Research Article

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Association of miRNA122 & ADAM17 with lipids among hypertensives in Nigeria

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Abstract: Background. Dyslipidaemia and hypertension are established major risk factors for cardiovascular diseases. The suggested roles of miRNA-122 and ADAM17 in lipid metabolism can therefore be applied in the management of metabolic disorders. The authors' aim was to determine the association between miRNA-122 and ADAM17, as well as the association between miRNA-122 and lipid fractions, in the study participants.

Method. A comparative cross-sectional study was conducted among 200 hypertensive patients and 100 non-hypertensive adult controls between May, 2015, and June, 2016, in Nigeria. Lipids were analysed with spectrophotometric methods whereas ADAM17 and miRNA-122 were analysed with enzyme linked immunosorbent assay and quantitative polymerase chain reaction, respectively.

Results. The mean (standard deviation [SD]) ages of 200 hypertensives and 100 controls were 56.3 (6.9) and 54.9 (8.3) years, respectively. miRNA-112 and ADAM17 had significantly higher values among dyslipidaemic individuvals compared with non-dyslipidaemic participants. The correlation between miRNA-122 and ADAM17 levels was strongly positive, r=0.82, p<0.05. LDL-cholesterol and total cholesterol also showed statistically significant positive correlation with miRNA-122, r=0.53, r=0.51, (p< 0.001) respectively.

Conclusion. In this study, miRNA-122 showed a strong correlation with ADAM17 and a positive correlation with LDL-cholesterol and total cholesterol. These findings support the stimulant roles of miRNA-122 and ADAM17 in lipid metabolism and thus could be used in the management of dyslipidaemia.

Keywords: miRNA-122; ADAM17; Lipids; Hypertension; Dyslipidaemia

1 Introduction

MicroRNAs (miRNAs or miRs) are small (approximately 22 nucleotides) non-coding RNAs that control gene expression [1]. They have recently emerged as key regulators of metabolism. Approximately 30% of all human genes are thought to be regulated by miRNAs [2]. Indeed, miRNAs control gene expression in diverse biological processes, including development, differentiation, cell proliferation, and apoptosis, such that dysregulation of miRNAs may contribute to metabolic abnormalities [1-2].

Since the discovery of miRNAs, extensive surveys have begun to identify miRNA biomarkers specific for tissue types or disease status. A liver-specific miRNA, miRNA-122, has been shown to be the most abundant miRNA in the liver, with suggested roles in cholesterol, fatty acid and lipid metabolism [3]. It also interacts with the hepatitis C virus genome facilitating viral replication in the host cell. A disintegrin and metalloproteinase17 (ADAM17), also known as tumour necrosis factor-□ converting enzyme (TACE), has been found to be a direct putative target for miRNA-122 [4]. ADAM17/TACE has been described simultaneously by two research groups as the enzyme that processes membrane-bound tumor necrosis factor (TNF)-α precursor into a soluble form [5-6]. This enzyme has been shown to critically regulate inflammatory processes as it cleaves not only TNF-α but also its receptors TNFR1 and TNFR2 [7]. Although other proteinases have been shown to be proteolytically active towards the membrane-anchored

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proTNF-α, ADAM17 appears to be the most efficient in vivo [5]. For this reason, ADAM17 is considered an effective therapeutic target in TNF-α mediated disorders.

Atherosclerosis, as one such disorder, could benefit from this approach as TACE expression has been shown to be associated with lesions in atherosclerosis-prone sites in apo-lipoprotein E-deficient mice [8].

Dyslipidaemia and hypertension are established independent major risk factors in the development of cardiovascular disease (CVD). Their coexistence has more than an additive adverse impact on the endothelium, which results in enhanced atherosclerosis, leading to cardiovascular disease.

Prevalence studies of hypertension among medical admissions in tertiary centres in Nigeria have been reported to be 18.4% in Enugu [9], 28.2% in Port Harcourt [10] and 21% in Benin City [11]. In the Enugu study, it was reported that hypertension with its complications contributed to more than two thirds (69.6%) of the admissions affecting the cardiovascular system.

