Biochemical Study on Endothelial Nitric Oxide Gene Polymorphism in Fatty Liver Patients

Rizk Ahmed El-Baz[1], Alaa Wafa[2], Nasser Mohammed Hosny[3], Sameh Refaat El-Saka[4]

[1]Children Hospital – Genetic Unit, Faculty of Medicine, Mansoura University
[2]Internal Medicine Department, Faculty of Medicine, Mansoura University
[3]Inorganic Chemistry Department, Faculty of Science, Port-Said University
[4]B.Sc. Zology& Chemistry, Faculty of Science, Mansoura University

Abstract: *Endothelial nitric oxide synthase (eNOS) gene polymorphism association with non-alcoholic fatty liver disease (NAFLD).*
Objective: *to check for association of polymorphism of eNOS gene with susceptibility and severity of NAFLD in cases from Egypt.*
Subjects: *This work included 100 cases with NAFLD from Internal,liver unit and 100 healthy individuals. The mean age of cases was 45.45 ± 15.29 (range: 18 – 80 years).*
Results: *Total cases showed significant frequency of G894T GG (P < 0.001, OR = 0.24), 894 TT (P < 0.001, OR = 4.68), T786C CC (P < 0.001, OR = 8.0), 786 CT (P < 0.05, OR = 0.52). 27bp bb (P < 0.001, OR = 4.2), ab (P < 0.001, OR = 0.30). These were considered risk genotypes for disease of NAFLD in Egypt.*
Conclusion: *This study suggest that eNOS homozygous 894TT, 786C genotype may be increase risk of NAFLD in Egypt.*
Abbreviations: *eNOS: Endothelial nitric oxide synthase, NAFLD: non-alcoholic fatty liver disease, PCR-SSP: polymerase chain reaction with sequence specific primers.*

I. Introduction:

Non alcoholic fatty liver disease (NAFLD) is progressively diagnosed worldwide and is considered to be the most common liver disorder in Western countries, estimated to affect at least one-quarter of the general population **(Rector, et al., 2008; Lazo; Clark, et al., 2008).** NAFLD used to be almost exclusively a disease of adults but is now becoming a significant health issue also in obese children. The prevalence of childhood obesity has significantly increased over the past three decades **(Janssen, et al., 2005; park, et al., 2005).** and boosted the prevalence of NAFLD in adolescents **(Barshop, et al., 2008).**

Lipid accumulation in the liver is the major hallmark of NAFLD. A comprehensive understanding of the mechanisms leading to liver steatosis and further transition to nonalcoholic steatohepatitis (NASH) still remains elusive. There is no simple solution to understand the multi-factorial nature of NAFLD appearance and progression, presumably due to the nonlinear interactions of those factors. Abnormalities in lipid and lipoprotein metabolism accompanied by chronic inflammation are considered to be the central pathway for the development of several obesity-related co-morbidities such as NAFLD and cardio-vascular disease (CVD) **(Schindhelm, et al., 2007; Loria, et al., 2007).**

NAFLD is also very common in type 1 diabetes and is strongly associated with increased prevalence of CVD independent of other confounding factors **(Targher , et al., 2010).** In addition to the liver-related causes, CVD represents the major survival risk of patients with NASH **(Ekstedt, et al., 2006).** However, the nature of the relationship NAFLD/CVD is still under debate. McKimmie and coauthors **(Mckimmie et al., 2008).**did not find independent association between hepatic steatosis and CVD in a subset of participants in Diabetes Heart Study. They suggested that hepatic steatosis is more a secondary phenomenon than a direct mediator of CVD. Even so, sufficient evidence exists that CVD risk assessment seems mandatory in NAFLD patients.

Non-alcoholic fatty liver disease (NAFLD) occurs across all age groups and ethnicities and is recognised to occur in 14%−30% of the general population (**Nomura, et al., 1988 ; Browning, et al., 2004).** Primary NAFLD is related to insulin resistance and thus frequently occurs as part of the metabolic changes that accompany obesity, diabetes, and hyperlipidaemia. However, it is important to exclude secondary causes of hepatic steatosis by clinical assessment. Treatment of these conditions differs and revolves around correcting the underlying cause **(Angulo , 2002).**

