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Effect of Insulin Resistance on Platelet Counts in Normal, Pre-Diabetic and Diabetic Human Subjects

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ABSTRACT

This study investigated the effect of insulin resistance on platelet (PLT) counts in normal, pre-diabetic and diabetic human subjects; using fasting blood sugar (FBS), glycated haemoglobin (HbA1c) and homeostasis model assessment of insulin resistance (HOMA-IR) as comparative indexes for the discussion. The study compared the PLT, FBS, HbA1c and HOMA-IR of the normal, pre-diabetics, and diabetics(three sets) so as to assess the effect of insulin resistance on platelets. One hundred and twenty adult male and female human subjects comprising forty subjects each for three sets matched for age and sex were recruited into the study based upon specified criteria. Of the sets of human subjects, twenty were males and females respectively. Group A subjects were the non-diabetic, Group B were the pre-diabetic while Group C subjects were the diabetic. Blood samples were analyzed using Randox and Accubind kits, and a Haematology analyser for the tests. The overall results revealed a significant difference at 95% level of confidence interval ($p<0.05$) in the parameters. PLT counts were significantly increased ($p<0.05$) in the pre-diabetic and diabetic groups showing values of 186.95±6.04 mL, 206.70±8.72 mL, and 229.97±11.21 mL respectively for the non-diabetics, pre-diabetics and diabetics. The FBS and HbA1c showed a significantly increasing trend with values of 4.49±0.08 mmol/l, 6.00±0.11 mmol/l, and 10.84±0.96 mmol/l for FBS; and 4.75±0.05 mmol/l, 5.73±0.08 mmol/l, and 9.74±0.47 mmol/l for HbA1c, for the non-diabetics, pre-diabetics, and diabetics respectively. All values were significantly higher ($p<0.05$)across the groups for both FBS and HbA1c. There was a significant difference ($p<0.05$) in HOMA-IR. This research revealed that insulin resistance has a significant effect on platelet counts in diabetic human subjects.

INTRODUCTION

Insulin resistance (IR) is a metabolic condition in which insulin dependent tissues become less sensitive to insulin action, leading to an imbalance in the metabolism of carbohydrates, lipids and proteins[1]. This condition is caused by the influence of different risk factors in the population, such as aging, alcohol consumption, smoking, hypercaloric diets, sedentary lifestyle and obesity[2].The role of insulin resistance in the development of different pathologies, as CVD[3]is now recognized, as well as, in the pathogenesis and clinical outcomes of type 2diabetes mellitus (DM2)[4].

Many mathematical models have been proposed in recent years with the objective of simplifying the measurement of IR, highlighting the HOMA-IR, a validated method to measure IR from serum glucose and fasting serum insulin[5].

IR, characterized by a decrease in cell sensitivity to insulin, is one of the leading causes of metabolic abnormalities. Considering that metabolic abnormalities at childhood may increase the risk of cardiovascular diseases during adulthood, it is critical to diagnose insulin resistance in adolescents[6].

Insulin resistance (IR) is characterized by inappropriate physiologic response in which insensitivity to insulin results in compensatory hyperinsulinemia[7]. It is regarded a major risk factor for type 2 diabetes[8,9,10]. Increased human population with chronic diseases associated with IR are reported globally[11,12]. Thus, early detection of IR is crucial to prevent the manifestation of clinical diseases.

Many studies have demonstrated that IR is one of the most important contributing factors to CVD[13,14]. Furthermore, given that insulin resistance is an important risk factor for development of type 2 diabetes and incident cardiovascular diseases, identification of subjects with insulin resistance is a strategy for identifying high-risk people for targeted preventive interventions[13,15].

HOMA-IR, derived from the product of the fasting levels of insulin and glucose, is a robust tool used as a surrogate measure for insulin resistance[16,17]. Several population-based studies were conducted to establish cut-off values of HOMA-IR using receiver operating characteristic (ROC) curves for metabolic syndrome for the clinical usefulness[17,18,19,20,21,22].

