VOLUME - 11, ISSUE - 11, NOVEMBER - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Medical Biochemistry

DO PLASMA e NOS LEVELS SHARE A RELATIONSHIP WITH DYSLIPIDEMIA TO PREDICT CAD IN YOUNG ADULTS WITH OR WITHOUT HISTORY OF CAD IN FIRST DEGREE RELATIVES?

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ABSTRACT

Coronary Artery Disease (CAD) is endangering the human health globally. Earlier considered to be the disease of elderly, now many young individuals are also suffering from it. There are many modifiable factors due to lifestyle changes but the non-modifiable risk factors of CAD which have genetic correlations are being labelled as culprits and to identify these at an earlier date might help to delay the events. e NOS levels have been identified as one such factor leading to early CAD. The present study was conducted to establish correlation, if any of e NOS with lipid profile and dyslipidemias thereby leading to early atherogenesis.200 apparently healthy adults (age 18-35 y) of either sex were enrolled. They were subdivided into two groups-100 adults with the family history of CAD (Group A) and 100 adults without the family history of CAD (Group B) in first degree relatives (FDR's). Plasma e NOS and serum lipid profile levels were estimated in both the groups. The mean plasma e NOS levels in healthy adults with history of CAD in FDR's (Group A) were 140.97±44.75ng/ml whereas individuals without history of CAD in FDR's had higher e NOS levels (Group B) (152.07±44.61ng/ml) (statistically insignificant p =0.080). However, Total cholesterol (p =0.001) and LDL-c (p= 0.002) levels were higher and statistically highly significant in group A in comparison to group B. There was a positive but statistically insignificant correlation between e NOS and total cholesterol, HDL-c and LDL-c levels in both groups.There is a positive correlation between e NOS and lipid profile. But probably due to a smaller sample size it was statistically insignificant. A larger population study can be done to utilize this marker with dyslipidemia as early predictor of CAD.

KEYWORDS : e NOS, lipid profile, healthy adults, family history, first degree relatives

INTRODUCTION:

Coronary artery disease (CAD) is endangering the human health globally, and over the years its incidence has increased.(1) Developed nations have shown a decline in its prevalence and mortality, but this doesn't stand true for developing countries, like India.(2)

There are many risk factors for CAD. Some are modifiable like Dyslipidemias, smoking, diabetes, abdominal obesity, unhealthy diet, and physical inactivity(3) whereas others are non-modifiable like age, gender, family history of coronary artery diseases.(4)

It is suggested that atherosclerotic process begins in childhood which is influenced by genetics, diet and life style. All these risk factors progress to measurable vascular changes in adulthood. (5) Dyslipidemia is labeled to be atherogenic in nature. (6) These factors including genetic correlation are affected by diet and life style like no exercise, excessive smoking etc.

Endothelial nitric oxide synthase is one of the three NOS isozymes that are encoded by three distinct genes which requires L-arginine for the production of nitric oxide (NO) (7). These three isoforms of nitric oxide synthase are known as: neuronal NOS (n NOS), inducible NOS (i NOS) and endothelial (e NOS). Under physiological conditions, NO is mainly synthesized from e NOS in endothelium. (8,9). This molecule is associated with the dynamics of endothelial function. Its impaired metabolism leads to decreased nitric oxide production which is an indicator of endothelial dysfunction. It plays an important role in the development of coronary artery disease (10). Lipids are thought to play a central role in cardiovascular disease development and are traditionally monitored as predictors of cardiovascular events. (11)

While evaluating preventive interventions, family history of premature coronary artery disease becomes an important non-modifiable risk factors as well as significant predictor of CAD. (12)

The present study was designed to evaluate relation if any; between plasma e NOS and serum lipid profile levels in young healthy adults with and without family history of CAD in first degree relatives (FDR's), with an aim to see if both parameters taken together can be used as predictor markers of CAD in younger population, so as to bring on a change in life style to prevent or delay CAD.

MATERIAL AND METHODS

An observational, cross-sectional study was conducted in Department of Biochemistry, in a tertiary care hospital. Study was done from 2018-2021 after taking approval by the Institutional Research and Ethical committee.

200 healthy individuals, who were fitting in inclusion criterion, in the age group of 18-35 years of either sex after taking the informed consent were included in the study.