Thus, cardiovascular disease or injury, the primary clinical outcome of hypertension, is no longer merely an emerging problem in Africa, but has become firmly established, and its magnitude is approaching that of an epidemic [12].

Hence, therapeutic strategies targeted towards prevention and treatment of atherosclerosis that may ultimately lead to CVD need to be intensified. It is known that many patients find it difficult to adhere to counselling regarding exercise, low-fat diet and lifestyle modification as part of management of cardiovascular risk factors. Thus, new strategies are needed to complement these current options for management of cardiovascular disease. Evaluation of the role of miRNAs and their target proteins may provide further evidence to guide management of dyslipidaemia.

The authors' aim was to compare the plasma levels of miRNA-122 and ADAM17 in dyslipidaemic and nondyslipidaemic participants and to determine the association between miRNA-122 and ADAM17 and between miRNA-122 and lipid fractions in study participants.

2 Materials and methods

2.1 Study location

This comparative cross-sectional study was carried out at the University of Nigeria Teaching Hospital (UNTH) and Safety Molecular Pathology Laboratory, both in Enugu, Nigeria, between May, 2015, and June, 2016. UNTH is a 576-bedded tertiary health institution located in South-East Nigeria and serves Enugu, Anambra and Abia states, all in the South-East geopolitical zone of Nigeria. Farming and trading are the major occupations of this group of people. with a small percentage being civil servants.

2.2 Study population

Study participants included 200 hypertensive patients and 100 non-hypertensive adult controls (aged 18 years and older). Patients with essential hypertension attending the medical out-patient clinic at the University of Nigeria Teaching Hospital were recruited with systematic sampling. Healthy controls were recruited from hospital staff and patient relatives.

2.3 Sample collection and analysis

After a fast of 10-14 hours, blood samples were collected aseptically; 5 ml was placed in lithium heparin tubes for lipid analysis, and 5 ml was placed into EDTA tubes for miRNA-122 and ADAM17 analysis. Blood samples were centrifuged for 10 minutes at 1500 rpm and plasma separated into different plain tubes. These were stored at -18^{o}C. Lipid profile was analysed a day after collection, and miRNA-122 and ADAM17 were analyzed 2 weeks later.

Triglycerides [13], total cholesterol [14], high-density lipoprotein-cholesterol (HDL-c) [15] and low-density lipoprotein-cholesterol (LDL-c) [16] were determined with the methods referenced accordingly. ADAM17 was quantified with sandwich enzyme immunoassay [17] (Diagenics, Milton Keynes, England). miRNA-122 was extracted via Stratagene's Micro RNA Isolation Kit (Stratagene, California), which utilizes the guanidine isothiocyanate–phenol:chloroform extraction method [18] and quantified with quantitative polymerase chain reaction (qPCR). Lyophilized qPCR miRNA-122 template standards were obtained from Origene Technologies, Rockville, USA.

2.4 Inclusion criteria

Consenting adult hypertensive patients without any history of previous cardiovascular event were recruited for the study.

2.5 Exclusion criteria

Individuals less than 18 years of age, pregnant women, patients with hepatocellular carcinoma, individuals with any clinical or laboratory parameters suggestive of viral hepatitis or secondary hypertension, and those declining consent were excluded.

According to the National Cholesterol Education Program (Adult Treatment Panel III [ATP III]) [19], dyslipidaemia is defined as total cholesterol ≥5.2 mmol/L, low-density lipoprotein ≥3.4 mmol/L, triglycerides ≥1.7 mmol/L and high-density lipoprotein < 1.0 mmol/L.

2.6 Ethical considerations

Ethical clearance was obtained from UNTH Health Research Ethics Committee after review and approval of study proposal. Informed consent was obtained from participants after the purpose of the study was explained to them.

2.7 Statistical analysis

Data was double-entered into a Microsoft Excel spreadsheet, and analysis was carried out with Epi Info 3.5.1(CDC, Atlanta, GA, USA). Continuous variables were summarised as means (standard deviation [SD]) and categorical variables as counts and percentages. All tests were two-tailed, with $p < 0.05$ taken as statistically significant. Student's t-test was used to compare ADAM17 and miRNA-122 values for dyslipidaemic and nondyslipidaemic groups, and Pearson's correlation test was used to determine correlation.

