Research Paper

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Adiponectin receptor gene polymorphism in insulin Resistance, Metabolic syndrome and Male factor infertility.

Medical Science

KEYWORDS :

Sonam Bhatia	Rama Medical College and Research Hospital, Kanpur
Lal Chandra	Maulana Azad Medical College, New Delhi
Alpana Saxena	Maulana Azad Medical College, New Delhi
Dr. Ramchandra Murti Kaza	Maulana Azad Medical College, New Delhi
Amit Madaan	Ganesh Shankar Vidyarthi Medical College, Kanpur

ABSTRACT

Background: Metabolic syndrome (MetSyn) is a cluster of metabolic abnormalities -high blood pressure, high serum triglyceride, low serum high-density lipoprotein cholesterol & abnormal fasting plasma glucose levels. Male factor infertility represents perturbation in male patients with MetSyn. Adiponectin is a hormone, is a potential indicator of metabolic complications. This study evaluates MetSyn and insulin resistance to establish a paradigm with Genetic variation in adiponectin gene receptor (ADIPOR1 and ADIPOR2) polymorphism in male factor infertility.
Objective: 1.To estimate insulin resistance in male factor infertility.
2. To correlate metabolic syndrome with male factor infertility.
3. To find out the status of adiponectin gene receptor polymorphism in male factor infertility.
Methods: Total 52 cases and 54 controls fulfilling inclusion and exclusion criteria were included with their informed consent. The blood samples were collected for estimation of triglycerides, HDL-C, fasting plasma glucose & adiponectin receptor gene polymorphism. Triglycerides, HDL-C, fasting plasma glucose estimation were done by Clinical Chemistry Analyzer. Serum Insulin was measured by Electro-chemiluminisence. Adiponectin gene receptor polymorphism by ASO-PCR.
Results: In present study occurrence of MetSyn following IDF criteria in Male factor Infertility was found to be more than controls. There were significant differences between all the parameters of metabolic syndrome in cases and controls except HDL-C and systolic blood pressure. +219 (A/T) is a polymorphism in adiponectin receptor gene polymorphism in intron 2 which had been associated with T2DM.We found+ 219 (A/T) polymorphism with male factor infertility. The Odd's ratio in AA/AT and AA/TT genotype was found to be significant in male infertile patients. However, relationship between +219(A/T) polymorphism and metabolic syndrome was also found to be significant.
Conclusion: Occurrence of metabolic syndrome and its components to be higher in such patients compared to population of controls. And single nucleotide polymorphism +219 A/T adiponectin gene receptor is positively related to male factor infertility.

INTRODUCTION:

A cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus, which occur together more often than by chance alone, have become known as the metabolic syndrome. The risk factors include raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised fasting glucose, and central obesity[1]. Various diagnostic criteria have been proposed by different organizations over the past decade. Most recently, these have come from the International Diabetes Federation (IDF) and the American Heart Association/National Heart, Lung, and Blood Institute. The main difference concerns the measure for central obesity, with this being an obligatory component in the International Diabetes Federation definition, lower than in the American Heart Association/National Heart, Lung, and Blood Institute criteria, and ethnic specific[2]. The present article attempts to study occurrence of Metabolic Syndrome following IDF criteria in Male factor Infertility. It is a very common public health issue in developing countries like India. Approximately 15 percent of couples are infertile, and among these couples, male factor infertility accounts for approximately 50 percent of causes. Male infertility is a multi-factorial syndrome encompassing a wide variety of disorders. In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. Infertility in men can be diagnosed initially by semen analysis. The current estimate is that about 30 percent of men seeking help at the infertility clinic are found to have oligozoospermia or azoospermia of unknown aetiology. Therefore, there is a need to find the cause of infertility.

The numerous deleterious effects of MetSyn are being investigated throughout the medical community, as MetSyn may potentially affect many aspects of human physiology due to its systemic nature. Male factor infertility may represent one such perturbation in male patients with MetSyn. It is estimated that 15% of couples attempting to conceive are not able to do so within 1 year. Male factor infertility is present in 20–50% of these couples, either independently or in conjunction with female factor infertility issues. In the setting of an increasing prevalence and understanding of MetSyn, investigators are actively studying the potential relationship between male factor infertility and MetSyn. Insight gained from this innovative work may provide increased therapeutic options for male partners in affected infertile couples.

