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**Original Research Article**

# To estimate the levels of serum AMH, LH/FSH ratio and Prolactin in women with PCOS and find the correlation of AMH with LH/FSH in PCOS Patients with controls

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**Abstract**

**Background**

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy in women of reproductive age group and an incompletely understood enigmatic disorder of heterogenous nature. It starts appearing at 18 to 45 years of age and it may take years for its clinical presentation to appear. Anti-Mullerian hormone (AMH) has a glycoprotein dimer structure and is a member of the transforming growth factor-β (TGF-β) family. AMH is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles. Ovulatory women with the polycystic morphology can have increased LH/ FSH ratios; however, a single blood sample can fail to detect an increased ratio. With this background, the present study was undertaken to identify and assess the biochemical test in the form of antimullerian hormone, LH/FSH ratio, serum prolactin levels in patients with clinical features of polycystic ovarian syndrome.

**Aim and Objectives**

1. The objective of this study is to estimate the levels of serum AMH, LH/FSH ratio and Prolactin in women with PCOS.
2. To find the correlation of LH/FSH, with AMH in PCOS Patients and compare with controls.

**Materials and Methods**

This hospital-based cross-sectional study was carried out by the Biochemistry Department in conjunction with the Department of Medicine at SGT Hospital, Budhera, Gurugram's Faculty of Medicine and Health Sciences. 100 age matched healthy volunteers from general population will be taken as control. After explaining the purpose details of the study to all the subjects of both the groups, a written and informed consent will be taken. Ethical clearance will be taken from the Institutional Ethical Committee before the start of collecting the samples. In order to estimate the

levels of serum AMH, LH/FSH and Prolactin levels will be estimated by Chemiluminescence based immunoassay (CLIA) on fully automated Biochemistry analyzer MAGLUMI 1000.

**Results**

The parameters such as Serum AMH and Prolactin were significantly increased in patients withPCOS having p value <0.0001. Similarly the mean and standard deviation of LH/FSH ratio in cases was significantly higher i.e 2.73±1.06 and in control groups it was 1.60±0.59. The p value was notes <0.001, which is statistically very significant. It means there was significant change in LH/FSH level among both the groups. A significant correlation between AMH and LH/FSH ratio having pearson correlation coefficient r =0.77

**Conclusion**

In our study we found significant increased level of serum AMH, Prolactin, LH/FSH ratio, Antimullerian hormone independently in PCOD patients in comparision of normal control. We also noted positive correlation between AMH vs LH/FSH. So Multifaceted approach is required to diagnose and monitor disease of PCOD by utilizing all the hormone profile so that timely intervention can be done at optimum level. Measuring all these hormone along with radiological correlation can be utilized for diagnosing PCOD and management of this disease at various stages.

**Keywords:** Anti-Mullerian hormone (AMH), Prolactin, Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH)

## 1. INTRODUCTION:

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy in women of reproductive age group and an incompletely understood enigmatic disorder of heterogenous nature. It starts appearing at 18 to 45 years of age and it may take years for its clinical presentation to appear. The incidence of polycystic ovarian syndrome is 4% to 22% of women overall and 50% of women seen at infertility clinics (1,2). Polycystic ovarian syndrome was first described by Irving Stein and Michael Leventhal in 1935 in a group of patients presenting with amenorrhea, bilateral polycystic ovaries, and masculinising changes which might, according to them be due to the result of some hormonal stimulation very likely related to the anterior lobe of pituitary. Even after seventy years, the underlying cause for its heterogeneity and the development of signs and symptoms is not identified, the diagnostic criteria have yet to be universally agreed upon and the pathophysiology remains a point of intense research and debate (3). PCOS is characterized by low follicle stimulating hormone levels resulting in anovulation, elevated luteinizing hormone levels, resulting in hyperandrogenism, and insulin-resistance symptoms which may range from simple cystic acne, cephalic hair loss, or mild facial hirsutism to instances of oligomenorrhoea or amenorrhoea, sterility and severe generalized hirsutism. Classical Stein Leventhal syndrome which manifests as amenorrhoea, hirsutism, Anti-Mullerian hormone (AMH) has a glycoprotein dimer structure and is a member of the transforming growth factor-β (TGF-β) family. AMH is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles (4). AMH production by granulosa cells in the polycystic ovary is 75 times higher compared to healthy women. AMH levels in the plasma of PCOS patients are two or three times higher than average and begin to decline five years later than healthy women (5). Weerakiet's et al. stated that AMH plasma levels can be a marker of the degree to which folliculogenesis is impaired in patients with PCOS (6).

Prolactin is a bioactive polypeptide hormone synthesized by lactotrophs of pituitary of molecular weights 22,500 Daltons while the diamers or tetramers of the PRL display immune reactivity but not bioactivity may account for some cases of hyperprolactinemia not accompanied by galactorrhea. PRL secretion is episodic with a circadian sleep entrained increase generally lowest in the morning. About 20 to 30 % of PCOS women have hyperprolactinemia, 3 reproductive processes have been demonstrated to be impaired A study performed by Waldstreicher et al measured frequent (every 10 minutes) and prolonged (12-24 hours) serial blood samples which revealed a significant increase in the frequency and amplitude of LH release with normal FSH release in PCOS (7). The increased LH pulse frequency reflects an increase in GnRH release and suggests the presence of a hypothalamic defect. Ovulatory women with the polycystic morphology can have increased LH/ FSH ratios; however, a single blood sample can fail to detect an increased ratio. With this background, the present study was undertaken to identify and assess the biochemical test in the form of antimullerian hormone, LH/FSH ratio, serum prolactin levels in patients with clinical features of polycystic ovarian syndrome.

