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Evaluation of Plasma Homocysteine Levels in Type II Diabetes and Hypertensive Patients Attending University of Port Harcourt Teaching Hospital, Nigeria

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Abstract

Background: Homocysteine is an important non-protein amino acid, very useful in all methylation reactions occurring in the body as the precursor of the sole methyl group donor S-Adenosyl-methionine (SAM). However, elevated plasma homocysteine levels have been reported to contribute to epithelial damage leading to coronary artery disease and other metabolic syndromes. This study was aimed at evaluating the concentration of plasma homocysteine in diabetics and hypertensive patients in Port Harcourt, Nigeria. **Methods:** The study population included 60 Type II diabetes mellitus and Hypertensivesubjectsas group (I), 60 Type II diabetes mellitus and Normotensive subjects as group (II), 60 Hypertensive subjects as group (III), and 60 healthy subjects as control group within the age range of 30 - 70 years. An enzyme-linked immunosorbent assay (ELISA) method was used to quantitatively measure homocysteine in the serum sample, glycated haemoglobin were determined quantitatively using sandwich immunodetection and blood pressure was determined using mercury sphygnanometer. **Statistics:** The statistical analysis was done using GraphPad Prism version 9.4.1, and statistical significance was determined by a $P < 0.05$. **Results:** The results showed significantly higher plasma homocysteine levels in diabetics and hypertensive comorbidity patients when compared to healthy controls, $P < 0.013$. There was also a significant increase in plasma homocysteine levels in the comorbidity and hypertensives single morbidity, $P < 0.0001$ and also diabetes single morbidity, but there was no significant difference from the levels in the diabetics and hypertensives single morbidity. Furthermore, there was no significant difference between groups in terms of age range or duration of diagnosis. **Conclusion:**

Our result shows an increase in plasma homocysteine levels in diabetics and hypertensives when compared to controls, and comorbidity instigates a higher increase in plasma levels when compared with the single morbidity.

Keywords

Hypertensives, Diabetes Mellitus, Homocysteine, Hyperhomocysteinemia

1. Introduction

Homocysteine is a sulphur-containing non-protein amino acid, an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine [1]. It can be produced via demethylation of the terminal carbon of dietary methionine, which is abundant in animal protein [2]. This amino acid is present in plasma in different forms; 1% circulates as free thiol, while 70% - 80% is present as a disulphide bound to plasma proteins, mainly albumin and 20% - 30% combines with itself to form the dimer homocysteine [3]. Homocystéine has mol.wt of 135.18 g/mol, and it is a homologue of the amino acid cysteine, from where it derived its name because of its molecular similarities to cysteine. Homocysteine can also be produced by heating the amino acid methionine with sulphuric acid [1] [4].

The availability of homocysteine in the body is the key determinant of all methylation reactions in the body. Homocysteine can be methylated to methionine, which can undergo S-adenosylation to form S-Adenosyl-L-Methionine (SAM). SAM is the principal methyl group donor for all methylation reactions occurring in the cell [5], hence the biological importance of this amino acid. Condensation of methionine with ATP, also leads to the formation of S-Adenosylmethionine (SAM) [6]. Physiologically, homocysteine can be recycled by reconversion to methionine or conversion to cysteine with the presence of certain B-vitamins (Vit.B_6, B_{12} and Folic acid). Hence the suboptimal levels of these vitamins can lead to accumulation of homocysteine in the plasma, (hyperhomocysteinamia). High levels of plasma homocysteine have been reported as a marker of cardiovascular disease [4]. This is because homocysteine is said to induce oxidative injury of vascular endothelial cells. In the heart, this can lead to increased endothelial-myocyte uncoupling resulting in diastolic dysfunction and hypertension. Some authorities believe that hyperhomocysteinamia is an independent risk factor for various vascular diseases, such as atherosclerosis, hypertension, vascular calcification and aneurysm. It has also been implicated in Diabetes mellitus [7].

Diabetes is a long-term metabolic condition marked by high blood glucose which over time can seriously harm the heart, blood vessels, eyes, kidneys, and nerves. Diabetes mellitus has a significant impact on human health that is regrettably growing progressively in terms of general health, mortality, and economic effects. In Nigeria, the prevalence of diabetes mellitus is 3.7% with about

3.6 Million adults suffering from this disease [8]. Hypertension, is another long-term medical disorder characterized by persistently high blood pressure in the arteries [9], which if untreated can lead to a wide range of metabolic disorder including renal damage, visual impairments among others. In Africa, it is a prominent cause of illness and mortality, and Nigeria is no exception [10].