3 Results

Two hundred (200) adult hypertensive patients and 100 non-hypertensive controls were included in the study. All patients and controls were of Igbo extraction. The mean (SD) age was 56.3 (6.9) years and 54.9 (8.3) years with a range of 41-68 years and 44-69 years for patients and controls, respectively. Male-to-female ratio was 1:2 for patients, and 1:1.5 for controls. The baseline characteristics of the study groups are shown in Table 1. All hypertensive patients who participated in the study were receiving antihypertensive medications either singly or in combinations. The distribution of the patients receiving antihypertensive therapy is outlined in Table 2.

Based on ATP III criteria, 88 (44.0%) hypertensive patients and 23 (23.0%) of the healthy controls were found to be dyslipidaemic for one or more lipid component(s). The pattern of dyslipidaemia is depicted in supplementary Figure 1.

miRNA-112 and ADAM17 had significantly higher values among dyslipidaemic hypertensives when compared with non-dyslipidaemic hypertensives (Table 3).

Dyslipidaemic controls were also found to have significantly higher values when compared with non-dyslipidaemic controls (Table 4).

Correlation between ADAM17 and miRNA-122 levels in the subjects was strongly positive, $r = 0.82$ as shown in Figure 1.

Table 1: Baseline Characteristics of Study Groups. This table describes the age, male-to-female ratio, basal lipid values and systolic and diastolic blood pressure values of the study participants.

S/N	Baseline Characteristics	Hypertensives N = 200	Controls N = 100
1	Age Mean (SD)	56.3 (6.9)	54.9 (8.3)
2	Male:Female ratio	1:2	1:1.5
3	Basal lipid values: Mean (SD)		
	Total Cholesterol (mmol/L)	5.1 (1.3)	4.2 (1.3)
	Low density lipoprotein (mmol/L)	3.6 (1.1)	2.5 (1.2)
	Triglycerides (mmol/L)	0.7 (0.5)	0.6 (0.4)
	High density lipoprotein (mmol/L)	1.1 (0.4)	1.4 (0.4)
4	Systolic Blood Pressure Mean (SD) (mmHg)	151.6 (23.9)	113.5.6 (6.7)
5	Diastolic Blood Pressure Mean (SD) (mmHg)	91.9 (9.8)	79.0 (7.4)

As depicted in Figures 2-4, LDL-cholesterol and total cholesterol showed a statistically significant positive correlation with miRNA-122, whereas the correlation with triglycerides and HDL-cholesterol (supplementary Figure 2) was weak and not significant. Supplementary Figures 3

Table 2: Distribution of Patients Receiving Antihypertensive Therapy. All participating hypertensive patients were receiving antihypertensive therapy either singly or in combination. The majority (88) (44%) were receiving Vasoprin, followed by 48 (24%) on hydrochlorothiazide alone.

Antihypertensives	Number of patients (%)
Nifedipin	12 (6)
Frusemide	8 (4)
Lisinopril	32 (16)
Vasoprin	88 (44)
Hydrochlorothiazide alone	48 (24)
Hydrochlorothiazide + Losartan	12 (6)
Hydrochlorothiazide + Amlodipine	8 (4)
Losartan alone	20 (10)
Amlodipine alone	28 (14)
Aldomet	28 (14)
Irbesartan	8 (4)
Hydrochlorothiazide + Ramipril	8 (4)
Hydrochlorothiazide + Telmisartan	8 (4)
Hydrochlorothiazide + Irbesatan	12 (6)
Hydrochlorothiazide + Amiloride	20 (10)