## 2. MATERIALS AND METHODS:

The present Hospital based observational cross sectional study was conducted in Departments of Biochemistry and Obstetrics and Gynaecology, Faculty of Medicine and Health Sciences, SGT Medical College, Hospital & Research Institute (SGT University), Budhera, Gurugram, Haryana. Sixty diagnosed patients with "polycystic ovarian syndrome by using Rotterdam criteria"(8)between age group of 18-45 years, attending the Obstetrics and Gynaecology OPD of SGT Hospital Budhera, Gurugram, were included for the study. Two criteria were required for diagnosis:
Number of follicles ≥ 12 having diameter 2-9 mm diameter. The volume of each ovary > 10cc with peripheral volume of follicles. 100 age matched healthy volunteers from general population will be taken as control. After explaining the purpose details of the study to all the subjects of both the groups, a written and informed consent will be taken. Ethical clearance will be taken from the Institutional Ethical Committee before the start of collecting the samples.

After explaining the purpose and details of the study to all the subjects, a written and informed consent was taken prior to the sample collection and an ethical clearance was obtained from the Institutional Ethical Committee of SGT University, Gurugram. Patients with Other endocrinal disorder, Patients on Vitamin D drugs, Pregnant women, any other chronic diseases were excluded from the study. A total of 5 ml venous blood was obtained between days $2^{nd}$and $3^{rd}$ day of the menstrual cycle. Samples were taken in capped vacutainers and were centrifuged at 3500 rpm for 15 minutes. Serum AMH, LH/FSH and Prolactin levels will be estimated by Chemiluminescence based immunoassay (CLIA) on fully automated Biochemistry analyzer MAGLUMI 1000.

**Statistical analysis**
Statistical analysis was done on SPSS (statistical package for social science) software. All the data was expressed as mean ±SD/±SE of the mean .The p value ≤ 0.05 was considered as significant. The data obtained was compared between two groups by student t–test. Pearson's correlation coefficient was applied for correlation between two quantitative variables. Chi square test was applied for non-parametric variables.

## 3. RESULTS:

**Tables:**

| Age group(yrs) | Cases | Control |
|---|---|---|
| 18-22 | 7 | 7 |
| 23-26 | 10 | 10 |
| 27-30 | 25 | 25 |
| 31-34 | 12 | 12 |
| 35-38 | 23 | 23 |
| 39-42 | 16 | 16 |
| 43-46 | 7 | 7 |

Table 1. Distribution of cases and control based on age group

| | Cases | Control | p value |
|---|---|---|---|
| Mean± SD | 32.91±6.71 | 33±6.69 | 0.927 |
| | | | Not significant |

Table 2:- Showing mean and standard deviation of age in cases and controls

| S.NO | Parameters | Mean±SD in cases | Mean ±SD in control | P value |
|---|---|---|---|---|
| 1 | AMH | 7.49±4.31 | 3.14±1.06 | <0.001 |
| 2 | LH | 16.01±7.06 | 6.99±2.20 | <0.001 |
| 3 | FSH | 6.06±2.28 | 4.72±1.70 | <0.001 |
| 4 | LH/FSH | 2.73±1.06 | 1.60±0.59 | <0.001 |
| 5 | Prolactin | 18.14±7.27 | 9.93±4.11 | <0.001 |

Table 3 :- Represents mean with SD values of serum AMH, Prolactin, LH ,FSH and LH/FSH ration levels among PCOD cases and control groups.