Insulin resistance is decreased biological response to normal concentration of circulating insulin and is found in both obese, non-diabetic individuals and patients with type 2 diabetes mellitus[3]. Insulin resistance is hypothesized underlying pathogenic mechanism of metabolic syndrome. Insulin resistance is likely in patients with metabolic syndrome but not necessarily present in all the cases.

Adiponectin a hormone mainly produced from adipose tissue[4, 5]. It is a protein complex which has potential anti-diabetic, anti-atherosclerotic and anti-inflammatory property. Hypoadiponectinemia is associated with increased body mass index, decreased insulin sensitivity, less favourable plasma lipid profile, increased level of inflammatory markers and increased risk for the development of cardiovascular disease. Hence, it is a potential indicator of metabolic complications. This study evaluates MetSyn and its components in order to establish a paradigm with male factor infertility along with Genetic variation in adiponectin gene receptor (*ADIPOR1* and *ADIPOR2*) polymorphism which could have effects on insulin resistance-related phenotypes and male factor infertility[6]. The association of insulin resistance, metabolic syndrome having polymorphism in adiponectin gene receptor (*ADIPOR1* and *ADIPOR2)* and its components with male factor infertility is largely unknown in spite of the fact; male factor infertility and MetSyn are common increasing problem in Indian urban population.

MATERIALS AND METHODS:

Design, setting and participants

We conducted Hospital based case control study in the Department of Biochemistry, Maulana Azad Medical College in collaboration with Department of Surgery, Lok Nayak Hospital, New Delhi. Total one hundred and six (52 cases and 54 age and sex matched controls) fulfilling inclusion and exclusion criteria were included with their informed consent.

Anthropometric measurements

Waist circumference (WC) was measured to the nearest 0.1cm at theat level of anterior superior iliac spine.Blood pressure was measured from the right arm after the subject had been sitting for at least five minutes. The average of two readings taken five minutes apart was recorded.

Biochemical measurements

The blood samples were collected for estimation of serum triglycerides, HDL-C, insulin, fasting plasma glucose & adiponectin receptor gene polymorphism.

After overnight fasting, blood sample was collected by venipuncture in all subjects in plain vial (3ml), fluoride vial (2ml) and EDTA vial (3ml). It was allowed to clot for 30-60 minutes. Serum was separated by centrifuging for 5 minutes at room temperature. Some of the serum was used for insulin, HDL-C and triglycerides. Fluoride vial sample was used for fasting plasma glucose estimation.

Definition of the metabolic syndrome using IDF criteria3

According to the new IDF definition, for a person to be defined as having the metabolic syndrome they must have: central obesity (defined as waist circumference >90 cm for south Asian men and >80 cm for women) plus any *two* of the following *four* factors:

- Raised triglyceride: >150 mg/dL or specific treatment for this lipid abnormality.
- Reduced HDL cholesterol: <40mg/dL in male and <50mg/dL in females or specific treatment for this lipid abnormality.
- Raised blood pressure: systolic BP>130mm Hg or diastolic BP>85mm Hg or treatment of previously diagnosed hypertension.
- Raised fasting plasma glucose (FPG) : >100 mg/dL or previously diagnosed type 2 diabetes mellitus.

Biochemical parameters:

1. Serum Triglycerides, HDL-C, fasting plasma glucose estimation were done by Clinical Chemistry Analyzer (Beckman Coulter DXC 800, USA) by standard methods.
2. Serum Insulin was measured by Electro-chemiluminisence.
3. HOMA–IR (homeostasis model assessment of insulin resistance) was calculated by: **HOMA –IR = serum glucose (mg/dL) × plasma insulin (µIU/mL)/405**
4. **SNP (+219) A/T intron 2 rs11061971 *ADIPOR2*** Adiponectin receptor gene polymorphism by Polymerase Chain Reaction.

It was done using ASO-PCR

DNA extraction:

Total genomic DNA was isolated from whole blood using Genomic DNA Mini Kit (Blood/Cultured Cell) from Geneaid.