The HOMA-IR was developed in 1985 and has been widely used for IR quantification. However, insulin measurement is still not readily available in many routine laboratories and this is due to standardization issues[23]. IR predisposes to several metabolic disorders including hyperglycemia, high blood pressure, and dyslipidemia, all of them strongly associated with diabetes, atherosclerosis, and cardiovascular disease. The evaluation of IR requires sophisticated methods which are not available for use in daily clinical practice[24].

Hyperinsulinemic-euglycemic clamp is the direct method to measure IR and is considered the ‘‘gold standard’’ procedure, but it is difficult to perform in daily practice. Several surrogate markers have therefore been proposed, including the homeostatic model assessment of IR (HOMA-IR), one of the most widely used. HOMA-IR is calculated based on the measurement of fasting blood glucose and insulin levels[5].Thereare two issues to be considered with regard to insulin. On one hand, insulin has a high biological variability(within- and between-subject variability of 21.1% and 58.3%respectively)[25]and on the other hand, its measurement is yet to be standardized[26,27]. These two aspects have direct impact on the estimation of IR using the HOMA-IR index[28].

Having in mind the association between insulin and diabetes, and other metabolic conditions, and the importance of determining the level of insulin, this study investigated the effect of insulin resistance on platelet counts in normal, pre-diabetic and diabetic human subjects. The study was done on the premise that base-line data could be provided for physicians in the assessment of insulin resistance on platelet counts. This is by proffering some scientific information on insulin resistance, PLT counts, HbA1c, FBS, and HOMA-IR index of normal, pre-diabetic and diabetic human subjects. The study was limited to enrolling normal individuals, pre-diabetic and diabetic human subjects for the purpose of investigation IR and/or PLT counts, and FBS, HbA1c, and HOMA-IR.

METHODOLOGY

This study was conducted in the University of Port Harcourt Teaching Hospital (UPTH) in Obio/Akpor Local Government Area of Rivers State, Nigeria. The study area is located in the Niger Delta region, bordering the Atlantic Ocean. It was a cross-sectional study involving subjects that routinely visited the healthcare facility for their medical needs at the Out-Patient unit. They were grouped into three: control GROUP A, and test Groups B and C.

GROUP A: The control group consists of forty (40) normal (non-diabetic) subjects.

GROUP B: The test group consists of 40 pre-diabetic subjects.

GROUP C: The test group consists of 40 diabetic subjects.

Individuals aged between thirty six (36) to seventy six (76) years who agreed to participate in the study were included, while those with co-infection and other metabolic disorders were excluded. The minimum sample size was calculated by employing the formula below:

$N = Z^2(pq) / e^2$ [29].

Where N = minimum sample size, Z = 1.96 at 95% confidence limits, so that z^2 = 3.8416, p = prevalence of increased normal and diabetic subjects' percentage average, q = 1-p and e = error margin tolerated at 5% = 0.05 (e^2 = 0.0025).

6.80% was the prevalence of increased normal subjects and 10.20% the prevalence of increased diabetic subjects.

((6.80 + 10.20)/2)% = (17.00/2)% = 8.50% (8.50% as the prevalence of increased mean of normal and diabetic subjects), p = 8.50% = 0.0850, q = 1-p = 1-0.0850 = 0.9150

N = ((3.8416(0.0850 x 0.9150))/0.0025 = 119.51 = approximately 120.

Subjects were issued or given the informed consent form to complete or fill out after listening to a detailed explanation from the researcher. This is followed by obtaining five (5) ml of blood samples was collected from the phlebotomy department of UPTH using 5 ml syringe from each subject. Two (2) ml was put into Lithium heparin bottle, 2 ml into plain bottle, and one (1) ml into Fluoride oxalate bottle. The samples were placed in sample racks and left to stand for at least thirty (30) minutes at room temperature. The sample was centrifuged for 5 minutes using the centrifuge (Hettich Universal 320) at room temperature and a completely cell free non-haemolysed sample was obtained. The samples were then separated into a 1 ml sample container which was labeled with the serial number of the subject, and left to refrigerate before use.