**Figures:**

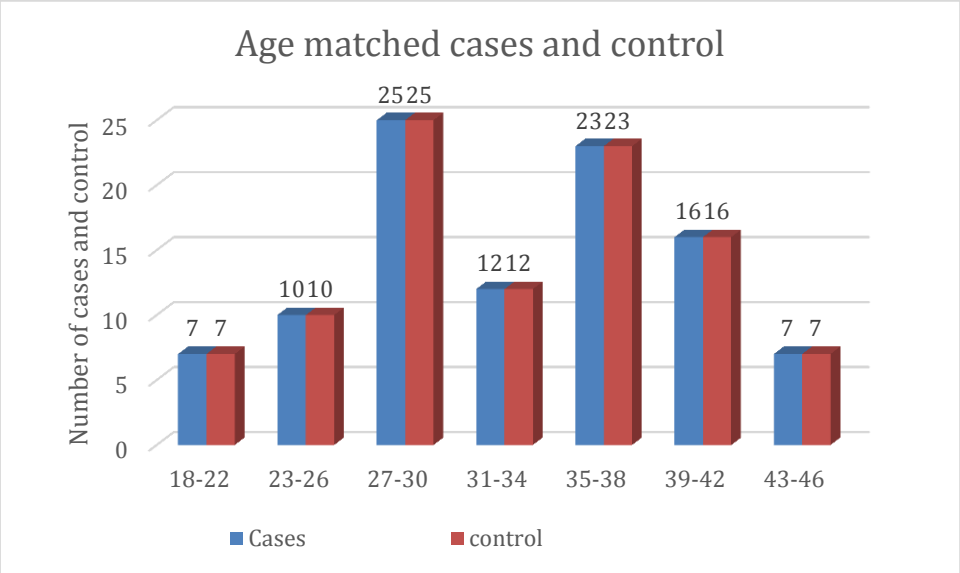


**Fig -1:** Graph showing Distribution of cases and control based on age group

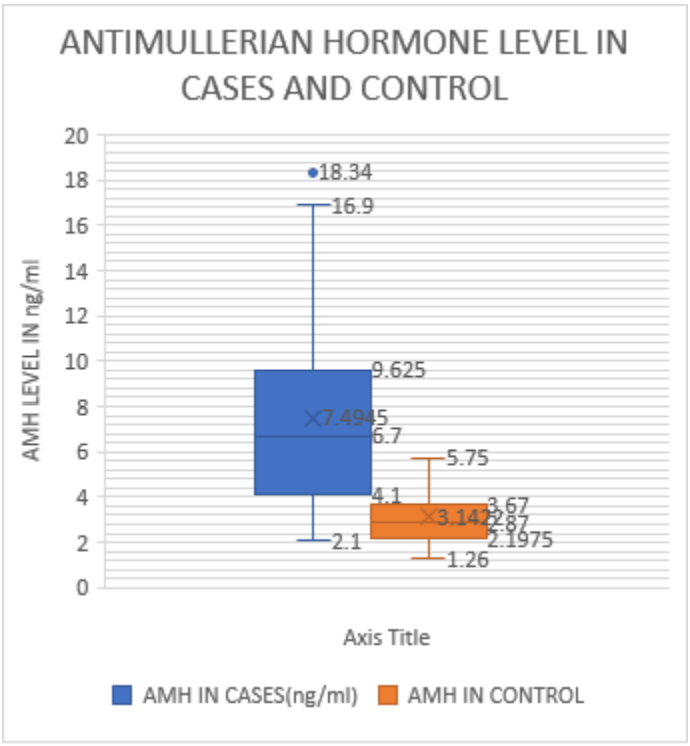


**Fig 2**: Graphical representation of serum Antimullerian hormone level in the form of box and whisker chart

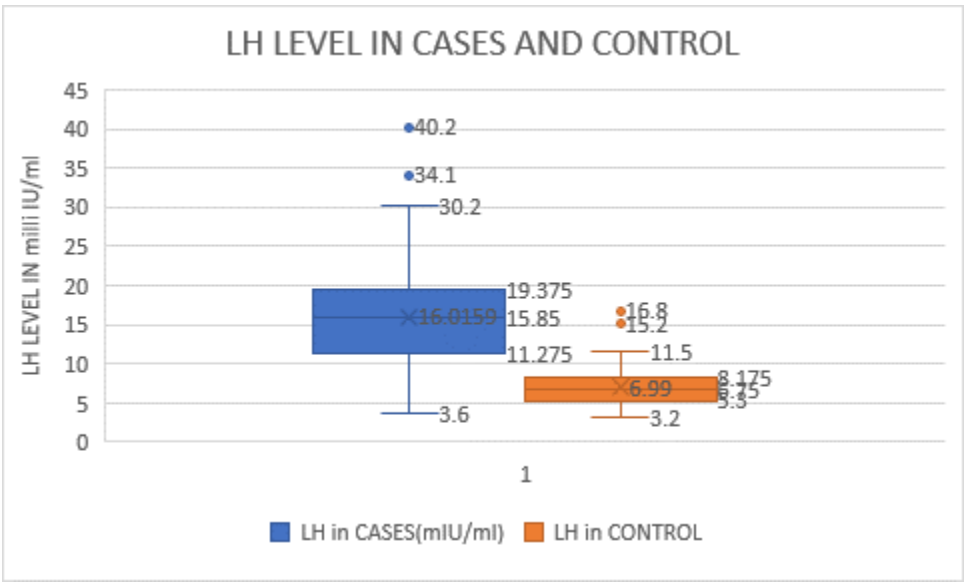


**Fig 3:-**Graphical representation of serum Leutenizing hormone in the form of box and whisker chart

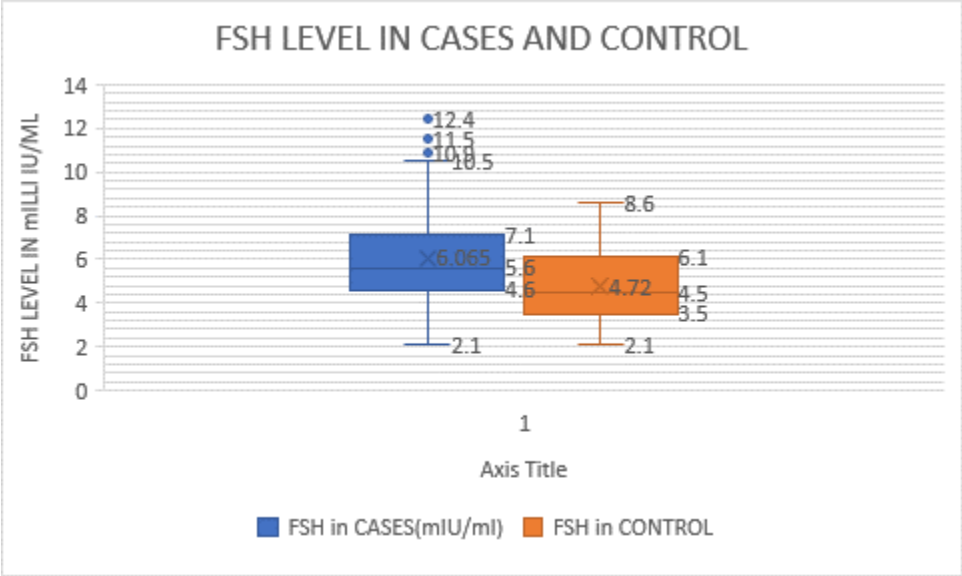


