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Influence of NADPH Oxidase (p22phox C242T) and Adiponectin (SNP +45) Gene Polymorphisms on Clinically Euthyroid Type 2 Diabetics- Fresh Insights into Oxidative Stress Mediated Insulin Resistance

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KEYWORDS

Diabetes mellitus, Euthyroid, ADIPO Q, NADPH Oxidase, Polymorphism

ABSTRACT:

Background: Diabetes mellitus is a chronic metabolic disorder with a pronounced risk of comorbidities including hypertension, thyroid and other endocrine, organ abnormalities, vastly attributable to its debilitating nature and impending ramifications. This study is an earnest attempt to bridge the gap between biochemical and clinical implications of NADPH Oxidase (p22phox C242T) and Adiponectin (SNP +45) gene Polymorphisms, and determine the association of oxidative stress with insulin resistance in clinically euthyroid Type 2 Diabetes mellitus.

Methodology: The study included 80 subjects (clinically euthyroid) comprising 40 type 2 diabetics (T2DM) and an equal number of healthy volunteers (both genders) in the age group 35 to 65 years. Data related to anthropometry measures was computed. Biochemical estimations were on the basis of established procedures with stringent quality control. Two ml of blood samples in EDTA tubes were aliquoted for polymorphism studies (C242T & SNP+ 45T/G)

Results: A positive association was found between the gene polymorphisms, namely ADIPOQ SNP + 45 T >G (TT & TG) in exon 2 and NADPH oxidase (C242T p22phox) (CC+CT & TT) in T2DM. Biochemical parameters, namely HbA1c and HOMA-IR were significant with p values of 0.014 and 0.017 respectively. Triglyceride/High Density Lipoprotein, Mg 2+, Thyroxine and Cortisol were significant with reference to NADPH oxidase and ADIPO Q polymorphisms (p values of 0.044, 0.05, 0.025 and 0.002 respectively).

Conclusion: SNP + 45 T>G and NADPH (C242T p22phox) gene polymorphisms in insulin resistant T2DM are characteristic in the clinically euthyroid population with reference to glycemic indices and lipid profile.

1. Introduction

There are cardinal evidences linking the pathogenesis of diabetes mellitus with oxidative stress [1,2]. Oxidative stress is aptly described as a culmination of increased production or decreased scavenging of reactive oxygen species (ROS). Despite multiple sources of ROS, a

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major role of Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase as a source of superoxide and hydrogen peroxide has been well documented [3]. Although NADPH oxidase has been primarily characterized in phagocytic leucocytes 4, its typical roles have been delineated in several cell types including endothelial cells, vascular smooth muscle cells and adipocytes [5–8]. This has prompted us to probe as to whether NADPH oxidase activity could lead to pathogenesis / progression of micro- and macrovascular complications of Type 2 Diabetes mellitus (T2DM).

NADPH oxidase (NOX) derived ROS plays a critical role in maneuvering endothelial function and vascular tone. It is of immense significance as to how the expression of genes associated with cellular defense against oxidative stress is altered in patients with T2DM [9]. NOX catalyzes the formation of a superoxide free radical by enabling the transfer of one electron to oxygen from NADPH. NADPH + $2O_2$ ↔ $NADP^+$ + $2O_2^-$ +H^+. Evidence based information depicts the fact that NADPH oxidase is a major source of intrarenal oxidative stress and is upregulated by a host of metabolic factors culminating in the overproduction of ROS in podocytes, endothelial cells, and mesangial cells in glomeruli. This has a nexus with the initiation and progression of glomerular diseases. A definitive role exists for the NADPH oxidase-induced oxidative stress in the pathogenesis of metabolic disease- related renal injury. Comprehending the mechanism would be of immense help in devising potential therapeutic strategies with greater focus [10, 11]. Lines of evidences cite the fact that the genes associated with the generation of ROS and the characteristic antioxidant defense mechanisms are altered in Asian Indians with T2DM, thereby eventually predisposing to microangiopathy. These changes are linked to the markers of oxidative stress, namely lipid peroxidation and protein carbonyls. This opens up newer vistas in therapeutic modalities that could modulate the changes in cellular ROS, thereby finding use in preventing vascular complications [12].