NB: Percentage above 100 due to patients on more than one drug component

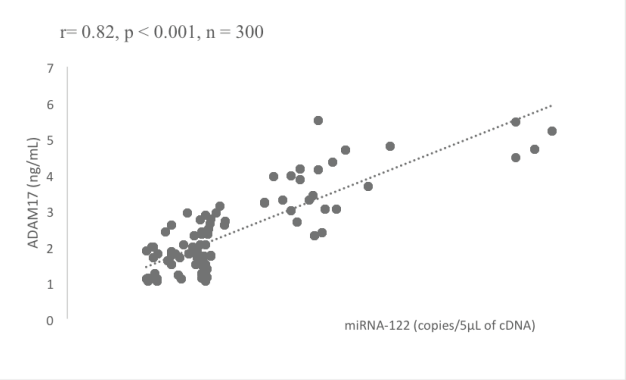

Figure 1: Correlation Graph of ADAM-17 and miRNA-122.
There was a strong statistically significant positive correlation between the two variables, r = 0.82, P < 0.001, n = 300

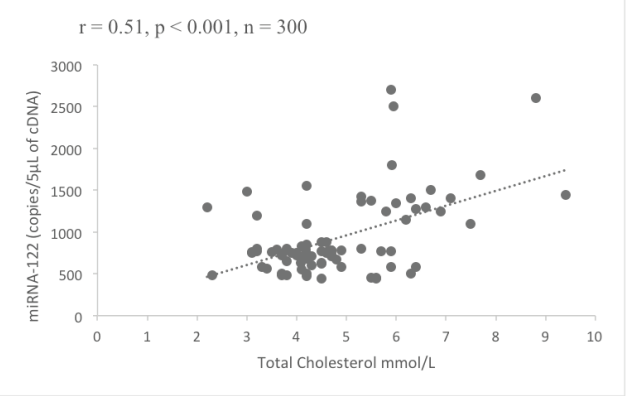

Figure 2: Correlation Graph of miRNA-122 with Total Cholesterol
There was a statistically significant positive correlation between miRNA-122 and Total Cholesterol, r = 0.51, p < 0.001, n = 300

Table 3: Assay Values of Dyslipidaemic and Non-dyslipidaemic Hypertensive. Dyslipidaemic hypertensives had significantly higher values of miRNA-112 and ADAM17 when compared with non-dyslipidaemic hypertensives.

Assays	Dyslipidaemic hypertensives N=88 Mean (SD)	95% CI	Non-dyslipidaemic hypertensives N=112 Mean (SD)	95% CI	p-value
ADAM17 (ng/mL)	3.7 (0.9)	3.5 - 3.9	1.9 (0.6)	1.8 - 2.0	P < 0.001
miRNA-122 (copies/ 5µL of cDNA)	1389.4 (578.2)	1286.0 – 1493.0	668.3 (135.6)	642.9- 693.7	P < 0.001

Table 4: Assay Values of Dyslipidaemic and Non-dyslipidaemic Controls. Dyslipidaemic controls were found to have significantly higher values of miRNA-122 and ADAM17 when compared with non-dyslipidaemic controls.

Assays	Dyslipidaemic Controls N=23 Mean(SD)	95% CI	Non-dyslipidaemic Controls N=77 Mean(SD)	95%CI	p-value
ADAM17 (ng/mL)	3.0 (0.8)	2.7 - 3.4	1.7 (0.5)	1.6 - 1.8	P= 0.005
miRNA-122 (copies/ 5µL of cDNA)	1351.4 (167.4)	1279.0- 1423.8	647.8 (110.0)	622.8 - 672.8	P < 0.001

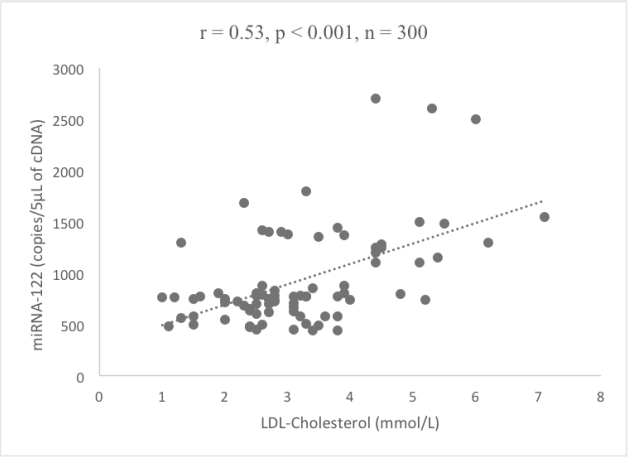

Figure 3: Correlation Graph of miRNA-122 with LDL-Cholesterol
There was also a statistically significant positive correlation between miRNA-122 and LDL-Cholesterol, r = 0.53, p < 0.001, n = 300

