DISCUSSION

Familial clustering of PCOS has been consistently reported, suggesting that genetic factors play a role in the development of the syndrome. Sisters, brothers, fathers, mothers, daughters and now even sons of women with PCOS have been found to have a higher risk for exhibiting either hyperandrogenic or metabolic (hyperinsulinemic) traits of the disorder and thus PCOS has become a 'family affair'.

Ours is the first Indian study to illustrate the association between $11BHSD1$ gene and PCOS. The results of our study show that there is a significant association between PCOS and $11BHSD1$ gene polymorphism. Apart from one genetic association study, no family studies regarding PCOS have been reported so far. Our study has shown that 37% of PCOS subjects and 36% of their family members are have 332-29T>G polymorphism, which is one of the major SNPs associated with PCOS in comparison to control (8%) with an odds ratio of 6.18(p<0.0001).

Alessandra et al also reported similar findings in their study when they examined 102 Caucasian women for $11BHSD1$ gene polymorphism and found frequency of heterozygosity in cases thirty three (33%) and in control eight (8%). The association of the $HSD11B1$ genotype with PCOS was mainly attributable to lean, rather than obese, PCOS patients.
San Millán et al. performed in another population of 116 Caucasian women, in which no association between \textit{HSD11B1} genotype and PCOS was found. In addition, ovarian morphology was not used in the diagnostic criteria by San Millán et al. suggesting that different populations of PCOS and control subjects were included in the two studies and reinforcing that \textit{HSD11B1} polymorphisms may be relevant only in some subgroups of this heterogeneous condition. Also, data from White et al did not show association between \textit{HSD11B1} genotype and PCOS, also reported lower prevalence of rs1208664 T$\rightarrow$G in the obese than lean patients. This suggests that this genotype either reduces the chances of becoming obese or reduces the chance of developing PCOS in the presence of obesity. This could be attributed to lower intracellular cortisol levels, particularly in adipose tissue and liver, which are protective against features of the metabolic syndrome.

Results of our study show that PCOS is transmitted as an autosomal dominant disorder. Our study revealed that thirty seven (37\%) of PCOS subjects had T$\rightarrow$G base pair substitution in 11B-HSD1 gene, while we found 36\% of family members also had the same complication. In our study we observed thirty one (31\%) of mother of PCOS subjects had the same basepair substitution, indicating a strong maternal inheritance.

When we genotyped for (tttta)\textsubscript{n} marker of \textit{CYP11A} gene, we observed a similar story of inheritance. Twelve (12\%) of PCOS subjects were positive while nine (9\%) of their family members showed the same. Neda gharani et al reported their association data when they examined 110 PCOS subjects with 97 controls,
demonstrate that allelic variants of CYP11a mediate the development hyperandrogenemia, which is in turn associated with PCOS and hirsuitism. In the same study they have shown association between CYP11A \((\text{tttta})_n\) marker and testosterone level. Apart from one genetic association study, no family studies regarding PCOS have been reported so far. Maitra et al. conducted mutational analysis of CYP11A1 and Leptin as genetic determinants of hyperandrogenicity and obesity in PCOS patients, although the study did not indicate any variations in the exons of the aforesaid genes with regard to the syndrome. The same group exhibited a trend towards dyslipidaemia among women with PCOS, particularly in parameters associated with cardiovascular risk.

A significant association of obesity rather than raised testosterone with dyslipidaemia was also confirmed by this study, in addition to this, Kalra et al. concluded that insulin resistance is associated with dyslipidaemia in women with PCOS, independent of obesity. The previous findings in this subject on \(\text{CYP11a gene}\) was conducted in Tamil Nadu, where populations are typically south Indians. We have carried out our research in Dakshinakannada(DK) district where the samples were collected from multigeneous population. This is why the difference of frequency of positive results and biochemical variables.