Taking cognizance of the fact that free radicals mediated biochemical metamorphosis is associated with T2DM as well as adiponectin status and also in view of the association between insulin resistance (IR) and thyroid status, the need arises to visit holistically the commonly observed polymorphisms of the genes (NOX and adiponectin), namely NADPH Oxidase (p22phox C242T; rs4673) and Adiponectin (SNP +45; rs2241766) concurrently in the given setting. The significant biochemical events linking IR, ADIPOQ a secreted protein that shows conspicuous homology to subunits of complement factor C_1q, besides containing a collagenous structure at the N-terminal and a globular domain at the C-terminal and the two enzymes are schematically depicted **(Fig.1).** It is also well documented that oxidative stress induces thyroid disorders.

Though these biochemical events are considered common to both insulin resistant T2DM and thyroid disorders, as exemplified by previous studies, 13 very few studies are available in the literature that addresses the special group, namely clinically euthyroid type 2 diabetics. Hence, we undertook this study to elucidate the role of NADPH oxidase and ADIPOQ in clinically euthyroid type 2 diabetics.

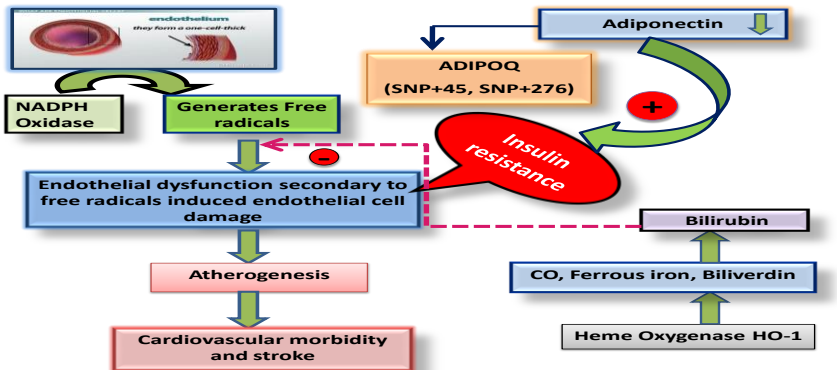

Fig.1: Proposed mechanism to portray the link between NADPH oxidase and ADIPOQ in T2DM

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2. Methods

Inclusion criteria:

40 consecutive controls (adults: 35-60 years, age and gender matched) with no obvious clinical manifestations of T2DM and thyroid disease were included for the study, namely Group 1-Control.

40 consecutive patients with T2DM (FBS≥126 mg/dl) (adults: 35-60 years of age and gender matched) with no obvious clinical manifestations of thyroid disease and who had visited the diabetic clinics housed at a tertiary health care establishment in Pondicherry, South India were included for the study, namely Group 2 – Case

Exclusion criteria: Diabetic patients with a previous history of thyroid diseases such as hypothyroidism, hyperthyroidism, Goitre were excluded (pertaining to Case) and those with T2DM and Thyroid diseases were also excluded (pertaining to Control). Patients with a previous history of cardiac, liver, muscle and other chronic illness were also excluded.

Biochemical assessments:

All the estimations were enabled by established methods/procedures duly approved by the International Federation of Clinical Chemistry and laboratory Medicine (IFCC). The Internal quality Control was maintained through samples provided by M/sBiorad USA. External Quality Assessment, was facilitated through the Clinical Biochemistry laboratory of Christian Medical College (CMC), Vellore which has been accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL). Venous blood was collected from the subjects, following an overnight or 12 hours of fasting and the samples were analyzed for fasting plasma glucose, Insulin, HOMA-IR (a measure of insulin resistance), lipid profile, glycated hemoglobin, T3, T4 and TSH. Fasting and postprandial glucose were estimated, based on glucose oxidase-peroxidase method. Fasting insulin was enabled by automated chemiluminescence. Glycated hemoglobin (HbAIC) was quantitated by HPLC method. Fasting insulin (venous plasma) levels was determined by automated electro Chemiluminescence. The insulin resistance index was assessed by the homeostatic model assessment of Insulin resistance (HOMA-IR) and computed using the formula: Fasting insulin (mU/L) x fasting glucose (mmol/L)/22.5. Triacylglycerols (TAG) in serum was measured by glycerol kinase method. Total cholesterol was quantitated by the enzymatic method. HDL cholesterol was measured by polyanion precipitation. LDL cholesterol was computed using Friedwald equation i.e., LDL cholesterol= Total cholesterol- (HDL cholesterol + VLDL) where VLDL = TAG/5. Care was taken to apply the Friedewald formula within the prescribed limits of Triacylglycerol. Small dense LDL particles was quantitated using the surrogate marker (TAG/HDL) and the thyroid hormones, T3, T4 & TSH estimated by automated electrochemiluminescence method. Free Triiodothyronine (FT3), Free Thyroxine (FT4), Thyrotropin (TSH) and Cortisol in serum were quantitated based on automated electrochemiluminescence method. Total and Direct bilirubin were estimated by established methods. Total protein in serum was enabled by spectrophotometry (Biuret method). The transaminases (aminotransferases), namely Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), as well as alkaline phosphatase (ALP) was quantitated spectrophotometrically by UV Kinetic methods.

Genomic DNA was extracted from whole blood using commercial kit, and genotyping for ADIPOQ gene and NADPH Oxidase were enabled, as per the established protocol. These two functional single nucleotide polymorphisms (SNPs) were used to study the effect on T2DM, with reference to clinically euthyroid cases. The entire procedure has been explained to the patients along with the risk and benefits in their language and written informed consent was obtained. The study was approved by the duly constituted Research Advisory committee and Institutional Human Ethics Committee (MGMCRI/IRC/04/2020/11/IHEC/124).

Statistical analysis

Epi Data and MS excel were deployed for the purpose of enabling statistical analysis. As per the convention, Mean and Standard deviations (SD) were computed with reference to the numerical variables. Independent t-test was used to determine the significance between the mean values of two groups. Analysis of covariance (ANCOVA) was employed for the purpose of testing the main and interaction effects of categorical variables on a continuous dependent variable. Proportions of genotypes of alleles was compared through Pearson $\chi 2$

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analysis, odds ratios (ORs) and 95% confidence intervals (CI). The genotype frequencies were tested for Hardy–Weinberg equilibrium using the $\chi 2$ test.

3. Results

The patient's demographic characteristics are presented in **Table 1** that reveal the differential characteristics of history of patients and shows strong association with respect to adiponectin and NADPH oxidase gene polymorphisms in clinically euthyroid T2DM.

Table 2 represents additional biochemical tests in T2DM to examine the role of adiponectin in the modulation of vascular NADPH oxidase, namely waist-hip ratio, HbA1c, TAG/HDL, HOMA-IR, T4, Zn^{++}, Mg^{++} and cortisol. These portray a pronounced association among healthy and clinically euthyroid type 2 diabetic patients.

Table 3 represents the details pertaining to descriptive statistics for HbA1C. Tables 3,3.1.3.2,3.3,3.4,3.5,3.6 exhibit the association of HbA1c, TAG/HDL, HOMA-IR, Zinc, Magnesium, T4 and Cortisol with ADIPO Q and NADPH Oxidase gene polymorphisms.

Table 4 represents the association of biochemical parameters with adiponectin and NADPH oxidase gene polymorphisms in clinically euthyroid type 2 diabetic subjects based on the analysis of covariance