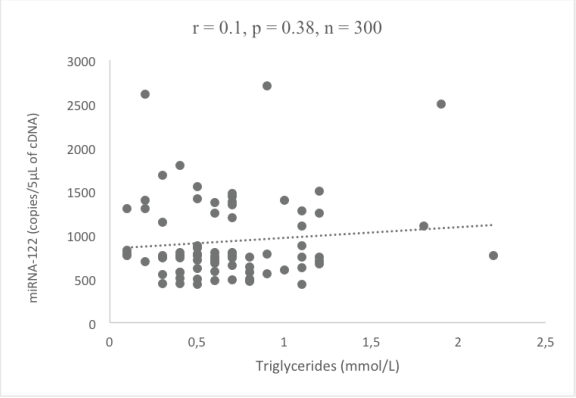

Figure 4: Correlation Graph of miRNA-122 with Triglycerides
Correlation between miRNA-122 and Triglycerides was weak and not significant, r = 0.1, p = 0.38, n = 300

and 4 show the 95% confidence interval (CI) plots for miRNA-122 and ADAM17, respectively.

4 Discussion

Dyslipidaemia and hypertension remain established independent risk factors for cardiovascular disease, and their coexistence represents a synergy that enhances the development of atherosclerosis. In this study, the authors demonstrated higher levels of miRNA-122 among dyslipidaemic hypertensives and controls when compared with non-dyslipidaemic individuals. This corroborates earlier work by Esau et al [3] and Illiopoulis et al [20]. Illiopoulis et al demonstrated that miRNA-122 promoted lipogenesis directly, whereas miRNA-370 promoted lipogenesis indirectly via upregulation of miR-122. Esau et al [3] documented that miRNA-122 inhibition in normal mice resulted in reduced plasma cholesterol levels, increased hepatic fatty-acid oxidation, and decreased hepatic fatty-acid and cholesterol synthesis rates, whereas miRNA-122 inhibition in a mouse model for diet-induced obesity resulted in decreased plasma cholesterol levels and a significant improvement in liver steatosis, accompanied by reductions in several lipogenic genes. The current study, however, differs from the results of Cheng et al [21] that suggested that nonalcoholic steatosis in humans was associated with decreased levels of miR-122. ADAM17 levels were also seen to be elevated in the dyslipidaemic groups, in agreement with reports of Satoh et al [22] and other previous studies [7,23], which demonstrated increased expression of ADAM17 in association with hypertension and atherosclerosis in animal models.

This finding was expected because miRNA-122 exerts some of its actions via regulation of ADAM17. Together with the significant positive correlation seen between miRNA-122, LDL-cholesterol and total cholesterol, this observation further suggests that miRNA-122 may have a role in lipogenesis.

The high proportion of dyslipidaemia seen among hypertensives in this study points to the fact that dyslipidaemic hypertension is still inadequately treated among patients in the study environment. This observation suggests that either the clinicians were not adequately targeting lipid reduction or the patients were not compliant with medication, lifestyle modification or dietary counselling. Due to the synergistic effect of dyslipidaemia and hypertension on the cardiovascular risk profile, other strategies are necessary to complement existing management protocols. Therapeutically targeting miRNA-122 and ADAM17 with RNA-based gene silencing strategies in the form of antagomirs may be useful in ameliorating dyslipidaemia, thereby reducing the risk of atherosclerosis and cardiovascular diseases. A recent report by Zhao et al [24] indicated that gene silencing of TACE in a rabbit model enhanced plaque stability and improved vascular positive remodeling. Krutzfeldt et al [25] also demonstrated that plasma cholesterol measurements showed reduced levels in antagomir-122-treated mice, suggesting that antagomirs may be powerful tools to silence specific miRNAs *in vivo* and may represent a therapeutic strategy for silencing miRNAs in disease.

