Imbalance of Serum estrogen and progesterone concentrations in puberty age girls suffering from sickle cell anemia in tribal population

Abstract

About 7% of the world's population is carriers of some form of haemoglobin disorder. There are about 270 million carriers of sickle cell anemia and/or thalassemia. The level of ovarian steriods alters in girls suffering from sickle cell anemia which causes complications such as hypogonadism which includes amenorrhea or delayed menarche etc. by determining the level of these hormones in sickle cell patients we can avoid many complications associated with the SCD thus present study will help in prognosis of the disease in tribal indivisuals. There is no significant difference between mean age of menarche in sickle cell trait (AS) and normal individuals (AA), supporting the fact that the sickle cell trait lead an normal life and seldom require treatment for their genetic condition.

Key words: - Puberty age, SCD, estrogen, sex-hormones, tribes.

INTRODUCTION

The sickle cell disease is a widespread genetic disorder characterized by red blood cells assuming an abnormal, rigid, sickle shape that results in a risk of serious complications. It occurs in high frequency in many tropical countries of the world[1]. The sickling occurs due to a mutation in the haemoglobin gene. Homozygosity of the gene, contributed by both the parent, result in to sickle cell anaemia, whereas person with one recessive gene are called carrier or trait shows asymptomatic sicklemia[2]. Sickled haemoglobin polymerizes under deoxy condition and therefore obstructs small blood vessels. While the carrier leads a normal life, the diseased person suffers from various complications such as anaemia, frequent infection, fever, hand-foot syndrome, stroke, acute chest pain, vasoocclusive crisis, spleenomegaly, renal failure, leg ulcers, hypogonadism etc[3].

In India, sickle cell gene is mainly restricted to tribal and scheduled caste population. In our country, tribals with sickle gene are mainly concentrated in Madhya Pradesh, Orissa, Chhattisgarh, Jharkhand, Gujarat, Andhra Pradesh etc[4]. Hypogonadism is one of the most prevalent endocrinopathies in subjects with SCD. Delayed onset of puberty is a frequent finding in girls and boys with SCD.Menarche is delayed by a mean interval of 2-3 years. A case control study performed by Soliman *et. al.* found that two-thirds of girls with SCD have delayed breast development (mean age of thelarche at 13.5 years), and the mean age of spontaneous menarche is 15.6 years . Reasons for decreased growth are multi-factorial with contributions from abnormal endocrine function.Estradiol is a steriod hormone (molecular weight of 272.3daltons), which are secreted principally by ovarian follicles and aso by adrenals, corpus luteum and placenta and in males by testes estrogenic hormones are secreted at varying rates during the menstrual cycle throughout period of oarian activity[5,6,7].Progesterone is a steriod hormone, which plays an important role in the preparation and maintenance of pregnancy. it is synthesized from cholestrole via pregnenolone then rapidly rapidly metabolized to pregnanediol primarily in the liver the ovary and placenta are major sites of production but small amount is also produced by adrenal cortex in both men and women. Circulating progestrone levels, which are characteristically low during follicular phase and sharply increase during luteal phase of menstrual cycle,reaching a maximum approximately 5 to 10 days after midcycle LH peak[8].The level of these ovarian steriods alters in girls suffering from sickle cell anemia which causes complications such as hypogonadism which includes amenorrhea or delayed menarche etc. by determining the level of these hormones in sickle cell patients we can avoid many complications associated with the SCD thus present study will help in prognosis of the disease[9,10]. Therefore the present proposed research aimed compare estradiol and progesterone level of sickle cell patients (SS) as well as trait (AS) patients with normal individuals (AA)

MATERIALS AND METHODS

Materials:

All chemical procured by standard analytical and preparative grade.