Table1: Depiction of demographic and baseline parameters

		Group		Total	Chi-Square Tests	Odds Ratio	95% Confidence Intervals		p-value
		Case	Control				Lower	Upper	
ADIPO-Q	TG	16 (40%)	13 (32.5%)	29 (36.3%)	0.487	-	-	-	0.485
	TT	24 (60%)	27 (67.5%)	51 (63.8%)		1.384	0.554	3.459	
NADPH oxidase	CC	21 (52.5%)	19 (47.5%)	40 (50%)	0.200	-	-	-	0.655
	CT+TT	19 (47.5%)	21 (52.5%)	40 (50%)		1.221	0.508	2.939	
History	Y	26 (65%)	15 (37.5%)	41 (51.3%)	6.054	-	-	-	0.014*
	N	14 (35%)	25 (62.5%)	39 (48.8%)		3.096	1.244	7.706	
Gender	F	16 (40%)	13 (32.5%)	29 (36.3%)	0.487	-	-	-	0.485
	M	24 (60%)	27 (67.5%)	51 (63.8%)		1.384	0.554	3.459	

ADIPOQ (TT –wild type TG-heterozygous) NADPH oxidase (TT-wild type CC-homozygous CT-heterozygous) Y-Yes; N-No; F-Female; M-Male

Table2: Comparison of baseline parameters between case and controls

Parameters	**Group**	**Mean**	**SD**	**SE**	**Independent t-test**	**p-value**
Age(yrs)	Case	54.8	10.454	1.653	5.066	< 0.001#
	Control	40.825	13.967	2.208		
W/H	Case	0.964	0.065	0.01	7.159	< 0.001#
	Control	0.88	0.035	0.006		
FBS(mg/dl)	Case	175.175	57.862	9.149	8.624	< 0.001#
	Control	94.55	12.157	1.922		
PPBS(mg/dl)	Case	274.225	86.97	13.751	10.852	< 0.001#
	Control	120.925	20.467	3.236		

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HbA1c mmol/mol	Case	8.695	1.778	0.281	13.126	< 0.001#
	Control	4.872	0.479	0.076		
TAG(mg/dl)	Case	170.55	62.171	9.83	3.721	< 0.001#
	Control	124.025	48.883	7.729		
TAG/HDL	Case	3.687	1.563	0.247	3.541	< 0.001#
	Control	2.603	1.143	0.181		
T3 ng/dL	Case	2.725	0.669	0.106	-0.083	0.934
	Control	2.736	0.41	0.065		
T4 mcg/dL	Case	1.259	0.354	0.056	3.541	< 0.001#
	Control	1.016	0.25	0.04		
TSH mIU/L	Case	3.293	1.881	0.297	0.591	0.556
	Control	2.938	3.304	0.522		
Insulin (mIU/L)	Case	12.464	5.362	0.848	13.864	< 0.001#
	Control	0.665	0.468	0.074		
HOMA-IR	Case	5.332	2.871	0.454	11.397	< 0.001#
	Control	0.155	0.111	0.018		
Zinc mcg/dL	Case	41.9	5.737	0.907	-15.617	< 0.001#
	Control	61.825	5.674	0.897		
K^+ mEq/L	Case	4.735	0.372	0.059	3.046	0.003*
	Control	4.48	0.376	0.06		
Mg++ mg/dl	Case	1.445	0.353	0.056	-2.347	0.021*
	Control	1.613	0.281	0.044		
Uric acid mg/dl	Case	3.933	0.965	0.153	1.971	0.052
	Control	3.547	0.772	0.122		
Alb g/dL	Case	4.529	0.378	0.06	4.900	< 0.001#
	Control	4.185	0.233	0.037		
ALP IU/L	Case	83	21.455	3.392	-2.010	0.048*
	Control	96.75	37.582	5.942		
Cortisol mcg/dL	Case	19.423	6.393	1.011	6.676	< 0.001#
	Control	11.408	4.099	0.648		

FBS- Fasting Blood Glucose, PPBS- Post prandial Blood Glucose, Triacyl glycerol, High density Lipoprotein, TSH - Thyroid Stimulating Hormone, HOMA-IR- Homeostasis model Assessment- estimated Insulin resistance, K+- Potassium, Mg2+ - Magnesium, Alb- Albumin, ALP- Alkaline Phosphatase. *p* value is statistically significant. $p<0.01^{\#}$, $p<0.05$*