Components:

- RBC Lysis Buffer
- GT Buffer
- GB Buffer
- Wash Buffer
- Elution Buffer (10 mM Tris-HCl, pH8.5 at 25^{o}C)
- GD Columns
- 2 ml Collection Tubes

Procedure:

1. To 200 µl of the whole blood taken in a 2 ml eppendorf 20 µl of Proteinase K solution was added. Mixed in vortex for 10-15 sec.
2. 200 µl of Lysis solution was added to the sample and mixed in vortex to obtain a homogenous mixture. The sample was incubated in a water bath at 70°C for 45 minutes.
4. 200 µl of ethanol was added to the lysate obtained and mixed for 10-15 seconds.
5. The lysate is transferred to the GD column provided. Centrifuged at 12000x g for 2 minute. The flow-through liquid was discarded.
6. 400 µl of W1 Buffer solution was added to the spin column and centrifuged at 12000 x g for 2 minute. Flow-through liquid was discarded.
7. 600 µl of wash solution was added to the column and centrifuged at 12,000 x g for 3 minutes to dry the column. The collection tube was discarded and the column was placed in a new collection tube.
8. 200 µl of pre-heated (at 60-70^{o}c) elution buffer was added to the column. Incubated for 1 hour at room temperature. Centrifuged at 12000 x g for 1 minute to elute the DNA.
9. Agarose gel run was done with the elute to confirm the presence of DNA.

Agarose gel Electrophoresis:

Reagents:

1. Agarose
2. Ethidium bromide (10 mg/ml) - 10 mg of Ethidium Bromide dissolved in 1 ml of distilled water
3. Gel loading buffer 6x: 0.25% Bromophenol blue – 40 gm of sucrose and 0.25 gm of bromophenol blue dye dissolved in 100 ml of distilled water
4. TAE (Tris base, acetic acid and EDTA) buffer- 24.2 gm of Tris acetate, 5.71 ml of glacial acetic acid, 10 ml of 0.5 M EDTA dissolved in 100 ml of distilled water to make 50x stock. pH adjusted to 8. 1 ml of stock is diluted with 49 ml of distilled water to obtain a working solution of 1x.

Procedure:

1. 0.7 % agarose gel was made by adding 350 mg of agar in 50 ml of 1x TAE buffer.
2. 2.5-µl ethidium bromide was added to it. The solution was heated till it forms a homogenous solution.
3. The warm gel was poured in the gel rack and allowed to solidify with a comb 5mm.
4. Cooled gel, was put together with the rack, in a horizontal electrophoresis chamber with TAE completely covering the gel. Comb was removed.
5. 10 µl of elute was mixed with 2 µl of gel loading dye was loaded in the well.
6. DNA ladder was loaded in one of the wells.
7. The electrophoresis was run at 16 mA current and 100 V for 45 minutes.
8. After the running electrophoresis, gel was visualised in gel dock and the image was stored.

The DNA solutions were stored in -80° C till further analysis.

Polymerase Chain Reaction:
The required region of *ADIPOR2* gene from the genomic DNA was amplified by Allele specific polymerase chain reaction using primers

Reagents:
1. Master mix
2. Forward Primer:

AA allele	AGGAAAAAATGTGGAGGAA
TT allele	AGGAAAAAATGTGGAGGAT

3. Reverse Primer:

REVERSE	TAGTAGTAGTAGTAGTAGTAGT

Reconstituted to make a stock of 100 pM.

Procedure: PCR reactions were carried out in a total volume of 23µl. The final concentration of the reaction mixture was 10µl of master mix, 0.25µl of forward primer, 0.25µl of reverse primer, 12.5µl of Depc water and 2µl of total genomic DNA.

PCR conditions were as follows:
Initial denaturation: 94° C for 10 minutes 1x
35 cycles of:
Denaturation at 94° C for 45 seconds
Annealing at 48° C for 1 minute
Extension at 72° C for 1 minute
Final extension: 4°C for 10 minutes 1x

The amplified products were resolved using electrophoresis in 2% agarose gel and were 184bp long.