In the present study we examined the prevalence of a polymorphism of gene CYP17 promoter and found no association as \(A1A2=0.0\) and \(A2A2=0.0\) genotype frequencies.
Barbara et al reported a frequency of 37% in PCOS subjects with A2 allele in Chilean population, when they examined 159 women with clinical and hormonal evidences of PCOS and they concluded the presence of this gene defect in PCOS seems to be associated with increase in body weight, abdominal adiposity and metabolic components.

Diamati-kandarakis et al describe the same gene cyp17 polymorphism in Greek population with 50 PCOS subjects and reported 58% were heterozygous carriers of the polymorphic allele and 8% carried A2 allele in homozygosity, they concluded although this base pair substitution is not a primary genetic defect in PCOS, it may aggravate in clinical picture of hyperandogenemia, particularly when homozygosity exist.

Gharani et al also reported a non significant association with cyp17 gene and PCOS, when they examined 96 PCOS women in white population.

In another study Marszalek et al illustrate a similar result in Poland population, while they genotyped 56 PCOS women and concluded the T>C polymorphism of cyp17 gene is not associated with steroid hormone synthesis in PCOS and it is not a primary genetic defect in this disease.

The high incidence of PCOS in first degree relatives of the affected members, in previous studies\textsuperscript{91-94}, suggests a dominant pattern of inheritance. This is based on the assumption that at least 50% of the siblings of the PCOS probands are affected with the disorder\textsuperscript{91}. Twin studies on PCOS have revealed an
incidence of 50% has suggested a complex pattern of polygenic inheritance\textsuperscript{92}. Other studies have reported that 50% hirsutism cases among the affected sisters of PCOS\textsuperscript{93}. They have shown that some characteristics of PCOS inherited differed in proportion; e.g. PCO 73\%, hyperandrogenemia 87\% and hyperinsulinemia 66\%. Another report has shown 22\% of PCOS in affected sisters of the proband\textsuperscript{94}.

However when we analyzed clinical conditions associated with PCOS, we found a very high association suggesting autosomal dominant transmission. Break up data of the family history data showed nearly 20\% of the fathers of pco probands having diabetes mellitus, hypertension obesity/dyslipidemia each. Also a similar percentage of mothers of the pco probands had the above metabolic syndrome characters. Among the siblings of pco probands, nearly 10\% of them had diabetes mellitus, hypertension, obesity/dyslipidemia. However among the uncles, aunts and grandparents of our pco probands, the percentage of diabetes mellitus, hypertension, obesity/dyslipidemia was less than 15\%. When any one of the metabolic syndrome character such as diabetes mellitus, hypertension or dyslipidemia was considered, we found prevalence of 50\% and 54\% among the first degree and second degree relatives of our PCOS subjects respectively.

One third of the PCOS subjects, we diagnosed, were found to be having metabolic syndrome characteristics, this was early consideration as autosomal
dominance inheritance pattern of the met-S leading to a conception the PCOS is metabolic syndrome equivalent. However obesity was high and very strong family history, PCOS subjects can develop dyslipidemia, hypertension and diabetes mellitus at later stages of life.

Hague et al,\textsuperscript{95} have reported a high prevalence of polycystic ovaries among siblings that could not be explained by a simple dominant model. A large family study (St Mary's family) from Franks group concluded that PCOS is inherited on an oligogenic basis\textsuperscript{96}. In another study on the inheritance of PCOS by Govind et al,\textsuperscript{91} that included 29 families of PCO probands were analysed. The results of the segregation analysis in that study showed that 52% of the mothers, 21% of the fathers, 66% of the sisters and 22% of brothers were affected with PCOS features that were consistent with an autosomal dominant inheritance pattern of PCOS in families, perhaps caused by the same gene\textsuperscript{91}. The fact that both PCOS and the metabolic syndrome have insulin resistance and obesity at the core of their pathophysiology and have heritable components provided a hypothesis that parental metabolic syndrome would be related to the PCOS phenotype in their offspring and that metabolic syndrome prevalence would be increased in adolescents with PCOS. Thirty-six adolescent girls with PCOS and their first degree relatives were evaluated for metabolic syndrome characteristics in a study which concluded that familial factors related to paternal metabolic syndrome seem to be fundamental to the pathogenesis of PCOS\textsuperscript{97}. 
Segregation analysis in a few PCOS studies have shown consistent results with autosomal dominant inheritance\textsuperscript{98,99} whilst one study suggested an X-linked mode of inheritance\textsuperscript{100}. In our study there was equal transmission of metabolic syndrome characters from the paternal and maternal side indicating that it is not X-linked inheritance.