They were subdivided into two subgroups of 100 apparently healthy individuals in each group. Group A had individuals with family history of CAD in first degree relatives (FDR's) and group B had individuals without the family history of CAD in FDR's. The first-degree relatives' term stands for parents, maternal and paternal grandparents, siblings, maternal and parental aunts and uncles. The blood samples were taken for estimation of plasma e NOS and serum lipid profile (Total

cholesterol, High density cholesterol, Low density cholesterol, Triglycerides) levels in both groups.

Exclusion criteria:

Following individuals were excluded from the study:

1. A known case of diabetes mellitus
2. A known case of hypertension
3. Individual on the drugs like statins and antidepressant medications.

EDTA plasma sample was used for e NOS estimations and serum sample in plain non gel vacutainer was collected for lipid profile estimation. Samples (both EDTA & plain) were separated and stored at - 20 degree Celsius for later estimations. Serum lipid profile including total cholesterol, triglycerides, LDL-c, HDL-c were estimated on automated analyser (Siemens Dimension RXL max) by kits from Siemens. e NOS was measured by the commercially available kit supplied by QAYEE-BIOCHEMICALS which is based on double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) using assay range: 9.3 ng/ml-300 ng/ml

Statistical Analysis:

It was done using SPSS version 23.0. Data was reported as number, percentage; mean ± standard deviation (SD). Continous data was compared between 2 groups using student't'- test unpaired. Pearson correlation coefficient was calculated to find correlation among different continous variables. A p value < 0.05 was considered significant for all the tests.

RESULTS:

All the 200 study participants were equally subdivided into the two sub groups. Group A (N=100) had apparently healthy young individuals with family history of CAD in first degree relatives (FDR's) and group B (N=100) had apparently healthy young individuals without the family history of CAD in FDR's.

Table1: Lipid profile and e NOS concentrations (mean± SD) according to family history of CAD in first degree relatives

PARAMETER	GROUPA (N=100)	GROUP B (N=100)	p VALUE
e NOS (ng/ml)	140.97±44.75	152.07±44.61	0.080
Total Cholesterol (mg/dl)	224.32±45.04	202.27±47.25	0.001*
Triglycerides (mg/dl)	160.25±72.25	142.31±62.96	0.063
LDL (mg/dl)	144.82±38.44	127.14±39.85	0.002*
HDL (mg/dl)	47.44±9.5	46.66±9.52	0.565

Student 't' test Unpaired; *p<0.05; Significant

In table 1:

1. Plasma e NOS levels were found to be higher in the group B (152.07±44.61) than group A (140.97±44.75) but statistically insignificant (p=0.080).
2. Levels of Total cholesterol were higher in group A (224.32±45.04) and statistically highly significant (p=0.001), when compared with group B (202.27±47.25).
3. Levels of LDL-c were also higher in group A (144.82±38.44) and statistically highly significant (p=0.002), when compared with group B (127.14±39.85).
4. Triglycerides and HDL-c levels were higher in group A than in group B though statistically insignificant.

The plasma endothelial nitric oxide synthase levels were found to be higher in the subjects without history of CAD in FDR's.

This is in correspondence with similar results where endothelial nitric oxide synthase leading to production of nitric oxide has been identified as cardiac saver molecule (13).

Table 2: The correlation between plasma e NOS and total cholesterol, triglycerides, HDL-c, LDL-c of healthy adults with and without history of CAD in FDR'S

PARAMETERS	Group A		Group B	
	r	p	r	p
e NOS vs Total Cholesterol	0.195	0.052	0.086	0.393
e NOS vs Triglycerides	0.107	0.291	-0.021	0.839
e NOS vs HDL -c	0.042	0.676	0.169	0.092
e NOS vs LDL-c	0.178	0.077	0.068	0.499

*p<0.05; Significant

In Table 2, endothelial nitric oxide synthase (e NOS) showed a positive but statistically insignificant correlation with total cholesterol, LDL -c and HDL-c levels in both groups.

2. S. triglycerides showed a negative association with endothelial nitric oxide synthase in group B (r = -0.021, p=0.839) but a positive association was observed in group A (r = 0.107, p=0.291), both were statistically insignificant.