RESULTS:
There were total of 106 eligible subjects in the study. The age range of the study group was 21-30yrs. Among 52 male factor infertility, 31 patients were in this age group which comprises of 59.6% of total cases. Mean age of male infertile patients for cases is 30.15(±4.06) yrs & 31.6(±6.6) yrs for controls (Table 1).

Prevalence of the metabolic syndrome and its components
The prevalence of the metabolic syndrome was 61.5% using the IDF criteria (Table 2).

We analysed all the components of metabolic syndrome in cases and controls. According to IDF criteria, all the metabolic syndrome patients must have increased waist circumference criteria and any *two* of other criteria systolic blood pressure>130 mmHg or diastolic blood pressure>85 mmHg, HDL-C <40 mg/dL in males or <50 mg/dL in females, triglycerides>150 mg/dL, fasting plasma glucose >100 mg/dL.Frequency of occurrence of components of metabolic syndrome in Male factor infertility and controls shown in Table 3 & 4.

We also measured Serum insulin and Insulin resistance was estimated by HOMA-IR and both were found to be significantly raised in metabolic syndrome cases in comparison to control groups (Table 5).

+219(A/T) *ADIPOR2* Gene Polymorphism SNP (rs11061971):
All the subjects were genotyped for a single nucleotide polymorphism +219(A/T) ADIPOR2 Gene Polymorphism in intron2. In the male factor infertility group, 5 patients had AA genotype (wild type), 28 had AT genotype (heterozygous) and 19 patients had the mutant TT genotype. In the control group, 23 had AA genotype, 19 had AT genotype and 12 had TT genotype. There was significant difference between the frequency of AA, AT and TT among cases and healthy controls as calculated using chi square test p- value <0.0005 (Table 6).

The Odd's ratio (OR) in AA/AT genotype was found to be 6.77 (95% CI- 2.19-20.96) in male infertile patients, was significant. And Odd's ratio (OR) in AA/TT genotype was found to be 7.2 (95% CI- 2.17-24.35) in male infertile patients, was significant.

The Risk Ratio (RR) in AA/AT genotype was found to be 3.3(1.45-7.64) in male infertile patients, was significant. And RR in AA/TT genotype was found to be 3.4(1.47-7.96) in male infertile patients, which was also found to be significant (Table 7).

How ever Odd's ratio (OR) in AA/AT genotype was found to be 1.4(95% CI- 0.19-9.96) in male infertile patients, which was not significant. And Odd's ratio (OR) in AA/TT genotype was found to be 0.74(95% CI 0.09-5.49) in male infertile patients, was also not significant.

The Risk Ratio (RR) in AA/AT genotype was found to be 1.24(95% CI 0.37-4.14) in male infertile patients, which was not significant. And RR in AA/TT genotype was found to be 0.84(95% CI 0.26-2.73) in male infertile patients, which was also found to be not significant (Table 8).

DISCUSSION:
About one in ten couples of reproductive age are infertile and male factor infertility accounts for half of these cases. Despite the relative importance of infertility due to male partner, infertility evaluations have traditionally focused on women. Male factor infertility has many causes; hormonal imbalances, physical problems, psychological and/or behavioural problems. Moreover, fertility reflects a man's "overall" health[7].

Metabolic Syndrome is linked to male factor infertility[8]. Metabolic syndrome represents a constellation of abnormalities, including dyslipidemia, hypertension, and impaired glucose metabolism, with insulin resistance as the hypothesized underlying pathogenic mechanism. Male factor infertility may represent one such perturbation in some male patients with MetSyn. MetSyn is an important medical and epidemiologic entity, as its deleterious effect on patients is firmly established. Male factor infertility may represent another aberration observed in some patients with MetSyn. Currently, there is sufficient evidence to suggest the MetSyn male factor infertility paradigm[9]. Obesity/overweight may result in hypogonadism, increased scrotal temperatures, impaired spermatogenesis, decreased sperm concentration and motility, and increased sperm DNA damage. Similarly, non insulin dependent diabetes mellitus (NIDDM)/insulin resistance may contribute to and compound this scenario. Dyslipidemia with increased oxidative stress in the testicular microenvironment and/or excurrent ductal system may further decrease fertility.