Of the 103 PCOS subjects studied, the family pedigree of 13 pco subjects with large families have been depicted. In picture 1, 2, 3, 4, 6, 7, 8, 9, 10 & 12, the mothers of our pco probands had PCOS. The segregation ratio (observed:expected) was consistent with autosomal dominant inheritance. We found that our PCOS probands came from large families with one or more metabolic syndrome characters being present in the parents and most of the second degree family members.

As mentioned above many studies have aimed at studying the pattern of inheritance of PCOS though case control studies, family based studies, most with small sample size. Few of the studies have concluded that PCOS is inherited in an autosomal dominant pattern. However they have done ultrasound or physically examined the first degree relatives which we could not do in our study.

In this context, the suggestion that PCOS should be treated as a quantitative trait disorder which does not necessarily imply a truly polygenic
aetiology because it would be possible to explain the variable phenotype on the basis of a small number of key causative genes (a so-called oligogenic basis for disease) involved in androgen secretion and insulin secretion/action in conjunction with environmental, particularly nutritional factors\textsuperscript{96}.

Also it confirms that PCOS daughters come from family of metabolic syndrome. Our study probably proves that PCOS could be considered as metabolic syndrome equivalent, however the manifestation of metabolic syndrome characters being modified by age, diet and environmental factors.

The results of our study showed the mean age of our subjects was (28.2±4.1) years of age. The average age of menarche in our study subjects was (13±1.5) years of age. In our study we did not find premature puberty ruling out the possibility of adrenogenital syndrome. It is well recognised that women born with low body weight have tendency to develop increased insulin resistance and increased secretions of dehydroepiandrosterone (DHEA) that leads to the development of PCOS\textsuperscript{101}.

In our study hirsutism was observed in 80\% of the subjects., Acanthosis nigricans was noticed in 61\% which is closely related to insulin resistance. Other features such as moon face, buffalo hump arid male baldness were also evident in a larger proportion (45\%) of our subjects. Hence the presence of acanthosis nigricans, DM in majority of our PCOS subjects and buffalo hump
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and moon face in 70% of our subjects strengthen the theories suggesting the role of insulin resistant genes and abnormal cortisol metabolism in the pathogenesis of PCOS. Mutations in steroidogenic enzymes gene and insulin receptor gene have been identified\textsuperscript{102,103}. Positive association and linkage have been reported with an insulin gene variable number of tandem repeats (VNTR) locus\textsuperscript{104}. Since we have found 11 beta HSD gene polymorphisms in nearly a third of our patients having Cushingoid features and insulin resistance secondary to cortisol excess considered.

Significant associations between PCOS and menstrual irregularity, hirsuitism, acanthosis nigricans and fasting blood sugar have been reported for Asian Indians\textsuperscript{105}. Hyperandrogenism is an important characteristic of PCOS that can be recognized with increased male hormone levels or clinically with features of hirsutism and acne. Prevalence of hirsutism varies between 17 and 83% and is recognized to be more common in women with dark skin\textsuperscript{106}. Hirsutism is a common problem in Kashmir valley of India and is reported to be 10.5%. Wijayaratne and team have reported a higher level of hirsutism in South Asian population\textsuperscript{107,108}. Hirsutism was of greater severity in a group of women with PCOS who had hyperinsulinemia and LH:FSH ratio >2\textsuperscript{109}.