Table 3: Descriptive statistics of genomic association with glycemic control

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	7.938 + 1.395	9.531 + 2.286
	CT+TT	7.95 + 1.062	8.8 + 1.445
Control	CC	5.167 + 0.441	4.885 + 0.526
	CT+TT	4.929 + 0.663	4.707 + 0.292

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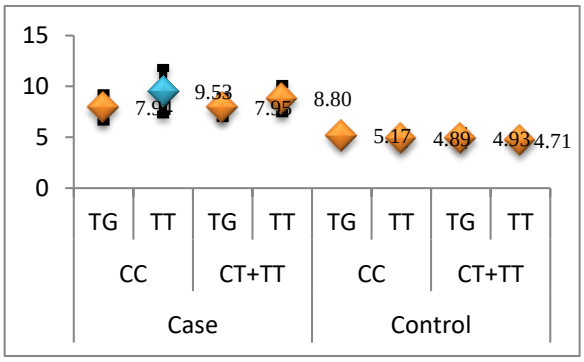

Table 3.1: Descriptive statistics of genomic association with Triacylglcerol/High Density Lipoprotein

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	3.621 + 0.982	4.3 + 1.88
	CT+TT	3.146 + 1.403	3.404+1.555
Control	CC	2.155 + 1.055	2.443+1.041
	CT+TT	4.011 + 1.054	2.239+0.808

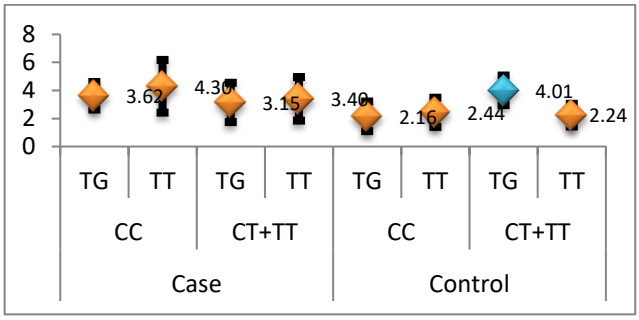

Table3.2: Descriptive statistics of genomic association with HOMA-IR

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	8.09 + 4.23	4.762 + 2.56
	CT+TT	4.954 + 1.117	4.277 + 1.742
Control	CC	0.085 + 0.035	0.212 + 0.151
	CT+TT	0.083 + 0.044	0.169 + 0.078

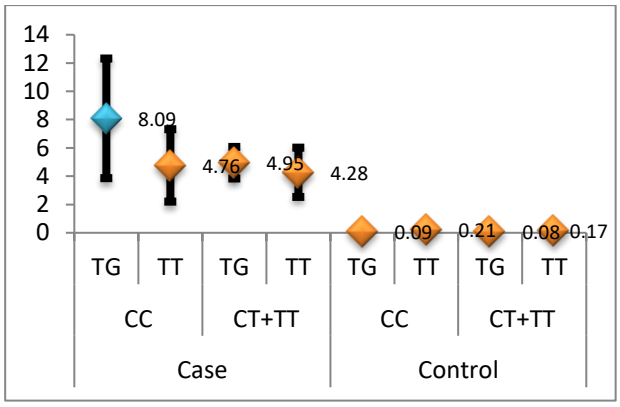

Table 3.3: Descriptive statistics of genomic association with Zinc

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	40.125 + 5.842	41.231 + 4.419
	CT+TT	43.25 + 6.042	43 + 7.043
Control	CC	61.833 + 5.419	59 + 4.761
	CT+TT	62.857 + 3.579	63.929 + 6.719

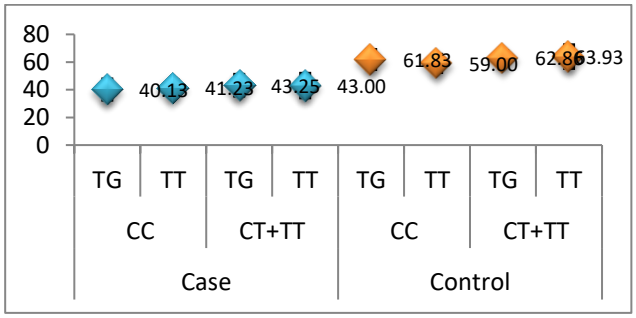

Table 3.4: Descriptive statistics of genomic association with Magnesium

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	1.163 + 0.403	1.546 + 0.293
	CT+TT	1.637 + 0.288	1.391 + 0.348