**Fig 4:-**Graphical representation of serum Follicle stimulating Hormone in the form of box and whisker chart

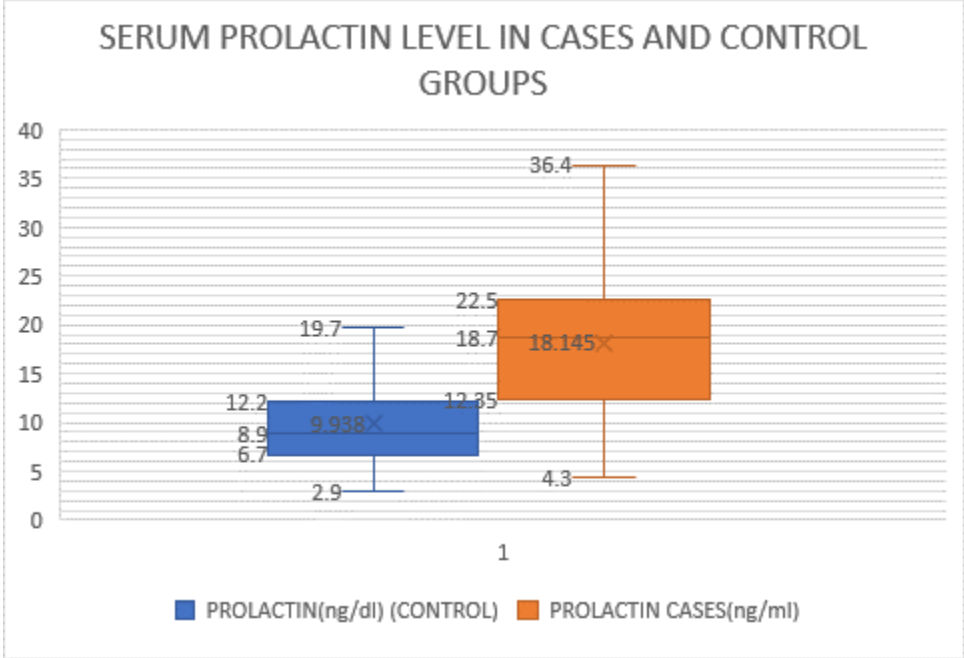


**Fig 5:-**Graphical representation of serum prolactin level in the form of box and whisker chart

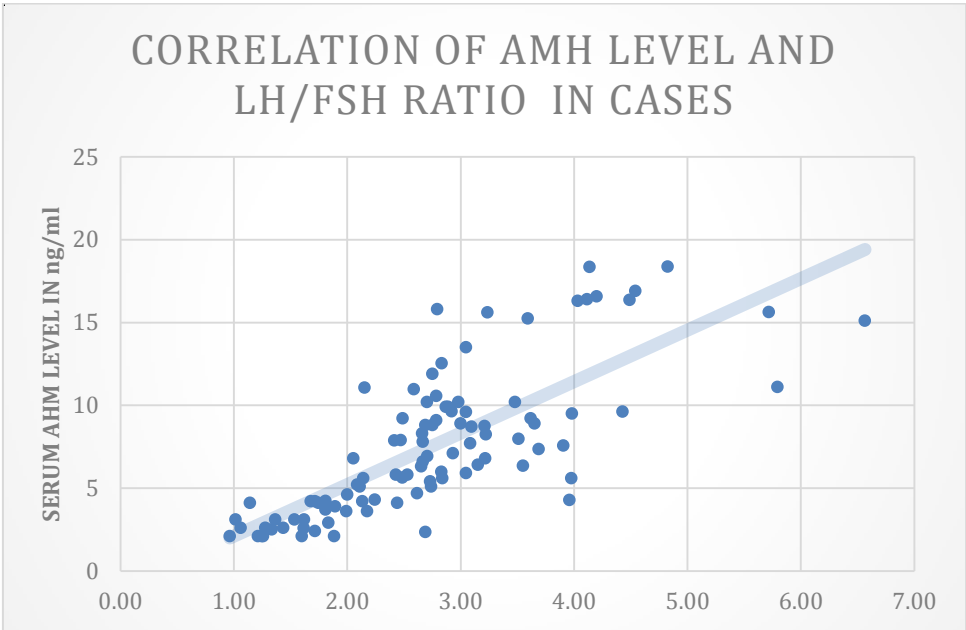


**Fig 6:**-Graph represent correlation between serum Antimullerian hormone level and LH/FSH ratio in PCOD cases- here pearson's coefficient noted r=0.77, which is positive correlation between these two parameters (LH=leutenizing hormone, FSH= Follicle stimulating hormone anf AMH= antimullerian hormone)