Whole blood sample collection from subjects was by intravenous means (collected intravenously) and the samples were collected into plain and heparinized bottles respectively, which were allowed to stand for 30 minutes to clot, centrifuged at 3,000 rpm for 10min for proper separation, separated into plain bottles and labeled accordingly. This was stored frozen, until when needed for biochemical and haematological analysis.

Sources of information was from published studies that assess insulin resistance on platelet counts in normal, pre-diabetic and diabetic human subjects were searched in MEDLINE, EMBASE and PubMed databases covering the period from year 2000 to 2018. Literature search was then carried out using the combination of terms "insulin", "insulin resistance", 'PLT', 'PLT counts', "HOMA-IR", "HbA1c", "Blood Sugar", "FBS", "diabetes", "diabetes mellitus", "type 2diabetes","T2DM", "type 2 DM", "epidemiology", and "review". The reference lists of the retrieved articles and reviews of this field[30,31,32] were also searched. The search was limited to human studies and English publications.

Fasting Blood Sugar (FBS) was analyzed using Randox Kits (RANDOX, USA). HbA1c test was analyzed using Wondfo Finecare System (WONDFO, CHINA). Insulin was analyzed using Calbiotech Inc., enzyme-linked immunosorbent assay (ELISA) Kit while HOMA-IR was also analyzed. Full blood count (FBC) which involves Platelet counts level was also analyzed.HbA1c was determined using the Finecare^{TM} HbA1c Rapid Quantitative Test which is a fluorescence immunoassay used for quantitative determination of HbA1c in human blood (Jeppsson *et al.*, 2002).The quantitative *in vitro* determination of FBS in serum and/or plasma was done on the Randox (Rx) Monza analyzer,

In determining the HOMA-IR, the IR Calculation: Insulin × Glucose÷405

Optimal Range: 1.0 (0.5 – 1.4). Less than 1.0 means one is insulin-sensitive which is optimal, above 1.9 indicates early insulin resistance and above 2.9 indicates significant insulin resistance. This calculation marks for both the presence and extent of any insulin resistance that one might currently express.

All data were subjected to statistical analyses. Statistical analysis was performed using SPSS version 21 (IBM, U.S.A). The data was analyzed using one-way analysis of variance (ANOVA) and significant differences were determined using post Hoc Duncan multiple comparison test ($p<0.05$). The results were considered significant at 95% confidence level. The values were represented as mean ± standard deviation (SD) and data obtained was analyzed using the SPSS. Data was shown as mean $\pm$ SD and displayed in figures. Qualitative variables of gender categories were summarized as proportions. Quantitative variables such as age were summarized as mean. Difference in mean of parameters was compared using ANOVA.

RESULTS

Glycemic indices, HOMA-IR and platelet counts level of subjects

The results obtained for the glycemic indices comprising the HOMA-IR and PLT counts level are shown in Tables 1-3.

FBS and HbA1c (Glycemic indices), and insulin of the subjects are shown in Table 1.

The FBS and HbA1c showed a significantly increasing trend with values of 4.49±0.08 mmol/l, 6.00±0.11 mmol/l, and 10.84±0.96 mmol/l for FBS; and 4.75±0.05 mmol/l, 5.73±0.08 mmol/l, and 9.74±0.47 mmol/l for HbA1c, for the non-diabetics, pre-diabetics, and diabetics respectively. All values were significantly higher ($p<0.05$) across the groups for both FBS and HbA1c.

Table 1 Fasting Blood Sugar and HbA1c (Glycemic indices), and Insulin of the subjects.