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Control	CC	1.75 + 0.378	1.662 + 0.25
	CT+TT	1.629 + 0.304	1.5 + 0.239

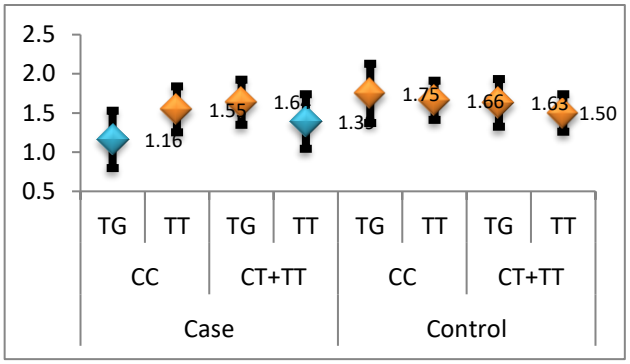

Table 3.5 Descriptive statistics of genomic association with T4

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	1.444 + 0.476	1.343 + 0.382
	CT+TT	1.059 + 0.144	1.17 + 0.252
Control	CC	1.222 + 0.272	0.964 + 0.163
	CT+TT	0.886 + 0.226	1.042 + 0.281

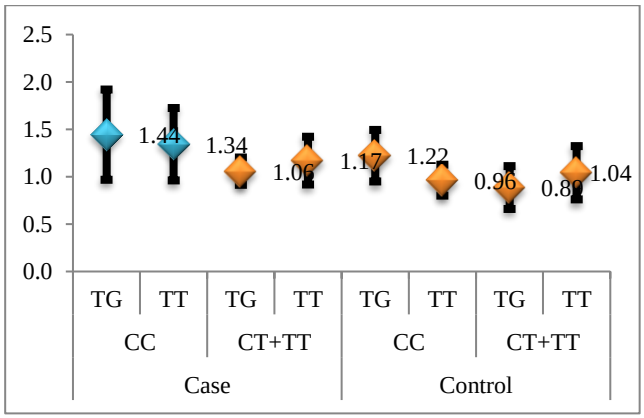

Table 3.6: Descriptive statistics of genomic association with Cortisol

Group	NADPH oxidase	ADIPO-Q	
		TG	TT
Case	CC	16.004 + 6.782	22.706 + 6.467
	CT+TT	20.899 + 6.307	16.956 + 4.168
Control	CC	9.453 + 3.047	13.602 + 4.539
	CT+TT	10.986 + 4.231	10.419 + 3.437

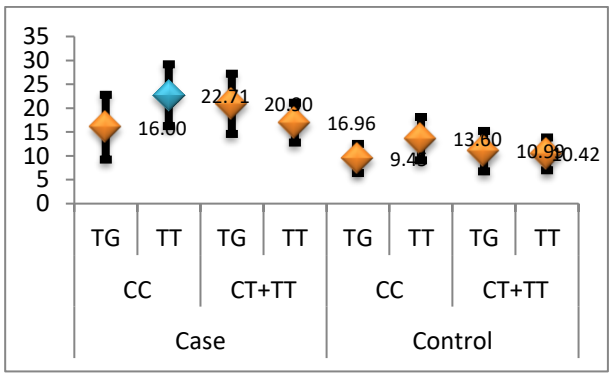

Table 4: ANCOVA for biochemical variables in association with ADIPO Q and NADPH Oxidase

Variables	HbA1c	TG/ HDL	HOMA-IR	Zinc	Mg	T4	Cortisol
Group	<0.001#	0.004	<0.001#	<0.001#	0.016*	0.001	<0.001#
NADPH oxidase	0.337	0.817	0.036*	0.044*	0.826	0.004*	0.597
ADIPO-Q	0.103	0.653	0.031*	0.865	0.531	0.737	0.183
Group Vs NADPH oxidase	0.797	0.015*	0.041*	0.842	0.083	0.277	0.867