**Figures:**

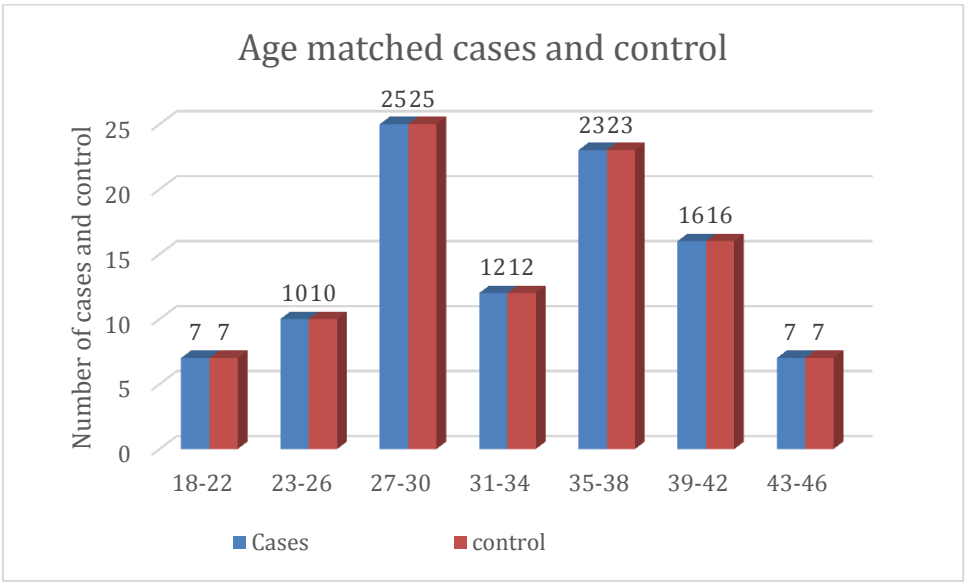


**Fig -1:** Graph showing Distribution of cases and control based on age group

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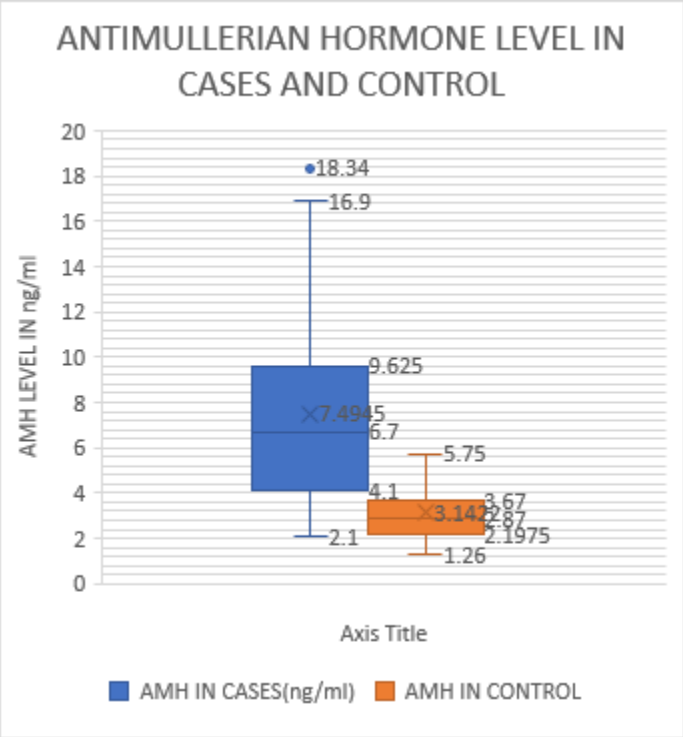