GROUP	FBSmmol/l	HbA1c mmol/l
NON-DIABETIC	4.49±0.08[bc]	4.75±0.05[bc]
PRE-DIABETIC	6.80±0.11[ac]	5.73±0.08[ac]
DIABETIC	10.84±0.96[a]	9.74±0.47[a]

Data are expressed as Mean ± Standard deviation (SD), n=120 where n represents the number of subjects. Values in the same column with similar superscript letter a, were significantly higher (p<0.05) than that of the non-diabetic. Values with the superscript b, were significantly lower (p<0.05) than that of the pre-diabetic. Values with the superscript c, were significantly lower (p<0.05) than that of the diabetic group. FBS – Fasting Blood Sugar, HbA1c – Glycated Haemoglobin, INS – Insulin

The HOMA-IR index of the non-diabetic, pre-diabetic and diabetic human subjects is shown in Table 2.

The table reveals an increasing trend in the HOMA-IR index across the groups. HOMA-IR values were 0.94±0.04 for the non-diabetics, 2.28±0.17 for the pre-diabetics, and 3.25±0.44 for the diabetics. HOMA-IR index values of the diabetics and pre-diabetics were significantly higher than that of the non-diabetics as shown in Table 2 below.

Table 2 Homeostatic model index (HOMA-IR) of human subjects for the non-diabetic control, pre-diabetic, and diabetic groups

GROUP	HOMA-IR
NON- DIABETIC	0.94±0.04[c]
PRE-DIABETIC	2.28±0.17[ac]
DIABETIC	3.25±0.44[ab]

Data are expressed as Mean ± Standard deviation (SD), n=120 where n represents the number of human subjects. Value with similar superscript letter a, was significantly higher (p<0.05) than that of the non-diabetic. Value with the superscript b, was significantly higher (p<0.05) than that of the pre-diabetic. Value with the superscript c, was significantly lower (p<0.05) than that of the diabetic group. HOMA-IR – Homeostatic Model Assessment of Insulin Resistance

Table 3 below shows the platelets counts levels of the human subjects used in this study. Platelet (PLT) counts were significantly increased (p<0.05) in the pre-diabetic and diabetic groups showing values of 186.95±6.04 mL, 206.70±8.72 mL, and 229.97±11.21 mL respectively for the non-diabetics, pre-diabetics and diabetics.

Table 3 Platelet (PLT) count in Human subjects

GROUP	PLT ($\times 10^9$/L)
NON-DIABETIC	186.95±6.04[c]
PRE-DIABETIC	206.70±8.72[ac]
DIABETIC	229.97±11.21[ab]

Data are expressed as Mean ± Standard deviation (SD), n=120 where n represents the number of human subjects. Value with similar superscript letter a, was significantly higher (p<0.05) than that of the non-diabetic. Value with the superscript b, was significantly higher (p<0.05) than that of the pre-diabetic. Value with the superscript c, was significantly lower (p<0.05) than that of the diabetic group. PLT – Platelet

DISCUSSION

The hall-mark of Type 2 diabetes is an abnormally high glucose that is unresponsive or only slightly responsive to insulin regulation.

Analysis of the platelet counts in the human subjects in this study showed that there were alterations in the platelet levels in the diabetic state. Platelet counts were also found to be elevated in the pre-diabetic and diabetic subjects relative to the normal control group. This is in agreement with findings reported by several previous studies and might be the indirect features of insulin resistance syndrome[33].

CONCLUSION

The study investigated the effect of insulin resistance on platelets counts and the findings largely corroborated previous studies. This study revealed that insulin resistance has a significant effect on platelet counts in normal, pre-diabetic and diabetic human subjects.

RECOMMENDATIONS

It is recommended that platelets counts should be checked routinely as it is significantly in diabetes. The research should also be conducted in various geographical locations as variations in different locations affect the genetic factor and limit the generalization of the research findings.

CONTRIBUTION TO KNOWLEDGE

Improvement in insulin resistance will help in ameliorating its effect on platelet counts and reduce the effect of diabetes on the subjects.

CONFLICT OF INTEREST

There was no conflict of interest

FUNDING

There was no funding for the research

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