Lipid transport in plasma utilizes highly specialized lipoprotein complexes. After a meal, dietary fat and cholesterol are absorbed into intestinal cells and incorporated in nascent chylomicrons. The liver is another important source of lipoproteins. A central metabolic role of the liver is to maintain plasma glucose within

narrow physiological limits regardless of the nutritional state of the animal. In energy excess, glucose is converted to fatty acids, which are further used to synthesize triglycerides. Triglycerides can be stored as lipid droplets within hepatocytes or incorporated into very-low-density lipoproteins (VLDL) and secreted into the blood. Once in the blood, triglyceride content of these particles is progressively reduced by the action of lipoprotein lipase (LPL), eventually resulting in intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDL) with relatively high cholesterol content **(Tulenko and Sumner, 2002).** LDL circulates and is absorbed by the liver by binding of LDL to LDL receptor **(Brown and Goldstein, ,1997).**

The blockade of hepatic VLDL secretion results in accumulation of triglycerides in the liver. Microsomal triglyceride transfer protein (MTTP) is essential for the formation of VLDL in the liver **(Olofsson, et al., 2000).** Mice that cannot secrete VLDL due to the conditional knockout of MTTP in the liver exhibit markedly reduced levels of triglycerides in the plasma and develop hepatic steatosis **(Raabe, et al., 1999; Bjorkegren, et al., 2002).**However, without insulin resistance and inflammation **(Minehira, et al., 2008).** In line with the rodent data, human MTTP polymorphisms lead to decreased MTTP activity and VLDL export and are associated with greater intracellular triglyceride accumulation. Altogether, this impacts NASH pathogenesis **(Namikawa, et al., 2004).**possibly through.

Abnormal lipoprotein concentration in plasma reflects disturbances in homeostasis of major lipid components of lipoproteins, triglycerides, cholesterol, and cholesterol esters. Excessive accumulation of triglycerides in the liver is the hall- mark of NAFLD. The potential sources of fat contributing to hepatic steatosis are dietary fatty acids through uptake of intestine-borne chylomicron remnants, increased lipolysis of peripheral fat store, and de novo synthesis. Tracer studies in obese humans with NASH demonstrated that 60% of triglycerides in the liver arose from free fatty acids, 25% from de novo lipogenesis, and 15% from the diet **(Donnelly, et al., 2005).**

Recent evidence suggests that NAFLD might be a mitochondrial disease. Mitochondrial dysfunction contribute to the pathogenesis of NAFLD since it affects hepatic lipid homeostasis, promotes ROS production and lipid peroxidation, cytokine release and cell death **(Begriche, K.; Igoudjil, A.; Pessayre, D.; Fromenty, B. 2006).** Selected recent published studies linking mitochondrial dysfunction to NAFLD. NAFLD has been shown to be associated with mitochondrial paracrystalline inclusions **(Caldwell, S.H.;. 2004 - Sanyal, A.J.;. 2001).** These paracrystalline inclusions have been observed in many mitochondrial myopathies **(Lammens, M.; Laak, H. 2005)** Uncoupling of the oxidation and the phosphorylation and increased free radical production and lipid peroxidation causes cell injury .**(Lammens, M.; Laak, H. 2005).**ROS production causes lipid peroxidation of mitochondrial membranes which can contribute to impaired mitochondrial function and perpetuate the ROS generation. Oxidative stress also triggers production of inflammatory cytokines, causing inflammation and fibrogenic response. This ultimately results in the development of NASH **(Rolo, A.P.; Teodoro, J.S.; Palmeira, C.M. 2012)**.

II. Subject and Methods

This study included 100 cases with (NAFLD) recruited from hepatology Department of Internal Medicine, Mansoura university hospital with an age ranging between 19-67 years

Case genotypes were compared to 100 healthy unrelated volunteers from the same locality DNA extraction and purification

Fasting blood samples (3mL) were collected into two sets of tubes: the first set was ethylene diamine tetra acetic acid (EDTA)–coated vacuum tubes stored at 4°C for DNA extractionand purification was performed using QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany).

Screening for the eNOS gene by PCR-restriction fragment length polymorphism

A- Detection of G894T Mutation using PCR Amplification/RFLP (Restriction Fragment Length Polymorphism)

Estimation of G894T gen mutation was described by (Papazoglou et al. 2004). PCR is based on the enzymatic of fragment of DNA that is flanked by two primers (short olignucleotide)

G894T genotype was performed using PCR amplification. Briefly, primer sequences were: Forward: 5`-CAT GAG GCT CAG CCC CAG-3` and reverse 5`-AGT CAA TCC CTT TGG TGC TCA C-3`.