**Fig 2**: Graphical representation of serum Antimullerian hormone level in the form of box and whisker chart

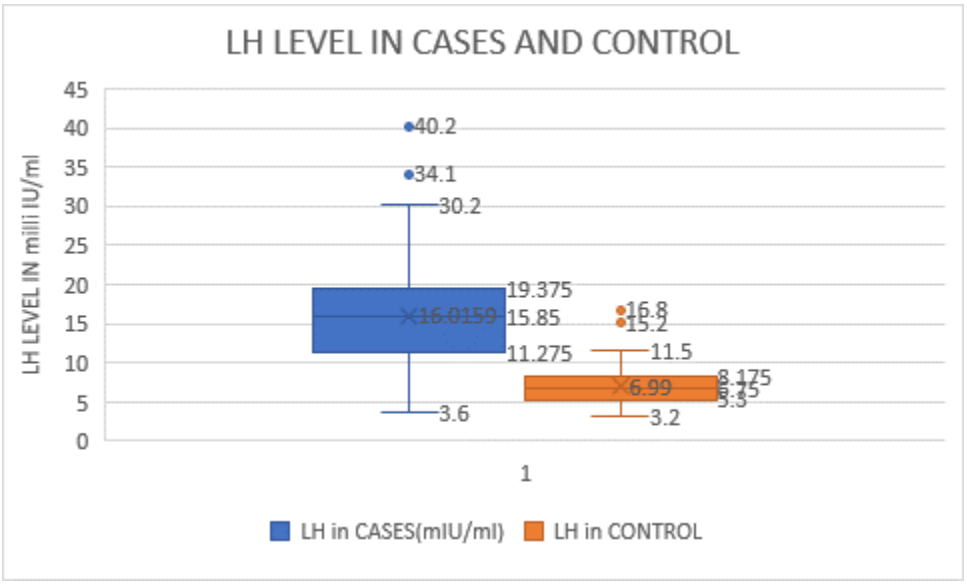


**Fig 3:-**Graphical representation of serum Leutenizing hormone in the form of box and whisker chart

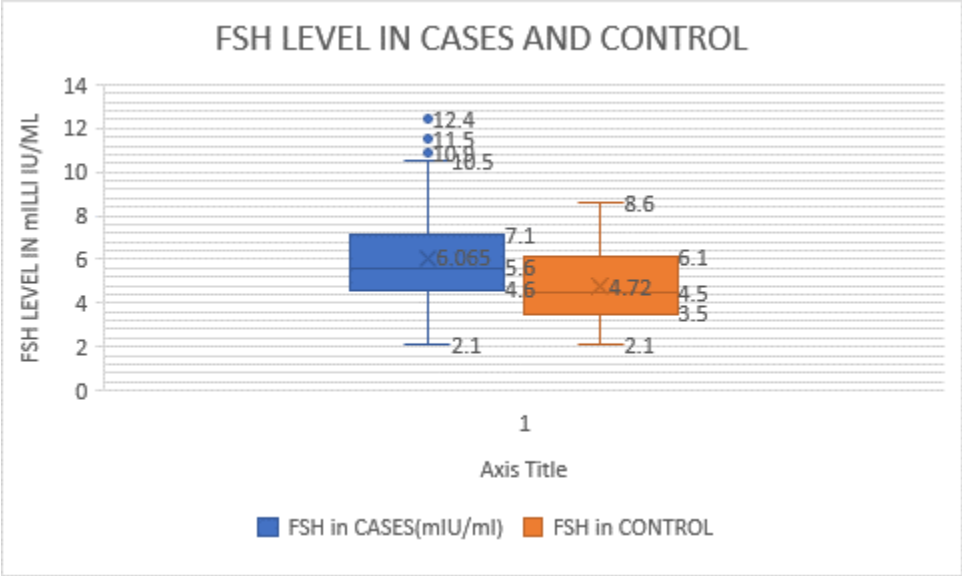


**Fig 4:-**Graphical representation of serum Follicle stimulating Hormone in the form of box and whisker chart

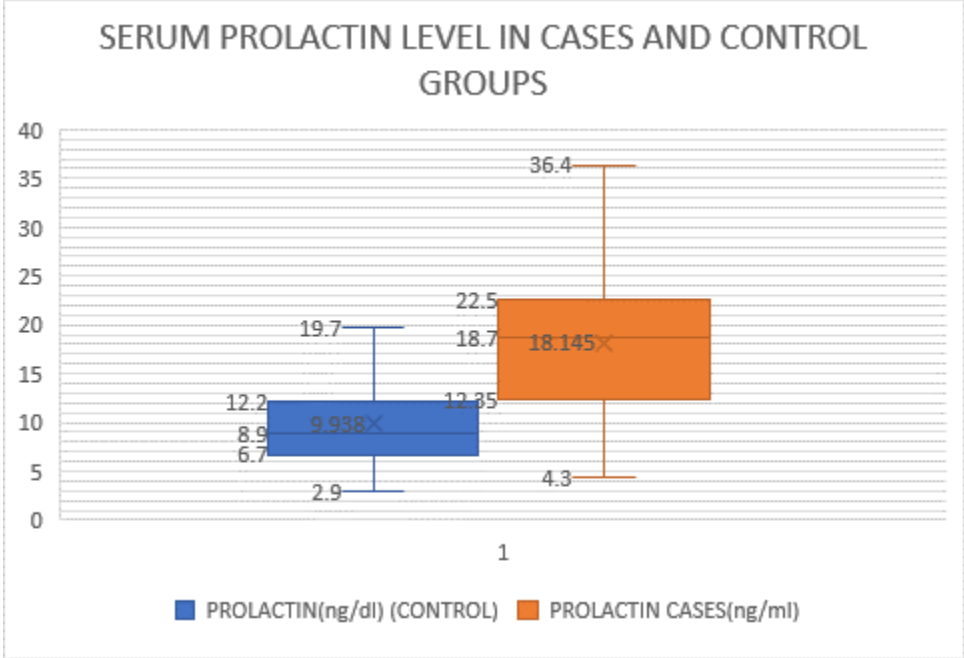


**Fig 5:-**Graphical representation of serum prolactin level in the form of box and whisker chart

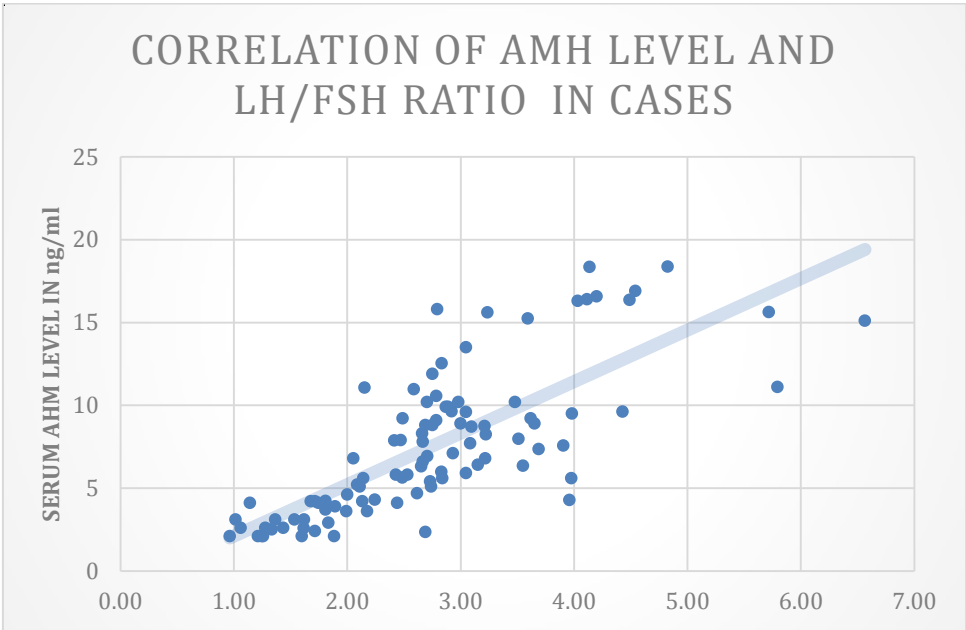


**Fig 6:**-Graph represent correlation between serum Antimullerian hormone level and LH/FSH ratio in PCOD cases- here pearson's coefficient noted r=0.77, which is positive correlation between these two parameters (LH=leutenizing hormone, FSH= Follicle stimulating hormone anf AMH= antimullerian hormone)

The goal of this research was to determine the levels of serum AMH, Prolactin and LH/FSH ratio and correlate LH/FSH ratio with AMH.

Demographic Parameters:

In this study 100 PCOD cases were included and these cases were compared with 100 age matched control group. Table 1 and Figure 1 shows that In this group maximum cases i. e 25 cases were in age group of 27-30 years followed by 23 cases in age group of 35-38 years. Minimum number of cases i.e 7 were in age group of 18-22 years and 43-46 years. In age group 39-42 years there were 16 cases, there were 12 and 10 cases in age group 31-34 years and 23-26 years respectively.