Study Population-

Blood samples were collected from subjects and volunteers (sickle cell patients) of age between four and above years at the department of biochemistry centre for genetic disease and molecular biology of Pt.J.N.M. Medical College and associated Dr. B.R.A.M. hospital Raipur (C.G.).for this study control and study group was taken from paediatrics and medicine O.P.D. of Dr. B.R.A.M. hospital and sickle cell O.P.D. of Pt. J.N.M. medical college Raipur. Patient's samples were identified after counselling and genotyping to determine their genotype group and some already known sickle cell patients that attended the clinics and sickle cell centers for routine medical check. No patients had any severe infection nor were on any sort of medication. The volunteers were maintained as per norms of center ethics committee on human research (CECHR) and local ethics committee

Selection of cases:

Control group: Control group consist of 50 normal puberty age girls and had no clinical evidence of any sickle cell disease or other disorder.

Study group : Study group consist of 80 patients of puberty age girls attending O.P.D. or admitted in medical college hospital Raipur suffering from sickle cell disease out of 80 patients 30 were sickle cell disease (SS) and rest 50 were sickle cell traits (AS).

Biochemical tests

Collection of sample:

All possible aseptic technique is used to collect the sample of blood 2 ml. In sterile, dry, plain vial after which the blood is centrifuged for 15-20 minutes at 3000 rpm and supernatant (serum) is used for level of estradiol and progesterone estimation.

Solubility test:

Deoxygenated Sickle cell haemoglobin has an abnormally low solubility. A fibrous precipitate is formed when a concentrated solution of sickle cell haemoglobins deoxygenated (This precipitate deforms red cells and gives them their sickle shape. The rate of fibre formation is proportional to about the tenth power of the effective concentration of deoxyhemoglobin S. Thus, fibre formation is a highly concerted reaction) HbS is deoxygenated form and is insoluble in phosphate buffer (giving

turbidity to the solution) while other haemoglobins are completely soluble (giving clear solution)[11].

One ml of phosphate buffer reagent was taken in a glass tube and a small quantity of sodium dithionite was added to it and was mixed well to dissolve. A small drop of washed red cells was added and was mixed well to produce light pinkish violet colour. The test was read after 3 to 5 min. It was read as positive, if the turbidity impaired the visibility of dark, bold lines on a white paper held against bright source of light at one inch distance. Negative test was indicated by visible li

Hb Electrophoresis:

Each of the major haemoglobin types has an electrical charge of a different degree, so the most useful method for separating and measuring normal and abnormal haemoglobins is electrophoresis. This process involves subjecting haemoglobin components from dissolved red blood cells toan electrical field. The components then move away from each other at different rates, and when separated, form a series of distinctly pigmented bands. The bands are then compared with the other samples on the same membrane strip called as control[12].Combination of electrophoresis technique with solubility test is a golden standard for detecting sickle cell haemoglobin in carrier and sufferer state. It is very cost effective.

ELISA (Enzyme linked immunosorbent assay):

Serum estradiol and progesterone level is estimated by using enzyme linked immunosorbent assay (ELISA)[13,14].

Competitive enzyme immunoassay:

The interaction is illustrated by the followed equation:

$$^{enz}Ag + Ag + Ab_{btn} \rightarrow AgAb_{btn} + {}^{enz}AgAb_{btn}$$

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immoblized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$$AgAb + {}^{enz}AgAb_{btn} + \text{Streptavidin cw} \rightarrow \text{Immoblized complex}$$

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several diffferent serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

Statistical Analysis: The result and observation recorded in this study was done by using statistical model ANOVA (Analysis of Variance) and SEM. All data analyzing withy use of SPSS statistical software version -11[15]