These primers amplified 206 bpfragment . Cycling conditions were as follow: initial denaturation step 94°C for 5 min, 35 cycling of 94°C for 5 min, 95°C for 25 min, 64°C for 35 min, and 72°C for 40min, and final extension of 72ᵌC for 5min. The amplified product was digested with Mbo1 fragments separated on 2% Agarose gel (Laboratories Canda, Madrid, and Spain). The G894 allele remained uncut (20 6bp), while the 894T was cut into two fragments of 119 and 87 bp (Papazoglou et al. 2004).

B. Detection of T786C Gene Mutation

By applies the same mentioned before except for using the following primers and restriction enzyme.

Primers for T786C:

Forward primer: 5`-ATGCTCCCACCAGGGCATCA-3`

Reverseprimer:5`-GTCCTTGAGTCTGACATTAGGG-3`   Cycling conditions were as follow: initial denaturation step 94ºC for 5 min, 30 cycling of 94ºC for 20 sec, 57ºC for 30 sec, and 72ºC for 30sec, and final extension of 72ºC for 5min. The amplified product was digested with NgoMIV enzyme and fragments separated on 2% Agarose gel (Laboratories Canda, Madrid, and Spain)The T786C substitution at bp 786 creatsNgOMIV recognition sequence. By abolishing aNgOMIV restriction site, the T786C mutation results in the digestion of the 236 bpamplicon into 203- and 33 bp fragments. Wild types (786TT) produced a single band at 236bp, heterozygote (786CT) produced 236, 203-, and 33-bp fragments, and homozygous mutants (786CC) produced 203 and 33bp fragments (Banyasz et al., 2006).

C. Detection of interon 4b/a Gene Mutation using PCR Amplification
By applies the same mentioned before except for using the following primers and no restriction enzyme.
Primer for interon 4b/a:
Forward primer: 5`-AGGCCCTATGGTAGTGGCCTTT-3`
Reverse primer: 5`-TGCTCCTGCTACTGACAGCA-3`
Cycling conditions were as follow: initial denaturation step 94ºC for 5 min, 35 cycling of 94ºC for 1min, 56ºC for 1 min, and 72ºC for 2min, and final extension of 72ºC for 5min. The amplified product was separated on 2% Agarose gel (Laboratories Canda, Madrid, and Spain)Theinteron 4b/a genotype was determined using PCR analysis. The interon 4b/a variant was identified using PCR ,No restriction enzyme digestion of the amplified product.
The interon 4b/a substitution at bp 193,220. (interon 4b/a) produced a double band at 193bp, 220bpCarier heterozygote (ba), produced Single band at 193-bp (aa) Carier homozygous and 220-bp (bb) Carier homozygous (Banyasz et al., 2006).

III.   Result
Comparison between all cases with NAFLD and healthy control regarding to their genotype (T786C) distribution of (eNOS) gene polymorphisms.

T786C		Cases (n = 100)	Control (n = 100)	P	OR (95% CI)
		N (%)	N (%)		
Genotypes	TT	33 (33%)	38 (38%)	> 0.05	0.80(0.45 – 1.44)
	CC	25 (25%)	4 (4%)	<0.001*	8.0(2.67 – 23.98)
	CT	42 (42%)	58 (58%)	<0.05*	0.52(0.30 – 0.92)
Alleles	T	108 (54%)	134 (67%)	<0.01*	0.58(0.39 – 0.87)
	C	92 (46%)	66 (33%)	<0.01*	1.33(1.07 – 1.65)

* Significant P < 0.05

There were significant difference between two groups where CC homozygous mutated is 25% vs 4% (P < 0.001).

Comparison between all cases with NAFLD and healthy control regarding to their genotype (27pb) intron 4b/a allele of (eNOS) gene polymorphisms.

27pb		Cases (n = 100)	Control (n = 100)	P	OR (95% CI)
		N (%)	N (%)		
Genotypes	aa	9 (9%)	14 (14%)	> 0.05	0.61(0.25 – 1.48)
	bb	62 (62%)	28 (28%)	<0.001*	4.20(2.32 – 7.60)
	ab	29 (29%)	58 (58%)	<0.001*	0.30(0.16 – 0.53)
Alleles	a	47 (23.5%)	86 (43%)	<0.001*	0.41(0.27 – 0.63)
	b	153 (76.5%)	114 (57%)	<0.001*	1.51(1.26 – 1.83)

* Significant P < 0.05

There were significant difference between two groups where bb mutated homozygous is high significant (62% vs 28%, P < 0.001).