In this study the mean and standard deviation of age in year among cases and control analysed and it was calculated as 32.91±6.71 in years among cases and 33±6.69 in years among control groups. The p value was noted as 0.927 which symbolizes that there is no any significant difference between cases and control groups. Hence these are age matched groups shown in table 2.

In our study the mean and standard deviation of serum Antimullerian hormone level in cases was higher i.e 7.49±4.31 and in control groups it was 3.14±1.06. The p value was notes <0.001, which is statistically very significant. It means there was significant change in serum antimullerial hormone level among both the groups. ( Table 3, Figure 2)

Similarly the mean and standard deviation of LH/FSH ratio in cases was significantly higher i.e 2.73±1.06 and in control groups it was 1.60±0.59. The p value was notes <0.001, which is statistically very significant. It means there was significant change in LH/FSH level among both the groups. ( Table 3, Figure 3,4)

Also the mean and standard deviation of serum Prolactin level in cases was higher i.e 18.14±7.27 and in control groups it was 9.93±4.11. The p value was notes <0.001, which is

statistically very significant. IT means there was significant change in fasting serum insulil level among both the groups. ( Table 3, Figure 5)
In our study we found a strong positive correlation between AMH and LH/FSH ratio having pearson correlation coefficient r =0.77 shown in (Figure 6)

## 4. DISCUSSION

This study was undertaken on women aged between 18-45 years diagnosed with PCOS. We wanted to estimate the level of serum Antimullerian hormone , vit D level, LH/FSH level, and serum prolactin level and fasting insulin in the patients of Policystic ovarian syndrome, Antimullerian hormone level with LH/FSH ratio was done . In this study, polycystic ovarian syndrome patients were diagnosed according to Rotterdam European Society of Human Reproduction and embryology (ESHRE)/ American Society for Reproductive Medicine (ASRM) criteria.
In the present study we found altered AMH value between cases and controls having mean value in cases 7.49±4.31 and 3.14±1.06 in controls ($p<0.001$) Shahrzad Zadehmodarres et al (9) conducted a cross sectional study in 2015 in which 60 PCOD cases and 57 control were analysed for antimullerian hormone level in cases and control group as , 7.14 ± 6.53 in cases and 3.34 ± 3.45 respectively. The results were comparable to the present study Shahrzad Zadehmodarres et al was noted as 3.15 with 70.37% sensitivity and 77.36% specificity and positive predictive value (PPV)=76% and NPV(negative predictive value)=71.93. In present study the cutoff value was noted as 3.895 with 79 percent sensitivity and 79% specificity.
Also In an Indian study done by Nalini Mahajan et al (10) the AMH level in PCOD cases were 7.56 ± 4.36 and 2.25 ± 1.81 respoectively which is comparable with our study. The study done by Budi Wiweko et al in 2014 (11) the AMH level in PCOD cases and control was notes as 9.50 ±5.11 and 3.53 ±1.95 respectively. Yu Ran et al in 2021 (12) reported AMH level in PCOD cases and control 8.63±4.73 and 5.57±3.31 respectively.
In the present study the mean±SD level of serum LH was noted in cases and control group was as 16.01±7.06 and 6.99±2.20. Which is comparable with study done by vinay Kr et al (13) in which it was noted as 6.99±2.20. However the study conducted by Budi Wiweko et al (11) it was 10.41±8.12 and 4.37 in PCod cases and control respectively. Also in study conducted by Shahrzad Zadehmodarres et al (9) serum LH level were noted in cases and control group as 5.96 ± 2.93 and 5.58 ± 3.09. In Indian study by Nalini Mahajan et al it was noted as 6.86±4.72 and 4.22±2.26 in cases and control groups (10).
In the present study the mean±SD level of serum FSH was noted in cases and control group was as 6.06±2.28 and 4.72±1.70. Which is comparable with study done buy vinay Kr et al (12) in which it was noted as 5.78±2.42. However the study conducted by Budi Wiweko (11) et al it was 5.39±1.47 and 6.30 in PCOD cases and control respectively. Also in study conducted by Shahrzad Zadehmodarres et al (9) serum FSH level were noted in cases and control group as 4.53 ± 1.62 and 6.88 ± 5.56. In Indian study by Nalini Mahajan et al it was noted as 4.23±1.29 and 6.68±2.22 in cases and control groups (10).
In the present study the the LH/FSH ratio was noted in cases and control group as 2.73±1.06 and 1.60±0.59. Which is comparable with study published by Hilma Putri Lubis et al (14) in 2020 in which a case–control study was performed in women with PCOS and non-PCOS, in which they observed Lh/FSH level as 2.10 ± 1.16 and 0.65 ± 0.31 in cases and control groups. Similarly in study conducted by Li Wei Cho et al (15) in which LH/FSH ratio in cases and control group was noted as 1.2 and 1.6.