The other characteristics of PCOS include insulin resistance, hyperinsulinemia, abdominal obesity, hypertension and dyslipidemia (decreased HDL and increased triglyceride) that culminates as metabolic
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In our study the PCOS subjects had a mean BMI of \(27.8 \pm 3.9\) and based on BMI segregation up to 77% of the subjects were either overweight or obese with a BMI over 25. Obesity is a major feature of PCOS that may contribute to PCOS pathogenesis by aggravating the intrinsic insulin resistance\(^{111,112}\).

The history of weight gain frequently precedes the onset of oligomenorrhea and hyperandrogenism suggesting a pathogenic role of obesity in the subsequent development of PCOS. A majority of our subjects (>80%) had gained body weight at puberty and a considerable number of them had answered positive to queries on hypertension, impaired glucose tolerance, dyslipidemia. These features may be responsible for the development of Metabolic syndrome at later stages of PCOS.

As a result of classification of PCOS subjects on the basis of BMI (for Asian population) in the present study, it was noted that more than 77% were either overweight or obese respectively with a mean BMI of >25. This contributed to a high percentage of subjects in that category and the remaining were lean PCOS subjects.

We analysed further for correlation between BMI and biochemical and hormonal profile, we found FBS and PPBS were higher than the recommended values in overweight subjects \(99.9 \pm 33.1\) mg/dL and \(113 \pm 40.9\) mg/dL.
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respectively. HDL was lower in BMI >30 group whereas LDL was more than the recommended values (>100 mg%) in all the BMI groups. We did not find any association between BMI and hormonal profile. However we found low serum Cortisol levels in overweight PCOS subjects indicating abnormal Cortisol metabolism and implicating Cortisol metabolism gene polymorphisms this is probably due to negative feed by visceral cortisol excess.

Hormonal profile of our PCOS subjects recorded imbalance of sexual harmones indicating that it is a polyendocrinal disorder. We found reversal of FSH:LH ratio and elevation in male harmones. Our study has recorded LH:FSH ratio with a mean value at (1.8 ±1.1) for the whole population that underwent the investigation. A breakdown figure of fouty one (41%) clearly had a LH:FSH ratio of over 2 which confirms Banaszewska and colleagues' remarks that LH: FSH ratio is not a characteristic of PCOS women. Testrosterone levels were elevated in more than 50% of our pco subjects. Our subjects also recorded higher levels of male hormones in relevance to increased LH and more than one half of the pco subjects had hyperinsulinemia. LH:FSH ratio greater than 2 has been considered as 'gold standard' in PCOS diagnosis for a long time. However, there are some controversies over essential diagnostic investigations in PCOS. A study by Banaszewska and colleagues has reported that elevated LH:FSH ratio > 2 was found only 45% of their PCOS subjects studied (54/119). Overproduction of LH and consequently altered LH:FSH ratio nowadays is considered not to be a characteristic of PCOS in all
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patients. A report on north Indian women with diabetes and PCOS are reported to have significantly high plasma LH/FSH ratio and androgens\textsuperscript{114}. Other studies have shown increased LH:FSH ratio in 94% of their patients when PCOS was not considered with insulin resistance. Now a days it is believed that elevated LH levels occurs more rarely in patients with insulin resistance and hyperinsulinemia than in a group without hyperinsulinemia\textsuperscript{109}.

We also found one third of our pcO subjects having hypothyroidism. The high prevalence was because our study was hospital based. Patients with hypothyroidism and PCOS more or less have similar complaints and clinical picture resulting in diagnosis PCOS during work up. We have also found a normal insulin level of (19 microU/ml) in this study. Sundararaman \textit{et al}\textsuperscript{111} and his coworkers have reported in a study involving 40 subjects from South India (Madras/Chennai) with elevated fasting insulin level in PCOS subjects (36 microU/ml) higher insulin resistance and greater intimamedial thickness. Another study has shown 33% of their subjects had elevated serum insulin levels.