Comparison between two sex group of NAFLD subjects regarding to their genotypes distribution of (eNOS) gene polymorphisms.

		Male (n = 76)	Female (n = 24)	P
		N (%)		
27pb	(aa)	7 (77.8%)	2 (22.2%)	0.866
	(ab)	21 (7.24%)	8 (27.6%)	
	(bb)	48 (77.4%)	14 (22.6%)	
T786C	(TT)	26 (78.8%)	7 (21.2%)	0.882
	(CT)	31 (73.8%)	11 (26.2%)	
	(CC)	19 (76%)	6 (24%)	
G894T	(GG)	13 (86.7%)	2 (13.3%)	0.275
	(GT)	48 (78.1%)	14 (21.%)	
	(TT)	15 (65.2%)	8 (34.8%)	

This table show there are no any significant difference between two sex groups (P > 0.05).
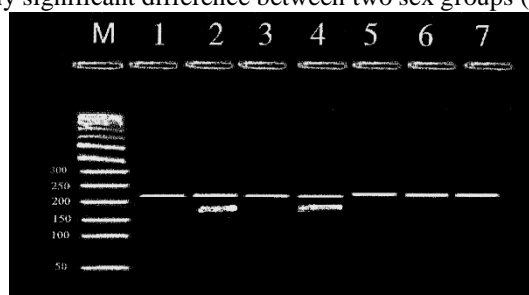

Fig (2): PCR amplification of intron 4b/a polymorphism of eNOS gene PCR product of intron 4b/a polymorphism have ban size (220) bp in bb carrier homozygous and ba carrier heterozygous which has (220, 193 pb fragments bane 2 and 4) (by using DNA size marker 50 bp).
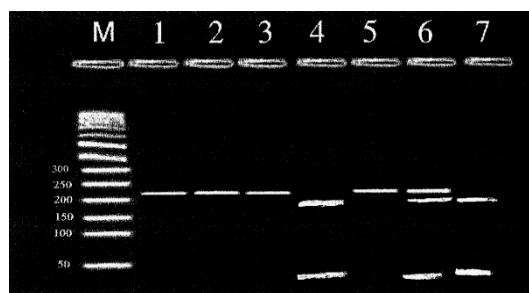

Fig (3): Enzymatic digestion of T786C polymorphism of eNOS gene. Wild type TT is found which appears at 236 bp in lanes 1,2,3& 5, digestion of PCR product of T786C polymorphism of eNOS gene using N90MIV enzyme. Which digests the 236-bp fragment into 203 and 33-bp fragments (heterozygous mutated genotype CT which has 236, 203, 33 bp fragments langes 6 only) (homozygous mutated genotype CC is found which has 203, 33 bp fragments lanes 4,7) (By using DNA size marker 50 bp).
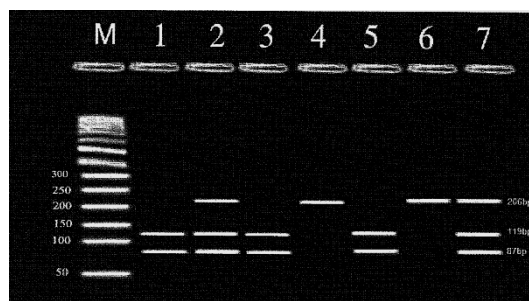

Fig. (4): Enzymatic digestion of G894T polymorphism of eNOS gene. Wild type GG is found which appears at 206 bp only lanes 4 and 6. digetion of PCR product of G894T polymorphism of eNOS gene using MboI enzyme. Which digests the 206-bp fragment into 119- and 87-bp fragments (heterozygous mutated genotype GT which has 206, 119,87bp fragments lanes 2 and 7) (homozygous mutated genotype TT is found which has 119, 87 bp fragments lanes 1,3,5) (By using DNA size marker 50 bp).