Type 2 diabetes mellitus and hypertension frequently coexist and 65% of diabetic patients also suffer from hypertension. Patients with diabetes have hypertension twice as often as those without the disease. Additionally, persons with hypertension are more likely than normotensive people to have insulin resistance and acquire type II diabetes mellitus [11]. As a result, due to shared risk factors such as endothelial dysfunction, vascular inflammation, arterial remodelling, atherosclerosis, dyslipidaemia, and obesity, diabetes and hypertension are strongly related to one another [11]. Hyperhomocysteinemia is a medical disorder marked by abnormally high blood homocysteine levels above 15 μmol/L, with therapeutic targets of plasma homocysteine levels as <6.3 μmol/l [12]. Plasma levels of homocysteine vary with age [13] and sex, [14]. Research suggests that hyperhomocysteinemia is strongly associated with other metabolic syndrome variables, such as hypertension, which is correlated with type II diabetes mellitus [15]. Interestingly, the most common inherited disorder leading to hyperhomocysteinemia is the 5-methylenetetrahydrofolate reductase (MTHFR) polymorphism [16] [17]. These are inherited abnormalities of genes responsible for the metabolism of folate and the B-vitamins. Consequently, individuals with dietary deficiencies of folic acid (B9), pyridoxine (B6), or cobalamin (B12) can as well develop hyperhomocysteinemia [18].

Numerous studies on the relationship of increased homocysteine levels to hypertension and Diabetes Mellitus have come with contradicting conclusions. It was against this backdrop that this study was designed to investigate the interaction between hyperhomocystienamia, hypertension and diabetes mellitus in Nigerian subjects living in Port Harcourt metropolis.

2. Materials and Methods

2.1. Study Population

The study was composed of a total of 240 subjects comprising 60 Type II diabetes mellitus and hypertensive patients (group I), 60 Type II diabetes mellitus and Normotensive patients (group II), 60 Hypertensive patients without any complications (group III), and 60 apparently healthy subjects (control group) and within the age range of 30 - 70 years. These subjects were recruited after ethical approval was obtained from the health facility and following the information given by their physician following patient's case file records. The subjects gave their informed consent to participate in the study and were residents of Port Harcourt metropolis for at least 5 years and attending University of Port Harcourt teaching Hospital in Nigeria. A diagnosis of T2DM was confirmed when a patient had a glycosylated haemoglobin (HbA_1C) value of 48 mmol/mol

(6.5%) or higher as reported by [17].

2.2. Homocystein Assay

Plasma homocysteine concentration was measured using Human Homocysteine (HCY) ELISA kit, Catalogue No. EH4011 from Fine Test. China, following manufacturers instruction. Briefly: after the frozen plasma samples were thawed, the micro titre plate was washed 2 times with PBS. Then 50 ul of Standard, Sample, control and blank were added into each well. Immediately, 50 ul of biotin-labelled antibody were added into each well, and the plate gently tapped to ensure thorough mixing, then the plate was incubated for 45 minutes at 37˚C in the dark. The reagents were aspirated and plate washed 3 times. Then 100 ul of SABC Working solution was added into each well and incubated for 30 minutes at 37˚C, followed by another 5-times wash step after aspiration. After this 90 ul of TMB Substrate solution was added. The plate was again incubated for 20 minutes at 37˚C, and finally 50 ul stop solution was added and absorbance of colour read at 450 nm immediately.

2.3. Estimation of HBAIc

HBA1C concentration was measured using a Finecare™ HbA1c Rapid Quantitative Test, China within 24 hours after sample was collected into EDTA specimen bottles, following manufacturer's instruction. Briefly ID Chip was inserted into the instrument. 10 μL of whole blood Sample were Drawn with a transfer pipette and added into the buffer tube. The specimens with buffer were mixed well for 1 minute by inverting the tube several times. The Test Cartridge was loaded with 75 μL of sample mixture and test Cartridge inserted into the Cartridge holder and the test button was clicked to display the results after 5 minutes.

2.4. Blood Pressure

Participant's blood pressure was determined using a mercury sphygmomanometer. Subjects were made to seat on a chair beside a table with both legs on the floor but not tangled; the subject's arm was made to rest on a table surface that is at level with their arm. Using a stethoscope with a proper-sized cuffs, properly place the cuff on the arm, place the stethoscope over the brachial artery (in the bend of the elbow) and inflate the cuff to a reasonable number digit eg.190, then gradually deflect the pressure in the cuff the while listening to hear the sound of the heat beat (Korotkoff). The first Korotkoff sound was noted, which is the systolic pressure, and the diastolic pressure also was noted, which is the last Korokoff sound before the sounds go silent.