DISCUSSION:

There is a continuous search for newer markers and risk factors to identify early cardiovascular injuries so as to prevent or delay the CAD incidence in young individuals. Conventional risk factors like dyslipidemia, smoking, diabetes mellitus and hypertension have been found to have decreased accuracy (14). e NOS has been found to have an important role in the endothelium vascular functioning, hence it may be considered in future as predictor marker for coronary artery disease.

Decreased bioavailability of nitric oxide, which is synthesized by the endothelial nitric oxide synthase (eNOS) leads to an imbalance between vasodilation and vasoconstriction in the vascular endothelium resulting in endothelium dysfunction (15)which is an underlying cause of coronary artery disease.

Many researchers reported that family history is another conventional risk factor for cardiovascular disease (16,17). They even showed that a positive history of premature CAD increases the risk for any cardiac event by 2 folds in the other family members. The younger populations with predisposing genetic factors are at higher risk of development of CAD (18).

In our study, plasma e NOS levels were higher in group B but statistically insignificant as compared to group A. (Table 1). It reflects that the apparently healthy young individuals without family history of CAD in their first-degree relatives were having a higher level of eNOS (a cardioprotective factor) as compared with the ones with family history of CAD, though the p value of 0.080 is statistically insignificant.

We also observed that total Cholesterol (224.32±45.04 mg/dl) (p=0.001) and LDL-c (144.82±38.44 mg/dl) (p=0.002) levels were high and statistically significant in group A. Serum triglycerides (160.25±72.25mg/dl) and HDL-c (47.44±9.5mg/dl) levels were also high in group A in comparison to group B (TG-142.31±62.96 mg/dl), (HDL-c46.66±9.52 mg/dl) though statistically insignificant. (Table 1) . Higher levels of lipid profile in the group A especially LDL-c are suggestive of increased cardiovascular risk in these individuals with family history of CAD in FDR's. According to the National Cholesterol Education Project (NCEP) Adult Treatment Panel III (ATP III), LDL-c levels less than 100 mg/dL are considered as optimal. If LDL-c levels are 160 mg/dL or greater they are considered as high. The desirable total cholesterol levels have been reported to be < 200 mg/dl and if the values exceeds 240 mg/dL it is regarded as high; HDL-c levels less than 40 mg/dL are considered as low; and elevated triglycerides are greater than 150 mg/dL(19). In both adult and pediatric patients high levels of total cholesterol, triglycerides, LDL-c, and low levels

of HDL-c have been observed in various studies thereby making such individuals more prone to CAD due to dyslipidemia (20,21). Researchers also supported that dyslipidemia is a common feature in children with a family history of dyslipidemia. They found a positive association between family history and the presence of dyslipidemia in children (22).

Table 2 showed a positive but statistically insignificant correlation of eNOS with total cholesterol(Group A r=0.195, p=0.052)(Group B r=0.086, p=0.393), HDL-c(Group A r=0.042, p=0.676)(Group B r=0.169, p=0.092) and LDL cholesterol levels(Group A r=0.178, p=0.077)(Group B r=0.068, p=0.499) in both groups A and B. Li et al,.have demonstrated that high density lipoprotein (HDL-c) stimulates endothelial nitric-oxide synthase (eNOS) activity by binding to scavenger receptors, class B, type I (SR-BI) in a ceramide dependent manner (23). It was also recently concluded that high cholesterol levels are well correlated with eNOS in CHD patients (24). But Letonja ,M observed no major effect on lipid profile by eNOS gene polymorphism in women of Caucasian with premature coronary artery disease. (25).

eNOS also showed a statistically insignificant negative correlation with Triglyceride levels in group B (r= -0.021, p=0.839) (table 2). These results were in accordance to that of Higashibata et al,. who discussed the effect of eNOS polymorphism on leisure time physical activity on serum triglyceride levels (26).

CONCLUSION:

In our study, sample size was less and we focused on age group of 18 -35 years that probably led to statistical insignificant results in the two groups. A larger study is expected to give better significant results. But still we can say that plasma e NOS have a relation with lipid profile which can become a significant indicator to predict CAD especially in individuals with family history of CAD in first degree relatives.

Abbreviations: CAD- coronary artery disease

FDR's- First degree relatives

e NOS – endothelial nitric oxide synthase.

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