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Group Vs ADIPO-Q	0.014*	0.05	0.017*	0.623	0.377	0.678	0.862
NADPH oxidase Vs ADIPO-Q	0.562	0.044	0.134	0.632	0.050*	0.025*	0.002*
Group Vs NADPH oxidase & ADIPO-Q	0.495	0.181	0.122	0.324	0.091	0.462	0.213

p value $\leq 0.05^{*} : \leq 0.001^{\#}$ statistically significant

4. Discussion

The role of thyroid as a bridge between adiponectin and NADPH oxidase among clinically euthyroid type 2 diabetics has been investigated in the current study. The available data points to the fact that the NOX family of NAD(P)H oxidases might acquire relevance with reference to the vascular generation of ROS in pathological conditions[14]. Thus, it should be possible to target therapies more effectively in order to mitigate the influence of oxygen free radicals. Promulgating therapeutic interventions on these lines could confer benefit with reference to the management of diseases associated with vascular damage.

As per a study on metabolic syndrome, a higher TSH within the euthyroid range is linked to atherosclerosis and adipocyte dysfunction [15]. As per a previous report from our laboratory, the relationship with insulin resistance, in the light of thyroid status, but with reference to Low density Lipoprotein particle size, T3, T4, TSH, Insulin, divalent cations and Adiponectin gene polymorphism could be considered cardinal[16]. Gene polymorphism of adiponectin that has been widely studied by other workers using insulin resistance as the point of focus was also taken up in this study. One such polymorphism is SNP+45 of adiponectin [16]. This study of ours had depicted the fact that even the wild type SNP+45 in exon2, namely TT could itself be used as a molecular indicator of altered thyroid status in insulin resistant type 2 diabetics, as studied in overweight and obese individuals who were apparently euthyroid. Our study further depicted differential effects of gene polymorphism with reference to adiponectin SNP + 45 in obese, non-obese and overweight type 2 diabetics who were found to be clinically euthyroid. One more study had also delineated the fact that LDL was statistically significant, while comparing non-obese with overweight T2DM [17]. A recent study has deciphered the fact that free triiodothyronine/free thyroxine (FT3/FT4) ratio is pronouncedly associated with insulin resistance in the euthyroid population, besides in adult hypothyroid individuals [18]. However, it may be worthwhile to study adiponectin gene polymorphism SNP+276 in the same setting. Yet another recent study has cited the link between thyroid hormone and lipid metabolism in type 2 diabetics who were euthyroid males [19].

Evidence based data is available on the role of NADPH oxidases not only from the standpoint of thyroid physiology but also from the perspectives of gland pathophysiology of thyroid. A study had particularly implicated these enzymes in the regulation of thyroid oxidative stress [20]. This study, in association with our present study would pave the way for more robust scientific studies in the realms of oxidative stress as linked to the type 2 diabetics, but from the point of view of adiponectin and divalent cations which are key players in insulin resistance.

A few other lines of evidences are presently available to portray the relationship among endogenous anti-oxidant, thyroid status and insulin resistance [21, 22]. The aetiology of vascular disease is significantly influenced by oxidative stress [23]. Patients with type 2 diabetes are potential candidates for exhibiting exaggerated vascular NADPH oxidase activity, synonymous with the generation of ROS [24,25], a finding that was confirmed in our population, as per the present study. Despite the fact that NADPH oxidase is a significant source of free radicals in the human system, it is difficult to quantitate its activity directly in the human vascular wall. It follows that any indirect estimation facilitated through the assessment of the

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levels of circulating oxidative stress indicators would prove to culminate in erroneous results [26]

Adipokines with pro- or anti-inflammatory potentials are elaborated by adipose tissue and the ratio of these molecules varies based on the adipose tissue depot [27] The very presence of T2DM itself is linked to adiponectin "resistance" in peripheral tissues including adipose tissue and skeletal muscle. This is attributed to the reduced expression of adiponectin receptors [28]. In our study, we observe that the ability of adiponectin gene (SNP+45) to overturn NADPH oxidase activity in patients with clinically euthyroid type 2 diabetics is conspicuous.