In present study the serum prolactin level in cases and control group was noted as 18.14±7.27 and 9.93±4.11 respectively . Our study was comparable with Latha P et al (16) in which 30 PCO cases and 30 age matched control women were analysed for serum prolactin level, it was noted as 14.08±6.87 and 10.78±6.72 respectively. Similar study was conducted by Kundu et al (17) in 2019 in which 50 PCOD and 50 age match control group were analysed for serum prolactin, and the level were 10.18±5.96 and 14.62±6.81 respectively

## 5. CONCLUSION

Polycystic ovary syndrome is a common endocrine disorder that affects 5-10% of women in their reproductive or premenopausal age. It is characterised by chronic anovulation (oligoovulation or amenorrhoea) and hyperandrogenism (hirsutism). The untimely and exaggerated effect of LH on PCOS granulosa cells has been associated in the arrest of follicular maturation. This may also be caused by LH-induced surge in ovarian androgen levels. Furthermore, the absence of the intercycle FSH peak also contributes, but most significantly the refractoriness of granulosa cells to FSH action, have been associated with anovulation in PCOS. In our study we calculated Lh/FSH ratio in 100 cses and fount significant rise in Lh/FSh ratio in PCOD cases. Hyperandrogenemia along with relatively high estrogen levels stimulate prolactin secretion, Antumullerian hormones are expressen in granulose cell of antral and preantral stage of graffian follicle and In Pcod there increase in number of follicle in ovary. These hormone may be good marker of detecting PCOD at early stage and for management of this condition. After analyzing serum antimullerian hormone and serum prolactin level in PCod cases it was noted that there was significant rise in these hormone in PCOD cases in comparision to control group. This proves that AMH level can play crucial role in diagnosing and monitoring the PCOD disease.

In our study we found significant increased level of serum AMH, Prolactin, LH/FSH ratio, Antimullerian hormone independently in PCOD patients in comparision of normal control. We also noted positive correlation between AMH vs LH/FSH.

So Multifaceted approach is required to diagnose and monitor disease of PCOD by utilizing all the hormone profile so that timely intervention can be done at optimum level. Measuring all these hormone along with radiological correlation can be utilized for diagnosing PCOD and management of this disease at various stages.

## 6. REFERENCES

1. Nagamani Peri, Deborah Levine. Sonographic evaluation of the endometrium in patients with a history or an appearance of polycystic ovarian syndrome. J Ultrasound Med 2007; 26: 55-58.
2. Belinda MS, Richard PD. Polycystic ovarian syndrome and the metabolic syndrome.Am J Med Sci 2005;330(6):336-342.
3. Abdel Gadir A, Khatim MS, Mowafi RS, Alnaaser HM, Muharib NS, Shaw RW. Implications of ultrasonically diagnosed polycystic ovaries. Its correlations with basal hormonal profiles. Human reproduction 1992;7(4): 453-7.
4. Bako AU, Morad S, Atiomo WA. Polycystic ovary syndrome: an overview. Rev Gynecol Pract. 2005;5:115–122.
5. Pellatt L, Hanna L, Brincat M, et al. Granulosa cell production of anti-Mullerian hormone is increased in polycystic ovaries. J. Clin. Endocrinol. Metab. 2007;92(1):240–245.

6. Weerakiet S, Lertvikool S, Tingthanatikul Y, Wansumrith S, Leelaphiwat S, Jultanmas R. Ovarian reserve in women with polycystic ovary syndrome who underwent laparoscopic ovarian drilling.Gynecol Endocrinol. 2007;23(8):455–60.
7. Filho RB, Domingues L, Naves L, Ferraz E, Alves A, Casulari LA. Polycystic ovary syndrome and hyperprolactinemia are distinct entities. Gynecol Endocrinol. 2007;23(5):267-272.
8. Daniel M, Fessler T, Natterson-Horowitz B, Azziz R. Evolutionary determinants of polycystic ovary syndrome: part 2. Fertility and Sterility 2016 July;106(1):0015-0282.
9. Shahrzad Zadehmodarres, Zahra Heidar, Zahra Razzaghi, Leili Ebrahimi, Kaveh Soltanzadeh and Farhang Abed; Serum lipid profile and insulin resistance in women with polycystic ovary syndrome; Iran J Reprod Med,2015: 13(4). 227-230.
10. Nalini Mahajan and Jasneet Kaur; Establishing an Anti-Müllerian Hormone Cutoff for Diagnosis of Polycystic Ovarian Syndrome in Women of Reproductive Age-Bearing Indian Ethnicity Using the Automated Anti-Müllerian Hormone Assay; Journal of Human Reproductive Sciences;2019; 12 (2) :104-13
11. Budi Wiweko, Mila Maidarti , M. Dwi Priangga, Nadia Shafira, Darrell Fernando, Kanadi Sumapraja et al Anti-mullerian hormone as a diagnostic and prognostic tool for PCOS patients. J Assist Reprod Genet (2014) 31:1311–1316
12. Yu Ran, Qiang Yi, Cong Li .The Relationship of Anti-Mullerian Hormone in Polycystic Ovary Syndrome Patients with Different Subgroups. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2021:14 1419–1424
13. Dr. Vinay Kumar and Dr.Rohit Kumar Singh; A cross-sectional study to investigate the prevalence and etiology of different thyroid disorders in PCOS patients: European Journal of Molecular & Clinical Medicine;2020;7(10):3744-48
14. Hilma Putri Lubis, Muhammad Fidel Ganis Siregar, Ichwanul Adenin, Binarwan Halim, Henry Salim Siregar and M. Oky Prabudi;Association between Luteinizing Hormone/Choriogonadotropin Receptor Ins18LQ Gene Polymorphism and Polycystic Ovary Syndrome: Open Access Maced J Med Sci. 2020 Aug 10; 8(A):517-520
15. Li Wei Cho, Vijay Jayagopal, Eric S Kilpatrick, Stephen Holding and Stephen L Atkin; The LH/FSH ratio has little use in diagnosing polycystic ovarian syndrome: Ann Clin Biochem 2006; 43: 217–219
16. Latha P, B V Ravi and Roshni Sadaria; Study of TSH and prolactin in PCOS subjects: A case control study: International Journal of Clinical Biochemistry and Research 2021;8(1):62–65
17. .Kundu D, Ghosh E , Baran Mandal A. and Basu S; Evaluation of prolactin and insulin resistance in women with polycystic ovarian syndrome; International Journal of Medical Research and Review 2019;7(3);200-205.