older adult male participants was significantly higher compared to the male control individuals (62.09 ± 7.87 Vs 23.40 ± 3.16 ; P-value= 0.000). The mean levels of fasting plasma glucose, glycated haemoglobin, Insulin and Homeostasis Model assessment insulin resistance (HOMA-IR) were significantly higher in the older adult male participants when compared to the observed values in the male control individuals (P-value <0.05) respectively (Table 2).

The mean age of the older adult female participants was significantly higher compared to the female control individuals (P-value= 0.000). Additionally, the mean levels of fasting plasma glucose, glycated haemoglobin, Insulin, and Homeostasis Model assessment insulin resistance (HOMA-IR) were significantly higher in the older adult female participants when compared to the observed values in the female control individuals (P-value <0.05) respectively (Table 3).

In the control subjects, there was statistically significant moderate positive correlation between the level of FBS Vs HbA1c ($r=0.484$, P-value = 0.000) and strong positive correlation between the level of Insulin Vs HOMA-IR ($r=0.980$, P-value = 0.000). (Table 4)

With regard to the test subjects, there were strong significant positive correlations between the variables of FPG and HbA1c ($r=0.704$, P-value = 0.000), FPG and HOMA-IR ($r=0.778$, P-value = 0.000), Insulin and HOMA-IR ($r=0.778$, P-value = 0.000) with a moderate significant positive correlation found between HbA1c Vs HOMA-IR ($r=0.557$, P-value = 0.000). (Table 5)

5. Discussion

The results of the current investigation showed that older adults had significantly higher mean plasma fasting glucose level than the control group. Likewise, it was discovered that the mean plasma glucose level were significantly higher in the male and female older individuals than in the corresponding controls. This demonstrates that plasma glucose levels rise with age and follow a similar pattern regardless of whether a person is male or female. This research supports the findings of Ko et al.³² on the effects of aging on plasma glucose levels in non-diabetic Hong Kong Chinese, which found that these individuals plasma glucose levels progressively increased with age. The lower insulin sensitivity that comes with aging, which may be caused by factors including an increase in abdominal fat mass, a decline in physical activity, and hormonal changes,

may be one explanation for the higher plasma glucose levels observed in this study among older persons. Similar findings were found by Chia et al.¹⁷ in their study on Age-Related Changes in Glucose Metabolism, Hyperglycemia, and Cardiovascular Risk, which demonstrated that as people aged, their plasma glucose levels in response to the oral glucose tolerance test (OGTT) increased steadily until they reached their peak at the seventh decade. Similar patterns were found in men and women; however men had higher glucose level than women.¹⁷ Age-related declines in insulin and other regulatory hormones, which are necessary to maintain the proper balance of glucose metabolism, contribute to the higher plasma glucose level observed in the older adults. Other comparable research supports the findings of the current report.³³

Interestingly, it was evident from the results of the current study that older people had mean serum insulin level that was statistically significantly higher than those of the control group. Also, the older male and female participants' mean serum insulin levels were significantly higher than those of the control groups. This might be brought on by hormonal changes, increased levels of abdominal fat mass, physical inactivity, mitochondrial dysfunction, and decreased insulin sensitivity, all of which raise the risk of obesity and insulin resistance. Also, the older persons in this study had higher mean insulin resistance (HOMA-IR) level than the control group, which may be the cause of the higher mean serum insulin level. Insulin is a polypeptide hormone that is primarily secreted by beta cells in the pancreatic islets of Langerhans.³⁴ Normally, insulin controls blood glucose levels and causes glucose storage in the liver, muscles, and adipose tissue, which leads to reduction in blood glucose levels with concomitant general weight gain.³⁵ However, as people age, insulin resistance often develops as a result of abdominal fat obesity, which increases the insensitivity of tissues to insulin. As a result, the beta cells of the pancreatic islet of Langerhans respond by producing more insulin, and as a result, higher concentrations of insulin are now needed for the uptake of plasma glucose into insulin-sensitive tissues like the liver, muscles, and adipose tissue. Hyperglycemia may eventually result from this if it is persistently present, and this can ultimately culminate in diabetes mellitus. A population-based study in Iranian subjects found that higher mean HOMA-IR levels in contrast with the present study.³⁶ Additionally, the same study obtained mean serum insulin level in older people similar to the observed results in this study. The mean insulin concentration and insulin resistance values found in this study among older people are consistent with the findings of Masoodian et al.³⁶

Furthermore, this study indicated that older male and female subjects had higher mean serum insulin level and insulin resistance than did the male and female control groups, respectively. The values obtained in older males

Table 1: Mean±SD serum levels of biochemical parameters studied in the older adult participants and control subjects

Parameters	Older-Adults (n=72)	Control Subjects (n=72)	t-value	P-value
Age (years)	59.96±8.47	23.21±3.41	32.191	0.000
FPG (mmol/L)	5.65±1.94	4.43±0.37	4.903	0.000
HbA1c (%)	6.91±1.56	4.67±0.80	10.177	0.000
Insulin(μIU/mL)	8.72±3.55	5.44±2.37	6.154	0.000
HOMA-IR	2.27±1.61	1.07±0.45	5.693	0.000