2.5. Statistics

The statistical analysis was done using GraphPad Prism version 9.4.1, Data generated was expressed as mean ± SD. Tukeys multiple comparison was used to determine differences between groups and statistical significance was inferred by a p value < 0.05.

3. Results

Table 1 presents a demographic picture of the study population, while table two shows the analysis of the test results. From our study, the plasma homocysteine level for DM and HBP comorbidity is 12.39 ± 6.73 μmol/L. DM single morbidity is 10.88 ± 2.61 μmol/L and HBP is 9.06 ± 4.56 μmol/L, while the control, apparently healthy subjects is 7.56 ± 4.41 μmol/L (Table 2). This study also evaluated the percentage level of HBAIc in the study populations. It was discovered that percentage level of HBAIc in the Diabetic and hypertensive comorbidity (9.94 ± 2.61%), diabetes mellitus single morbidity (9.43% ± 2.79%) and hypertensive single morbidity (7.3% ± 1.78%) are significantly higher when compared with the control (5.25% ± 0.625%). Furthermore Table 3 shows the comparison of

Table 1. Socio-demographic characteristics of the population.

Characteristics of the population	Diabetes + Hypertension	Diabetes	Hypertension	Control
Gender				
Male	30 (50.0)	30 (50.0)	24 (40.0)	33 (55.0)
Female	30 (50.0)	30 (50.0)	36 (60.0)	27 (45.0)
Age				
30 - 39	-	6 (10.0)	12 (20.0)	42 (70.0)
40 - 49	9 (15.0)	9 (15.0)	12 (20.0)	12 (20.0)
50 - 59	27 (45.0)	9 (15.0)	27 (45.0)	9 (15.0)
60 - 70	24 (40.0)	36 (60.0)	9 (15.0)	-
Foot ulcer	15	12	0	0
Alcohol intake				
Yes	24 (40.0)	21 (35.0)	21 (35.0)	27 (45.0)
No	36 (60.0)	39 (65.0)	39 (65.0)	33 (55.0)
Duration of diagnosis				
<6 months	-	9	-	Not Applicable-
1 - 10 years	48	30	54	-
11 - 20 years	12	12	6	-
21 - 30 years	-	9	-	-

Table 2. Comparative analysis of parameters between studied groups.

Parameters	Diabetes & Hypertensive mean ± SD	Diabetes mean ± SD	Hypertensive mean ± SD	Control mean ± SD	F test	P value	Remark
Homocysteine (μmol/l)	12.39 ± 6.73**a**	10.88 ± 2.61**b**	9.06 ± 4.56**b**	7.56 ± 4.41**c**	3.891	**0.0130**	S
HBAIc %	9.94 ± 2.61**a**	9.43 ± 2.79**a**	7.3 ± 1.78**b**	5.25 ± 0.625**c**	20.36	<**0.0001**	S
Blood Pressure							
Systolic mmHg	151.2 ± 17.45**a**	112.9 ± 7.13**b**	144.65 ± 17.44**a**	121.05 ± 5.89**b**	38.90	<**0.0001**	S
Diastolic mmHg	91.5 ± 8.51	71 ± 4.47	89.6 ± 8.50	79.3 ± 4.85	30.64	**<0.0001**	S

Key: S = Significant, NS = Non-significant Post-Hoc: Homocysteine values within same row with subscripts (a) are significant when compared to control group P ≤ 0.05. However, values within same row with subscript (b) when compared to control group is not significant.

Table 3. Comparative analysis of plasma homocysteine based on sex.

Homocysteine	Male	Female	t test	P value	Remark
Diabetes & Hypertensive	11.46 ± 7.66	13.32 ± 5.94	0.6068	0.5516	NS
Diabetes	10.39 ± 2.19	11.36 ± 7.74	0.3813	0.7074	NS
Hypertensive	8.89 ± 3.83	9.18 ± 5.17	0.1353	0.8938	NS
Control	7.07 ± 5.19	8.17 ± 3.44	0.5442	0.5930	NS

Key: S = Significant, NS = Non-significant.

the plasma homocystein levels with respect to sex. There was no difference in the plasma homocystein levels in both sexes.