IV. Discussion

NAFLD is defined as an excess of fat in the liver in which at least 5% of hepatocytes display lipid droplets **(Neuschwander-Tetri, 2005)** that exceed 5%-10% of liver weight **(Adams et al., 2005 and Browning & Horton, 2004)** in patients who do not A currently favored hypothesis is that "two hits" are required for a subject to develop NASH **(Day and James, 1998).** The first hit leads to hepatic steatosis, and the second to hepatocyte injury and inflammation. However, the primary metabolic abnormalities leading to lipid accumulation within hepatocytes has remained poorly understood. Accumulating evidence indicates that mitochondrial dysfunction plays a central role in the pathogenesis of NAFLD, and that NAFLD is a mitochondrial disease **(Pessayre and Fromenty, 2005).**

The liver, as a super metabolic achiever in the body, plays a critical role in metabolism of carbohydrate, protein and fat to maintain blood glucose and energy homeostasis.

Mitochondria serve as the cellular powerhouse that generates ATP or heat by using substrates derived from fat and glucose. Hepatocytes are normally rich in mitochondria and each hepatocyte contains about 800 mitochondria occupying about 18% of the entire liver cell volume. Mitochondria play an important role in hepatocyte metabolism, being the primary site for the oxidation of fatty acids and oxidative phosphorylation. A mitochondrion contains inner and outer membranes composed of phospholipid bilayers and proteins. The outer mitochondrial membrane contains numerous integral proteins called porins, which contain a relatively large internal channel that is permeable to all molecules of 5000 daltons or less **(Yongzhong et al., 2008).**Hepatic free fatty acids sources: (a) dietary triglycerides (TG) as chylomicron particles from the intestine; (b) de novo synthesis in the liver; (c) Free fatty acids (FFA). influx into the liver from lipolysis of adipose tissue; (d) diminished export of lipids from the liver; and (e) reduced oxidation of fatty acids. Imbalance of these metabolic steps will increased TG accumulation within the cytoplasm of hepatocytes.

Fatty acid oxidation (FAO) is the major source of energy for skeletal muscle and the heart, while the liver oxidizes fatty acids primarily under the conditions of prolonged fasting, during illness, and during periods of increased physical activity **(Pessayre et al., 2005).**While long-chain FFAs entry into the mitochondria is regulated by the activity of the enzyme carnitinepalmitoyltransferase I (CPT-I). Glycolysis generates pyruvate, which is transformed by mitochondria into acetyl-CoA, an intermediate that goes through the citric acid cycle for the production of reducing agents and ATP. However, when glucose and energy levels are elevated, acetyl-CoA is converted to citrate which can leak out of the mitochondrial matrix into the cytosol through the tricarboxylate carrier. In the cytosol, citrate regenerates acetyl-CoA which is converted to malonyl-CoA by acetyl-CoA carboxylase. Malonyl-CoA plays important roles in both hepatic fatty acid oxidation and lipid synthesis. Malonyl-CoA is the initial component for fatty acid synthesis. High malonyl-CoA levels also actively inhibit CPT-I enzyme activity thus robustly decreasing fatty acid oxidation by reducing the rate of fatty acid entry into the mitochondria. Thus, periods of caloric overconsumption, and excessive energy supply, increase malonyl-CoA levels which promotes hepatic fatty acid synthesis (storage) and suppresses fatty acid oxidation (catabolism). Conversely, in the fasting state, hepatic malonyl-CoA levels are low, allowing extensive mitochondrial import of long-chain FFAs and high rates of β-oxidation. During β-oxidation in mitochondria, FFAs undergo a dehydrogenation, then hydration, followed by a second dehydrogenation, and finally thiolysis, releasing one 2-carbon acetyl-CoA molecule and a shortened fatty acid. The cycle is repeated to split the fatty acid into acetyl-CoA subunits. The acetyl-CoA units enter the citric acid cycle to produce reducing agents which can be converted to ATP in the electron transport chain. Under fasting conditions, acetyl-CoA moieties can be converted into ketone bodies (acetoacetate and β-hydroxybutyrate), which are released by the liver to be oxidized in peripheral tissues by the tricarboxylic acid cycle **(Caldwell et al.,1999).** Although the mechanisms responsible for fatty liver are still not fully elucidated, decreased capacity to oxidize fatty acids, increased delivery and transport of FFAs into the liver, and augmented hepatic fatty acid synthesis are likely to play a significant role in the pathogenesis of NAFLD. We and others have shown that mitochondrial abnormalities are closely related to the pathogenesis of NAFLD which raised the possibility that NAFLD **(Ibdah et al., 2005).**

In this study we search about relation between CNOS polymorphism of NAFLD. So that we start with definition of NAFLD and causes and when we know that one hepatocyte have 800 mitochondria and the most of metabolism process occur in liver. So we sure that impairment of mitochondria and metabolism is the main cause of fatty acid deposition in hepatocyte the increase of synthesis of fatty acid or (FFA) up take and the decrease in oxidation which occur by mitochondria on the other hand we study the role of (eNO) in metabolism and mitochondria we obtain that eNO regulate metabolism and mitochondrial oxidation.