Makhside (2005) suggested the addition of hypogonadism to the constellation of aberrations seen in MetSyn. In their observational studies have reported that low levels of testosterone and Sex Hormone Binding Globulin (SHBG) are significantly correlated with MetSyn and its components (including measures of BMI, waist circumference, and waist-height ratio)[10-2].

The research design of the present study was a hospital based case-control study in which 106 subjects fulfilling inclusion criteria were recruited and WHO criteria were used to diagnose subjects with male factor infertility and criteria by IDF were used to diagnose subjects with metabolic syndrome. In the present study mean age of male infertile patients is 30.15(±4.06) yrs .This is in agreement with those documented by Birnie *et al* (2001) where it was 32.8 yrs.

The prevalence of metabolic syndrome in male infertile patients 32(61.5%) was found to be more as compared to cases (59.2%). Although the accumulation of visceral fat is an essential component of metabolic syndrome, and waist circumference measurement can be useful to estimate visceral fat, the reliability of waist circumference measurement has not been determined. The mechanisms whereby waist circumference is associated with risk in the presence of other risk factors are not clear. The IDF diagnostic criteria for metabolic syndrome emphasize central obesity as the core measure and use a waist circumference limit of 90 cm in men and 80 cm in women.

In present study we have used IDF criteria, so all the patients that we have included as cases are having higher waist circumference in comparison to patients whom we have enrolled as controls. Cut off value for waist circumference was used according to IDF criteria which have been already mentioned earlier. In present study the mean value of waist circumference in metabolic syndrome cases and control groups are 39.26±3.27 and 34.33±3.58 respectively.

Scarce data exist regarding the prevalence and frequency of the components of metabolic syndrome in male factor infertility in Indian urban population. As we have followed IDF criteria so most prevalent component of metabolic syndrome is increased waist circumference. And least prevalent component was increased triglycerides as well as decreased HDL-C component.

Ogbera *et al* (2010) also documented in a study on Nigerian people that increased waist circumference was most prevalent and raised triglycerides was least prevalent parameter in that population[13]. In present study maximum number of patients of metabolic syndrome are 3 components positive. The most frequent combination of 3 components is increased waist circumference, increased fasting blood sugar and increased blood pressure. The fourth most common component was decreased HDL and increased TG levels. Insulin resistance, which represents a reduced physiological response of the peripheral tissues to the action of the normal levels of insulin, is a major finding in several metabolic disorders, including type 2 diabetes and metabolic syndrome (MetSyn). Therefore, a reliable measure of insulin resistance is important for investigating the link between insulin resistance, MetSyn and male factor infertility. Furthermore, given that insulin resistance is an important risk factor for development of type 2 diabetes and incident cardiovascular diseases, identification of subjects with insulin resistance is a strategy for identifying high-risk people for targeted preventive interventions. The homeostasis model assessment of insulin resistance (HOMA -IR) , which is developed in 1985 by Matthews and coworkers, for application in large epidemiologic investigations , is an alternative to the glucose clamp and the most commonly used surrogate measure of insulin resistance in vivo[14]. In terms of precision (reproducibility of measure), HOMA-IR is comparable to the glucose clamp technique. HOMA-IR is inferior to the clamp technique in terms of accuracy, but using HOMA-IR makes it possible to study a large number of subjects and with a single glucose and insulin measurement in the fasting state[15]. In a study done by Esteghamati *et al* (2010) in Iran it was found that insulin resistance and MS were significantly associated, and HOMA - IR levels were directly related to the number of MS components. The prevalence of insulin resistance was notably higher among those who met either ATP III or IDF criteria of MS[16]. Highest level of HOMA-IR or insulin resistance is associated with group of patients with 5 positive components. It was observed that patients with 3 components had lower insulin values (mean=29.15μIU/ml) when compared to patients with 4 positive components (mean=30.25μIU/ml) and patients with 5 positive components (mean=31.55μIU/ml).So present study shows results in agreement to the study done by Esteghamati *et al* in 2010.