Results& discussion

Sickle cell disease (SCD) is an autosomal, recessive hemoglobinopathy characterized by haemolytic anaemia, intermittent occlusion of small vessels leading to acute and chronic tissue ischemia, and organ dysfunction. The result of the clinical study start with sickle cell solubility test is a simple method that detects the presence of sickle haemoglobin; of the 130 samples tested 80 samples were found to be positive..But the solubility test does not distinguish between sickle cell trait and sickle cell disorders. Positive samples are subjected to electrophoresis. Each of the major haemoglobin types has an electrical charge of a different degree, so the most useful method for separating and measuring normal and abnormal haemoglobins is electrophoresis. Combination of electrophoretic technique with solubility test is a golden standard for detecting sickle cell haemoglobin in carrier and sufferer state. The mean age of menarche were estimated in sickle cell disease (SS) girls, in sickle cell trait (AS) girls and control individuals (AS) .and mean age of menarche of study group were compared with control group.The general retardation of sexual development in girls with homozygous sickle cell (SS) disease has been widely recognised since early descriptions [16] [17]. Menarche was delayed by a mean interval of 1.7 years in Washington DC [18], and by 2.3–3.0 years in different studies in Jamaica [19-21]. Body weight and age were the best predictors of menarche in the Cooperative Study of Sickle Cell Disease in the USA, [22], A study in Jamaica identified fetal haemoglobin, and with less certainty, height and social class as the best predictors [23].In present study Mean age of menarche was found to be 13 years in normal individuals (AA), 13.08 years in

sickle cell trait (AS) and 15 years in sickle cell disease individual (SS). Mean age at menarche is delayed by 2 years in SS diseased individuals as compared with normal individuals.

The hormonal (estradiol and progesterone) level in sickle cell patients (In puberty age girls). In this study the correlation of hormonal (estradiol and progesterone) level with delayed menarche in girls with sickle cell anaemia was done by the detection of the level of estradiol and progesterone. Estradiol and progesterone are ovarian steroid hormones secreted principally by ovary. Its levels have been found to be markedly decreased in sickle cell anemia. A fall in estradiol and progesterone level extensively studied and demonstrated in sickle cell anaemia. The study involved hormonal level detection by using enzyme linked immunosorbent assay (ELISA) which is a very sensitive technique for hormonal level detection in serum and which helps in prediction for prognosis of many diseases like hypogonadism in sickle cell anaemia.There was general retardation of sexual development in girls with homozygous sickle cell (SS) disease. Menarche was delayed by a mean interval of 1.7 years in Washington DC, and by 2.3–3.0 years in different studies in Jamaica. Jamaica identified fetal haemoglobin, and with less certainty, height and social class as the best predictors. of menarche in the Cooperative Study of Sickle Cell Disease age of 13 years in AA controls [57].The Hgb, hematocrit, and hemoglobinF concentration were associated with weight, height, and BMI scores in females but not in males. In contrast, Zamel *et al(2007)* reported that Hgb concentration was associated with height and weight in prepubertal Jamaican males, but not females [63].The finding of this study is that the estradiol and progesterone level were found to be markedly decreased in sickle cell disease person (SS) as compared with sickle cell trait (AS) as well as with normal individuals (AA) . And Menarche was delayed by a mean interval of 2 years in sickle cell disease (SS) as compared to normal individuals. But there is no significant difference in mean age of menarche of sickle cell trait (AS) and normal individuals (AA).A feasible approach for detection of hypogonadism in sickle cell anaemia has been presented. This analysis has been conducted by using enzyme linked immunosorbent assay (ELISA) a very sensitive method; it has been able to give the result by using nano gram (ng) or pico gram (pg) quantity of the sample.The statistical results of estradiol and progesterone level were found to be

significant i.e. ($p<0.01$) .which shows that the result of this study is statistically significant.

Conclusion

Manifestations of endocrine and nutritional abnormalities are more common than once perceived in patients with SCD with and without evidence of iron overload. The current gaps in medical knowledge afford opportunities for future research investigating optimal approaches to the diagnosis, intervention, and prevention of these hormonal and nutritional dyscrasias. The present study concluded that the menarche was delayed by a mean interval of 2 years in sickle cell disease individuals (SS) as compared to normal individuals. estradiol and progesterone level were found to be markedly decreased in sickle cell disease person (SS) as compared with sickle cell trait (AS) as well as with normal individuals (AA) .There is no significant difference between mean age of menarche in sickle cell trait (AS) and normal individuals (AA), supporting the fact that the sickle cell trait lead an normal life and seldom require treatment for their genetic condition.