Our study suggest that (eNO) polymorphism dysfunction is the main cause of (NAFLD). In this study, when we extracted the (DNA) from whole blood and using (PCR) and primer specific for alleles of (eNOS) gene.

our study on three region of (eNOS) gene by three primer and using distinct enzyme then using gel electrophrosis, we obtained result show relation between this three area of genotypes.

G894T: We obtain thee form (a) TT homozygous mutant 23%, (b) GT heterozygous carrier 62%, and (c) GG homozygous normal 15%; **T786C:** (a) CC homozygous mutant 25%, (b) CT heterozygous carrier 42%, and (c) TT homozygous normal 33%; and **Intron 4b/a:** (a) bb homozygous carrier 62%, (b) aa homozygous carrier 9%, (c) and ba heterozygous normal 29%.

Our study verified that (eNOS) gene polymorphism is responsible for development of (NAFLD) and hope to use that in treatment of NAFLD by:

a- Regulation of (eNOS) gene or control on metabolism of (FA) by lipoic acid or propionyl-L-carnitine**(Nicole Cutler, 2011).**

b- Supplement (NO) by direct method or indirect by supplement of L arginine or C-activate (ePCs) endo-progenator cells by Phytosterol**(Da-long chen et al., 2015).**

This study showed an excess of homozygosity for the (TT),(CC) mutant excess among NAFLD cases by comparison of healthy.

Hence the results of this study suggest that eNOS homozygous (894TT), (786CC) genotype may be at increased risk of NAFLD in Egypt.

NAFLD is defined as an excess of fat in the liver in which at least 5% of hepatocytes display lipid droplets that excess 5%-10% of liver weight in patients who do not consume alcohol.

From this study we can conclude that G894T/T786C, intron 4b/a polymorphism of eNOS gene was found to be associated with development of NAFLD and mutant TT genotypes of G894T ,CC of T786C and bb, genotype of intron 4b/a may considered as genetic risk factors for development of NAFLD in Egypt.

Our result are in agreement with result of **(Petrtyl, 2013)** the study of Petrtyl that includes one hundred and thirty-two patients with liver cirrhosis (age 36-72 years) and 101 controls were examined for functional variants of eNOS (E298D, 27bpintr4, 786T/CThe frequency of E298D (GG 12%, GT 41%, TT 47%), 28bpintr4 (AA 6%, AB 28%, BB 66%), 786T/C genotypes (CC 17%, CT 45%, TT 38%)Examined eNOS variants have no relationship to pathogenesis of liver cirrhosis. Severity of portal hypertension was associated with the changes in endothelial activation in Scandinavian population .

Our result are in agreement with result of **(Vanessa Helena desouzazago, 2013),** LDL, nitrite and lipid-protein ratios (HDL2 and HDL3) ($p < 0.001$) in both genotypes. Interestingly, we observed genotype/drug interactions on CETP ($p < 0.07$) and lipoprotein (a) (Lp(a)) ($p < 0.056$), leading to a borderline This study aimed at evaluating if the T-786C polymorphism is associated with changes of atorvastatin effects on the lipid profile, on the concentrations of metabolites of nitric oxide (NO) and of high sensitivity C-reactive protein (hsCRP).

After each treatment lipids, lipoproteins, HDL2 and HDL3 composition, cholesteryl ester transfer protein (CETP) activity, metabolites of NO and hsCRP were evaluated.

The comparisons between genotypes after placebo showed an increase in CETP activity in a polymorphism-dependent way (TT, 12±7; CC, 22±12; $p < 0.05$). The interaction analyses between treatments indicated that (eNOS) gene polymorphism associated with lipid profile in brazil population.