4.1 Limitation of study

This is a hospital-based study, and thus study participants may not be representative of the entire adult pop-

ulation. Although our participants' body mass index may be important to know, it was not measured in this study. Despite these limitations, this study is relevant as it has documented salient findings that would contribute significantly to knowledge on this subject.

5 Conclusion/recommendation

The study showed a strong correlation between miRNA-122 and ADAM17 in the participants, and miRNA-122 showed a positive correlation with LDL-cholesterol and total cholesterol. The significantly higher levels of miRNA-122 and ADAM17 in dyslipidaemic subjects compared to those in non-dyslipidaemic subjects found in this study support their stimulant roles in lipogenesis. The high proportion of dyslipidaemic hypertensives seen in this study necessitates additional measures to combat dyslipidaemia, and the adoption of gene silencing of ADAM17 and/or miRNA-122 antagomir may fulfill this role. Hence, further research on the role of ADAM17 and miRNA-122 antagomirs in humans with dyslipidaemia is recommended.

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Contributorship: IAM and OBA conceived and designed the study protocol; IAM, OBA and JTE collected the data, performed data entry and data analysis. OBA and MCU interpreted the data. IAM and KNU prepared the manuscript. KNU and JTE edited the manuscript. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Conflict of interest: The authors report no conflicts of interest in this work.

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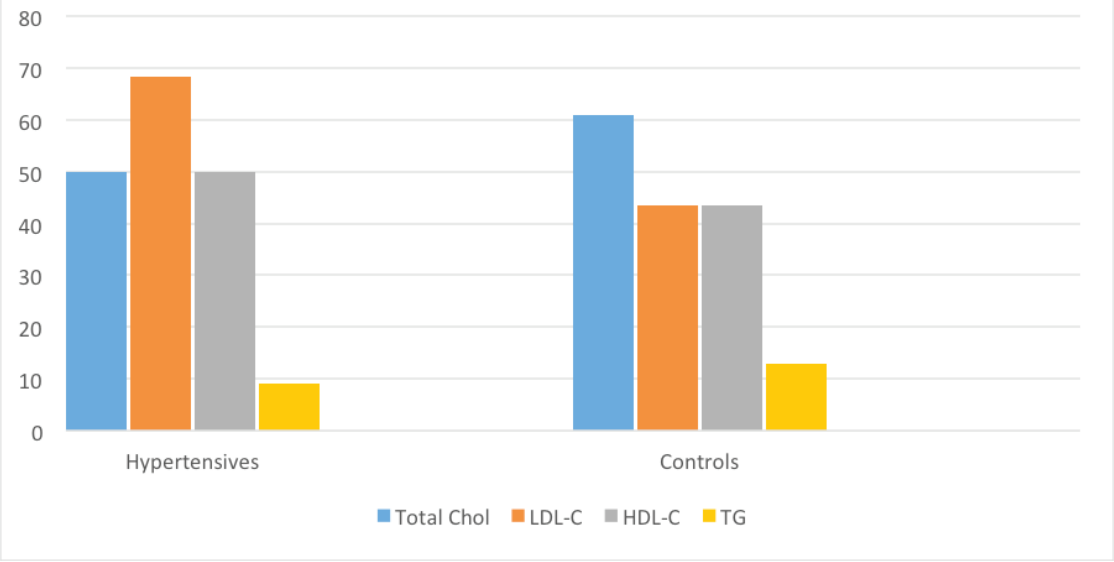
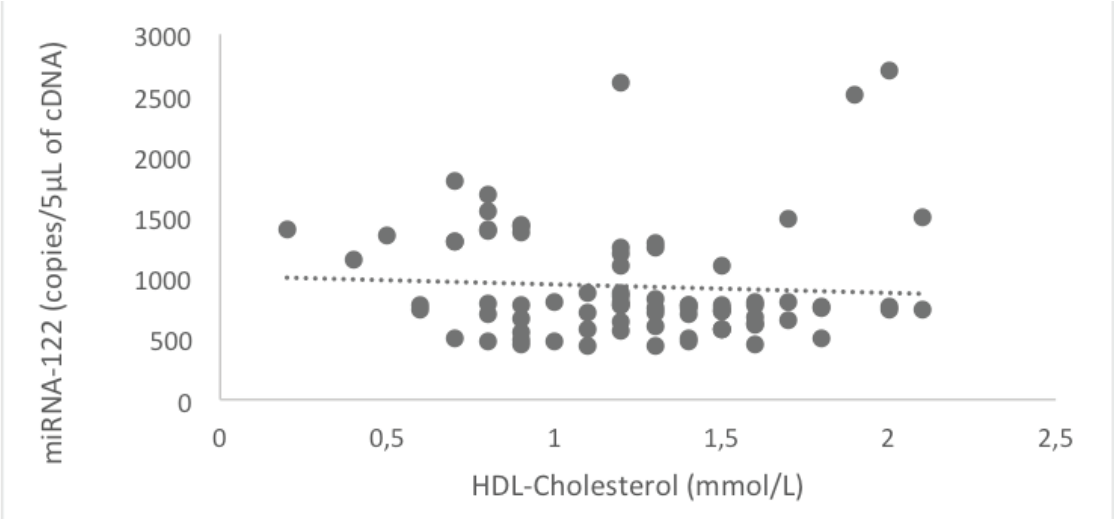