4. Discussion

Homocysteine, an important non-protein amino acid is very important in all methylation reactions occurring in the physiological processes as the precursor of the methyl group donor S-Adenosyl-methionine (SAM) which is involved in all methylation reations. However elevated plasma homocysteine levels have been reported to contribute to epithelial damage leading to coronary artery disease and other metabolic syndromes. This study was aimed at evaluating the plasma levels of homocysteine in diabetic (DM) and hypertensive (HBP) subjects attending University of Port Harcourt Teaching Hospital in Nigeria. Previous reports have implicated hyperhomocysteinamia in vascular and coronary diseases [19] [20]. Evidence also has shown that HBP patients are predisposed to insulin resistance and therefore type II DM, hence we set out to evaluate the plasma homocystein levels in these two disease conditions. Because the level of homocystein is determined by adequate folate and B vitamin metabolism, this work indirectly assess this metabolic pathway. However, the analysis of other cause of hyperhomocysteinamia, a mutation in MTHFR gene which has been found to be associated with hyperhomocysteinemia and coronary artery disease in some populations is beyond the scope of this work.

These results of plasma homocysteine levels for DM and HBP are in consonance with the reports of [19] [20], who reported similar plasma homocysteine level in the control subjects. Furthermore the results for all study groups are below the 15 μmol/l and therefore are not hyperhomocysteinemia, rather elevated plasma homocysteine levels (≥10 umol/L [15]. This finding is at variance with the report of [21], who reported 98% hyperhomocysteine in HBP residents in Northern Nigeria. The reason for this disparity may be due to geographical location of the residents in the northern Nigerian study which has a bearing with the dietary nature of the two populations. Another reason could be the age of the subjects used in the Northern Nigerian study [13]. However analysing our result with respect to the therapeutic targets of plasma homocysteine levels of <6.3 μmol/l [12], the subjects plasma homocysteine levels show increased trend (**Table 2**). This may suggest that the subjects in this study may not be having op-

timal folate and B vitamin metabolism. This study has also shown that the coexistence of DM with HBP instigate a greater rise in plasma homocysteine levels. This is because patients with the DM and HBP combined have significantly higher levels of plasma homocysteine compared with either DM or HBP as single morbidity.

This study also evaluated the percentage level of HBAIc in the study populations. HBA1C is a test that reveals diabetic control over a period of time, hence higher percentages depicting poor control. Surprisingly, the percentage HBA1C levels in hypertensive single morbidity is significantly higher than the control subjects. The reason for this finding is unknown, but the authors suggests that hypertension which predisposes to insulin resistance may alter the diabetic control over a long period of time before the patient is diagnosed for DM, since insulin resistance leading to type II diabetes mellitus is often discovered by chance. Higher percentages of HBA1C are obtained in subjects with high level of plasma homocysyetine, an indication that abnormal glucose metabolism may contribute to hyper homocysteinamia. This assumption is corroborated by the reports of [22] [23]. It is also a fact that both DM and HBP are often diagnosed by chance when the patient presents himself for a different medical condition, meaning that these comorbidities can exist long before they are diagnosed in most individuals.

Furthermore, we related the blood pressure with the plasma homocysteine level in various study populations. Our findings show that subjects with the comorbidities has a higher blood pressure and higher plasma homocysteine level than the single morbidity and control. This finding agrees with report of [19] [20], who reported association of hyperhomocysteine with hypertension. Our data is of the view that there is association of hyperhomocysteinemia with increased blood pressure.

Our result show that the homocysteine levels did not significantly differ in both sexes. This report is at variance with the report of [21] [24]. Homocysteine is known to mediate cardiovascular problems by its adverse effects on cardiovascular endothelium and smooth muscle cells by reducing the flexibility of vessels with resultant alterations in subclinical arterial structure, endothelial cell damage and function, thereby altering the process of haemostasis [5] [25] [26].

5. Conclusion

This study has demonstrated that HBP and DM patients have elevated plasma homocysteine levels than the control, in addition, HBP and DM comorbidity cause an enhanced elevation in the plasma homocysyetine levels than single morbidity. This study has also revealed that the control subjects have plasma homocysteine levels higher than the desirable therapeutic target. This implies that residents in these areas should consider the supplementation of their diets with adequate Folate and B vitamins in order to enhance the better metabolism of the amino acid homocysteine.

6. Limitations of the Study

This study did not include analysis for folate and B vitamins as well as other causes of hyperhomocysteinamia, such as a mutation in MTHFR gene which has been found to be associated with hyperhomocysteinemia and coronary artery disease was not done.

The authors hereby state that all human material or data were performed in accordance with the Declaration of Helsinki and Ethical approval for this study was obtained from the Rivers State Ministry of Health and the University of Port Harcourt Teaching Hospital with file no MH/PRS/391/VOL.3/646 and UTH/REC/2022043.

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Conflicts of Interest

Authors declare no competing interest in this study.

Author's Contributions

Author DOO designed the study, managed the data interpretation and wrote the draft. Author HAW reviewed the literature and analysed the data. Author EOA managed the data in conjunction with author LUN who performed the laboratory analysis and generated the data.

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