*P-value is statistically significant at <0.05

Table 2: Mean±SD serum levels of biochemical parameters studied in the older adult male participants and male control subjects

Parameters	Older AdultMales (n=36)	Male Control (n=36)	P-value
Age (years)	57.96±8.63	23.03±3.69	0.000
FPG (mmol/L)	5.49±1.10	4.39±0.33	0.000
HbA1c (%)	6.81±1.64	4.48±0.73	0.000
Insulin (μIU/mL)	8.69±3.63	5.88±2.87	0.002
HOMA-IR	2.19±1.45	1.14±0.54	0.004

*P-value is statistically significant at <0.05

Table 3: Mean±SD serum levels of biochemical parameters studied in the older adult female participants and female control subjects

Parameters	Older Adult females (n=36)	Female Control (n=36)	P-value
Age (years)	57.96±8.63	23.03±3.69	0.000
FPG (mmol/L)	5.80±2.50	4.47±0.41	0.001
HbA1c (%)	6.99±1.51	4.86±0.82	0.000
Insulin (μIU/mL)	8.76±3.52	5.00±1.66	0.000
HOMA-IR	2.34±1.78	1.00±0.34	0.000

*P-value is statistically significant at <0.05

Table 4: Levels of associations between parameters studied in control group (n=72)

Parameters	Pearson r coefficient	P-value
FBS Vs HbA1c	0.484	0.000
Insulin Vs HOMA-IR	0.980	0.000

*P-value is statistically significant at <0.05

Table 5: Levels of associations between parameters studied in test group (n=72)

Parameters	Pearson r coefficient	P-value
FPG Vs HbA1c	0.704	0.000
FPG Vs HOMA-IR	0.778	0.000
Insulin Vs HOMA-IR	0.778	0.000
HbA1c Vs HOMA-IR	0.557	0.000

*P-value is statistically significant at <0.05

and females in a previous study are comparable to the insulin resistance levels seen in both male and female older persons in this current investigation.³⁶ This demonstrates that insulin and insulin resistance appear to be affected similarly with increasing age in both males and females.

In this study, older adults had significantly higher mean glycated hemoglobin (HbA1c) value than did the control participants. Similarly, older males and females were found to have mean glycated hemoglobin level that was significantly higher than those of the control males and females, respectively. Given that these people have higher levels of blood glucose, insulin, and insulin resistance, this

is to be expected. Blood lipids, uric acid, age, weight, and gender have all been linked to an increase in HbA1c levels in the past.³⁷ HbA1c, as is well known, represents the average blood glucose over the previous three months and is directly associated to the problems of diabetes.³⁸ In people without diabetes, studies have shown that HbA1c rises with age also.³⁹

Expectedly, Insulin vs. HOMA-IR demonstrated strong significant relationships in the control group in the current investigation. Also, FPG vs. HbA1C, FPG vs. HOMA-IR as well as Insulin vs. HOMA-IR showed strong relationships in the older adults while HbA1c vs HOMA-IR was moderated

correlated in the older adult population. In line with the current study, Khan et al. conducted a study on the biomarker potential of C-peptide for screening of insulin resistance in diabetic and non-diabetic individuals.²⁶ They found highly significant correlations between HOMA-IR and insulin in both diabetic patients and control subjects. Insulin resistance is the result of the muscles, adipose (fat), and liver cells not responding to insulin as they should. This makes it difficult for the cells to absorb glucose from the blood, which means the pancreas must produce more insulin to allow for the uptake of glucose into the cells. Furthermore, glucose has been previously shown to be positively correlated with HbA1C levels in older adults.⁴⁰ Another previous study on Correlations between glycosylated hemoglobin and glucose levels in Chinese older adults with newly diagnosed type 2 diabetes mellitus found that glucose level was positively correlated with HbA1C.⁴¹ This agrees with this present finding. Also, Ozmen et al. showed that, while any single glucose value (e.g., fasting/postprandial) correlates with HbA1c, better correlations are achieved by averaging the glucose values of an individual.⁴²

6. Conclusion

This study demonstrates significant alterations in plasma glucose; glycated haemoglobin, insulin and insulin resistance as people age and this may predispose these individuals to developing diabetes mellitus and cardiovascular disease. Based on the findings in this study, routine monitoring of glycemic control markers in the older adults should be encouraged. It is strongly recommended that more research be done in this area with larger sample size and follow up.

7. Author Contributions

This work was carried out and approved in collaboration between all authors. Ogbodo EC, Onah CE, Meludu SC conceptualized and designed the study. Amah AK, Okeke CS, Obiorah MO, Okezie AO, Ogalagu RO, Iwuji JC and Mbam RE wrote the protocol and contributed in literature search. Ogbodo EC and Onah CE did the experiments. Meludu SC did statistical analysis. Ogbodo EC drafted the manuscript. Onah CE and Meludu SC supervised the study. Ogbodo EC wrote the final manuscript. All authors were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agree to be accountable for all aspects of the work.

8. Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

9. Sources of Funding

Self-funded.

10. Conflict of Interest

The authors declare no conflict of interest in the conduct and publication of this work.

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