Traditionally, adipose tissue was considered as a passive reservoir of energy in the form of triacylglycerol stores and as a place for the conversion of androgens to estrogens. In recent years, a number of papers have stated, that adipocytes produce many hormones and adipocytokines such leptin, TNFα, resistin, adiponectin, adipsin etc. These influence energy homeostasis, balance of fat stores and make possible adaptation of the organism to various situations (starvation, stress, infection and period of increased energy demand).[17]

Adiponectin is a recently described adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation. It is produced by adipose tissue (white and brown) and circulates in the blood at very high concentrations. It has direct actions in liver, skeletal muscle and the vasculature, with prominent roles to improve hepatic insulin sensitivity, increase fuel oxidation [via up regulation of adenosine monophosphate-activated protein kinase (AMPK) activity] and decrease vascular inflammation. Adiponectin exists in the circulation as varying molecular weight forms, produced by multimerization. Recent data indicate that the high-molecular weight (HMW) complexes have the predominant action in the liver. In contrast to other adipokines, adiponectin secretion and circulating levels are inversely proportional to body fat content. Levels are further reduced in subjects with diabetes and coronary artery disease. Adiponectin antagonizes many effects of tumour necrosis factor-a (TNF-α) and this, in turn, suppresses adiponectin production. Furthermore, adiponectin secretion from adipocytes is enhanced by thiazolidinediones (which also act to antagonize TNF- α effects). Thus, adiponectin may be the common mechanism by which TNF- α promotes, and the thiazolidinediones suppress, insulin resistance and inflammation. Two adiponectin receptors, termed Adipo R1 and Adipo R2, have been identified and these are ubiquitously expressed. AdipoR1 is most highly expressed in skeletal muscle and has a prominent action to activate AMPK, and hence promote lipid oxidation. AdipoR2 is most highly expressed in liver, where it enhances insulin sensitivity and reduces steatosis via activation of AMPK and increased peroxisome proliferator activated receptor a ligand activity. T -cadherin, which is expressed in endothelium and smooth muscle, has been identified as an adiponectin binding protein with preference for HMW adiponectin multimers. Investigations have begun to elaborate the mechanisms involved in the regulation of adiponectin expression and secretion. Studies have focused on the effects of hormonal and environmental factors on circulating adiponectin levels in the whole organism or on the expression and secretion of adiponectin at a cellular level. In vivo investigations found that circulating adiponecin levels per se are not subject to acute regulation. However, adiponectin expression may be regulated relatively acutely (4–6 h) through fasting and refeeding, by mechanisms thought to involve the nuclear receptors C/EBP b and nuclear factor Y[18]. In healthy normal weight subjects, circulating adiponectin levels appear to exhibit ultradian pulsatility and diurnal variation, with decline of up to 30% at night[19]. Such diurnal variations are lost in individuals with obesity or diabetes[20].

+219(A/T) ADIPONECTIN RECEPTOR GENE POLYMORPHISM:

SNP +219(A/T) Adiponectin receptor gene polymorphism has been studied for the in Relation to Type 2 Diabetes and Insulin Resistance-Related Phenotypes.

The prevalence of this polymorphism in Indian population is unknown. The association of this polymorphism with male factor infertility is unknown. We aimed at studying the occurrence of adiponectin receptor gene polymorphism A/T in intron 2 in cases of male factor infertility and healthy controls in Indian population.

In our study, we found in male factor infertility group, 5 patients had AA genotype (wild type), 28 had AT genotype (heterozygous) and 19 patients had the mutant TT genotype constituting 9.6%, 53.86% and 36.5% respectively. In controls, 23/54(42.5%) of controls had AA genotype, 19/54(35.18%) had AT genotype and 13/54 (22.22%) had TT genotype. There was significant difference in their frequency between the two groups. The Odd's ratio (OR) in AA/AT genotype was found to be 6.77 (95% CI- 2.19-20.96) in male infertile patients, which was statistically significant. And Odd's ratio (OR) in AA/TT genotype was found to be 7.2 (95% CI- 2.17-24.35) in male infertile patients, which was statistically significant. In a study done by Potapov, et al on type 2 diabetic patients in Russia, found that in adiponectin receptor gene +219(A/T) polymorphism, odds ratio was found to be significant for TT and not for AT[21]. Comparison of MetSyn in AA, AT and TT genotypes in Male factor infertility was found to be statistically non-significant.