The our result are in agreement with result of **(Farookthameem, 2008)** study participants (N = 670; 39 families) were genotyped for the three polymorphisms using polymerase chain reaction followed by restriction fragment length polymorphism assay. Association analyses between these polymorphisms and T2DM and its related phenotypes were carried out using a measured genotype approach as implemented in the computer program SOLAR. Of the variants examined, only the 27bp-VNTR variant exhibited significant association with high density lipoprotein cholesterol (HDL-C) (P = 0.04) and diastolic blood pressure (DBP) levels (P = 0.02) after accounting for trait- examined at the eNOS locus, only 27bp-VNTR appears to be a minor contributor to the variation in T2DM-related traits in Mexican Americans.specific covariates. The carriers of the rare allele (27bp-VNTR-4a) are associated with decreased HDL-C and increased DBP levels in Mexican American population.

Our result are in agreement with result of **(Liucs 2013)** this study investigated the role of three NOS3 polymorphisms (T-786C, intron 4b/a, and G894T) in the risk of MetS using a hospital-based case-control study.

We recruited 339 MetS cases and 783 non-MetS controls at a central Taiwanese hospital.

The T-786C TC+CC genotype was significantly associated with a decreased risk of MetS (odds ratio (OR), 0.63; 95% confidence interval (CI), 0.43-0.91), compared to the T-786C TT genotype, according to a logistic regression analysis. This beneficial effect was much greater for those who had ever smoked cigarettes (OR, 0.47; 95% CI, 0.26-0.87) or those who had not consumed alcohol (OR, 0.45; 95% CI, 0.26-0.77). In addition, the intron 4b/a variant genotype was marginally associated with a reduced risk of MetS (OR, 0.68; 95% CI, 0.47-1.00), compared to the intron 4b/a bb genotype, particularly for never alcohol consumers (OR, 0.56; 95% CI, 0.33-0.95). thisresults suggest that the NOS3 T-786C and intron 4b/a polymorphisms may contribute to the risk of MetS in taiwan population ..our result are in agreement with result of **(Imamura, 2008).** The allele frequencies of both polymorphisms showed no considerable differences in 233 non-diabetic subjects and 301 patients with Type II (non-insulin-dependent) diabetes mellitus. Non-diabetic subjects with the (-)786C allele had ($p<0.05$) higher fasting plasma insulin and homeostasis model assessment of insulin

resistance than those with the (-) 786T/ (-)786T genotype. Diabetic subjects with (-)786C allele showed higher HbA(1c) than those with the (-)786T/(-)786T genotype. A euglycaemichyperinsulinemic clamp study done on 71 of the 301 patients showed a lower glucose infusion rate in diabetic patients with the (-)786C allele than those without it. In diabetic patients with the (-)786C allele, plasma nitrate and nitrite concentrations were lower than in subjects without it (p=0.026). No differences were observed between mutant carriers of Glu298Asp and non-carriers among both non-diabetic subjects and Type II diabetic patients.

The (-)786T-C mutation of the eNOS gene is associated with insulin resistance in both Japanese non-diabetic subjects and Type II diabetic patients.

Our study in agreement with result of **(Miranda, 2013).**Haplotypes in the three groups of subjects. The CC genotype for the T(786)C polymorphism was more common in the MetS group than in the control group (OR = 3.27; CI 1.81-9.07; P < 0.05). However, we found no significant differences in the distribution of eNOS haplotypes (P > 0.00625; P for significance after correction for multiple comparisons). Our findings suggest that while eNOS haplotypes are not relevant, the CC genotype for the T(786)C polymorphism is associated with MetS in obese children and adolescents. in brazil

Our study in agreement with result of **(Alkharfy,2012)** in Saudi Arabia. (eNOS) gene polymorphisms and MetS in a Saudi Arabian Arabians (477 MetS and 409 Non-MetS). The genotype distribution (TT, p=0.001; TC, p=0.001; TC+CC, p=0.001) and allele (T, p=0.007; C, p=0.007) frequency of the -786T>C SNP were significantly different between Non-MetS and MetS subjects which remained significant after Bonferroni correction. Moreover: 1) the GT and GT+TT genotypes of the 894G>T SNP were associated with elevated blood pressure (p=0.017, and p=0.022, respectively); 2) the ab variant of 4a/b polymorphism was associated with decreased HDL levels (p= 0.044); and 3) the TC+CC genotype and C allele of the -786T>C SNP were associated with increased fasting glucose levels (p=0.039, and p=0.028, respectively). Also, G-a-C was identified as the risk haplotype for MetS susceptibility (p=0.034). The results suggest a significant association of 894G>T, 4a/b and -786T>C polymorphisms with MetS and its components is present in an Arab population.

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