The nexus between polymorphisms in the cytochrome b-245 alpha chain (CYBA) gene (that encodes p22phox) has been studied with reference to a wide gamut of physiological events including endothelial function, atherogenesis and oxidative stress in T2DM. Based on a previous study, it was concluded that C242T gene polymorphism possesses a direct link with endothelial function, thereby suggesting a possible role for the polymorphism of p22phox in the modification of vasculature in T2DM [22]. However, the research study did not particularly mention any designated role for adiponectin, a major player in IR mediated vascular abnormalities. Also, the same study did not make any reference to thyroid status, a major comorbidity frequently observed in T2DM, but disregarded on many an occasion in evidence based scientific studies.

A study has reported that C242T single-nucleotide polymorphism could cause myriad structural changes in p22(phox) which in turn is involved in the modulation of NOX 2 activation and oxidative changes -linked response to TNF-α or hyperglycemia. The same study had implicated C242T single-nucleotide polymorphism in the protective mechanism essentially aimed at attenuating cardiovascular diseases attributed to inflammation [8]. However, the present study of ours has earnestly attempted to relate the gene polymorphism of Adiponectin and NADPH oxidase with insulin resistance and oxidative stress, a point which is poised to acquire relevance and significance when considered in the context of association with clinically euthyroid type 2 diabetics.

It is of interest to note that effects of fatty acids and the glucolipotoxicity mediated β-cell failure during the progression of insulin resistance are linked to oxidative stress induced by NOX, the ER stress, and more significantly the molecular crosstalk involving NOX and ER stress [29]. It would be worthwhile to consider a setting that would take cognizance of the thyroid status, insulin resistance, activity of NOX, with reference to adiponectin leading to faithful considerations in precision medicine.

A study that is as recent as a year ago has established link between polymorphisms in NOX 5 and T2DM, thereby underlining yet again the role of oxidative stress associated genes in disease vulnerability [30]. Another interesting proposition is that the endogenous antioxidant, namely bilirubin acquires relevance in the context of obesity, insulin resistance, oxidative stress and thyroid status respectively [31,32].

Conclusion

It is concluded that thyroid hormones participate in molecular crosstalk involving ADIPO Q gene and NADPH oxidase gene in clinically euthyroid type 2 diabetics, a frequently ignored and less studied clinical population, thereby indicating that thyroid function/dysfunction and adiponectin levels are regarded as cardinal factors in the progression of vascular complications in T2DM. It is further concluded that the study has strived to implicate NADPH oxidase, independent of the antioxidant and pro-oxidant status in the given clinical scenario by justifying the fact that gene polymorphism of NADPH oxidase is much more reliable and objective in comparison to other frequently used markers of oxidative stress/lipid peroxidation, despite the inherent cost involved in the former.

Limitations of the study:

1. Small sample size
2. Gender specificity was not given impetus
3. Conventional markers of oxidative stress were not quantitated
4. Triglyceride Glucose index not computed which otherwise would have enhanced the objectivity of insulin sensitivity/insulin resistance

Future scope:

Oxidative stress is a condition that is largely considered as an upstream event linked to inflammation. This is exemplified by the fact that inflammation induces the activation of monocytes and macrophages, besides accelerating inflammatory response linked to insulin resistance and Diabetes mellitus. Hence, it would be

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worthwhile to study insulin resistance and diabetes mellitus in detail with reference to the conglomerate of NADPH oxidase, adipokines, inflammatory mediators Vis-à-vis the comprehensive package including lipid profile, glycemic control, anthropometry and thyroid profile. By enabling such holistic studies, it would be realistic to envisage therapeutic modalities based on precision medicine. Furthermore, the study should encompass other aspects including gender specificity and pre diabetes (impaired fasting glucose and impaired glucose tolerance) for generating well rounded data on the basis of evidence-based medicine.

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