CONCLUSION:
Findings of present study suggest presence of insulin resistance in male factor infertility than those without it. Occurrence of metabolic syndrome and its components to be higher in such patients compared to population of controls. And presence of single nucleotide polymorphism +219 A/T adiponectin gene receptor is positively related to male factor infertility.

COMPETING INTEREST: "The authors declare that they have no competing interests"

AUTHOR'S CONTRIBUTION:
"SB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. SB carried out the quantitative estimation. LC participated in the sequence alignment. AM participated in the design of the study and performed the statistical analysis.AS conceived of the study, and participated in its design and coordination and helped to draft the manuscript. RZ contributed in selection of cases. All authors read and approved the final manuscript."

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TABLE

Table 1: Distribution of Age in Male factor infertility and controls:

Age (years)	Male factor infertility (n=52) n (%)	Controls(n=54) n(%)
21-30	31(59.6%)	27(50%)
31-40	19(36.5 %)	20(37%)
41-50	1(1.9%)	7(12.9%)

Table 2: Occurrence of MetSyn in Male factor Infertility and controls:

	Male Factor Infertility	Controls	p value
MetSyn	32(61.5%)	5(9.2%)	<.0001

Table 3: Mean and S.D, in components of metabolic syndrome in Male factor infertility and controls (mean±S.D):

Components	Male factor infertility (n=52)	Controls (n=54)	p value*
Waist circumference (cm)	39.26±3.27	34.33±3.58	0.045
Fasting plasma glucose (mg/dL)	128.40±23.06	98.92±21.18	<.0001
Triglycerides (mg/dL)	172.75±39.99	136.59±24.22	<.0001
HDL-C (mg/dL)	42.21±8.8	41.12±8.35	0.51
Systolic blood pressure (mmHg)	125.38±5.66	122.37±10.51	0.070
Diastolic blood pressure (mmHg)	82±5.80	76.77±9.30	<.0001

***compared by Student's t-test**

Table 4: Frequency of components of metabolic syndrome in Male factor infertility:

Metabolic Syndrome defining criteria	Cases (n=52) frequency	Controls (n=54) frequency	p-value*
Increased Waist circumference(cm)	32(61.5%)	23(42.59%)	0.38
Increased Triglyceride (mg/dL)	33(63.46%)	11(20.37%)	<.0001
Decreased HDL-C (mg/dL)	19(36.53%)	17(31.48%)	0.72
Increased Blood pressure (mmHg)	27(51.90%)	14(25.92%)	0.010
Increased Fasting plasma glucose (mg/dL)	46(88.40%)	17(31.48%)	<.0001

*Using Chi-Square test

Table 5: Fasting serum insulin & HOMA-IR in Male factor infertility and controls (mean±S.D):

	Cases (n=52)	Controls (n=54)	p value*
Serum insulin (µIU/ml)	29.92±9.61	15.79±7.71	<.0001*
HOMA-IR	9.41±3.71	4.00±2.59	<.0001#

*compared by student's t-test
#compared by Mann Whitney-u test.U_A =371, Z=6.53

Table 6: Distribution of AA, AT &TT genotypes in Male factor infertility & controls:

	Male factor infertility n(%)	Controls n(%)	Total n(%)
AA	5 (9.6)	23(42.5)	28(26.4)
AT	28(53.86)	19(35.18)	47(44.33)
TT	19(36.5)	12(22.22)	31(29.24)

Table 7: Relationship between +219(A/T) polymorphism and Male infertility:

Genotype	OR(CI)	RR
AA	1	-
AT	6.77(2.19-20.96)*	3.3(1.45-7.64)
TT	7.2(2.17-24.35)*	3.4(1.47-7.96)

*p < 0.001

Table 8: Relationship between +219(A/T) polymorphism and Metabolic Syndrome:

	O.R(CI)	R.R
AA	1	-
AT	1.4(0.19-9.96)*	1.24(0.37-4.14)
TT	0.74(0.09-5.49)#	0.84(0.26-2.73)

*p =2.1
#p =1.0

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