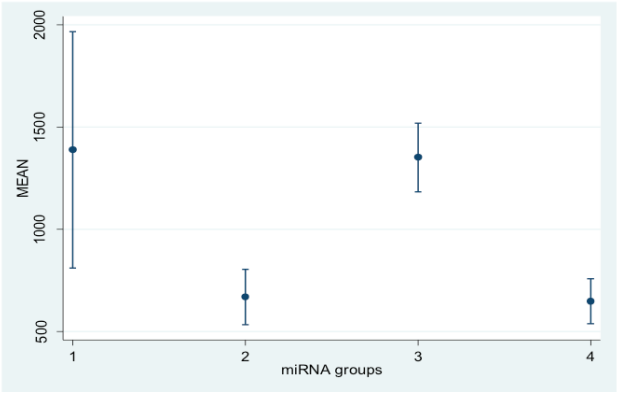
Supplementary Figure 1: Pattern of Dyslipidaemia among Hypertensives and Controls

More of the hypertensive patients (60) (68.2%) had elevated LDL-cholesterol ≥3.4 mmol/L, whereas more of the control participants (14) (60.9%) had elevated total cholesterol ≥5.2mmol/L.

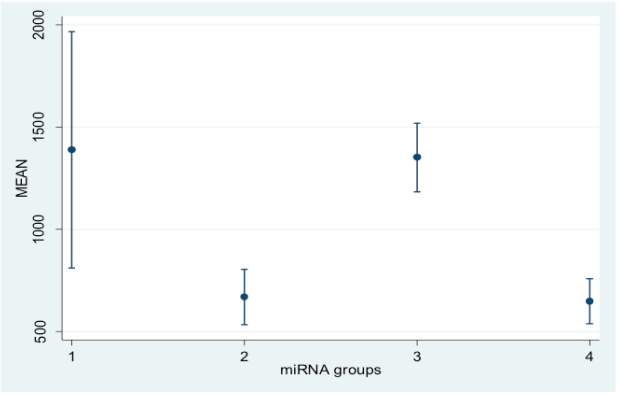


Supplementary Figure 2: Correlation Graph of miRNA-122 with HDL-Cholesterol

The correlation between miRNA-122 and HDL-cholesterol was negative, weak and not significant, r = -0.1, p = 0.60, n = 300



Supplementary Figure 3: 95% Confidence Interval Plot for miRNA-122



Supplementary Figure 4: 95% Confidence Interval Plot for ADAM17

miRNA-22 and ADAM17 95% CI groupings in supplementary figures 3 and 4:

1= Dyslipidaemic hypertensives

2= Nondyslipidaemic hypertensives

3= Dyslipidaemic controls

4= Nondyslipidaemic controls