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Table No. 1: Shows the number of sickle cell Diseased, trait patient and control with their percentage.

Groups	No. of patients	Percentage (%)
Control group (AA)	50	39
Study group (AS)	50	38
Study group (SS)	30	23
Total	130	100

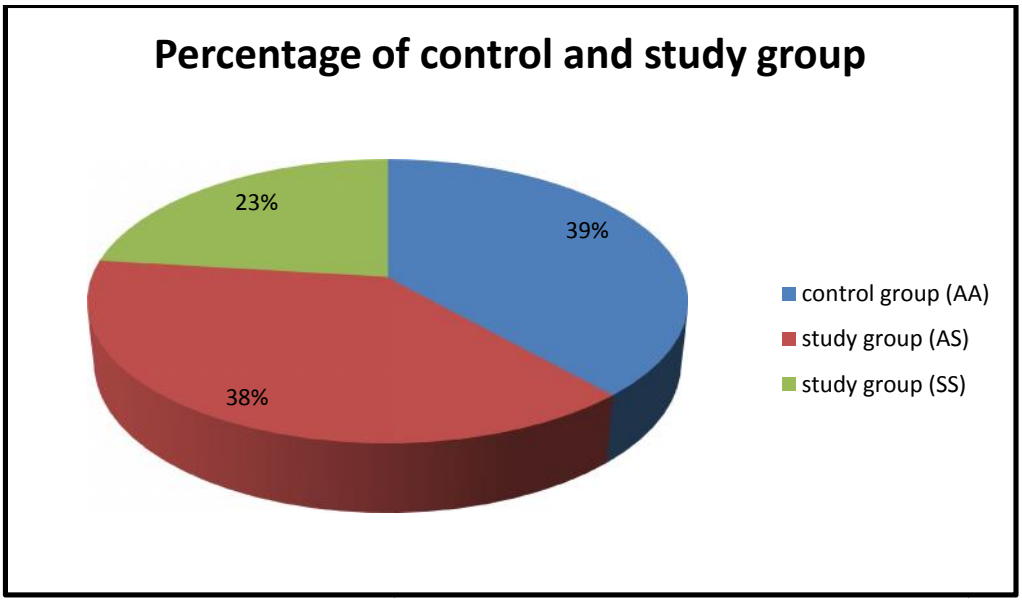

Figure 1: shows percentage of Control and Study group.

There are 130 individuals in which 50 were control group which consist of 39% of total, other 50 were study group (AS) which consist of 38% of total and remaining 30 were study group (SS) which consist of 23% of total.

Table No. 2: Shows mean age of menarche in control and study groups.

Group	No. Of cases	Mean age of menarche (yrs.)	S.D.
Control group (AA)	50	13	0.3112
Study group(AS)	50	13.08	0.1852
Study group (SS)	30	15	0.252

Age of menarche in control and study group were summarised by using mean and standard deviation. Age of menarche in control group was found to be 13 yrs. 13.08 yrs. In sickle cell trait patients (AS) and 15 yrs. In sickle cell diseased patients.

Table No. 3: shows mean serum progesterone level in controls.

Age groups (yrs.)	13-14	15-16	17-18	19
No.of cases	16	13	9	12
Serum progesterone level (ng/ml)	13.90	12.90	12.11	10.28

Mean serum progesterone level was 13.90 ng/ml in 13-14 years, 12.90 ng/ml in 15-16 years, 12.11 ng/ml in 17-18 years and 10.28 ng/ml in 19 years age.

Table No. 4: shows mean serum progesterone level in study group (AS)

Age groups (yrs.)	13-14	15-16	17-18	19
No. of cases	23	8	14	5
Serum progesterone level (ng/ml)	20.63	17.31	29.92	32.43

Mean serum progesterone level was 20.63 ng/ml in 13-14 years, 17.31 ng/ml in15-16 years, 29.92 ng/ml in 17-18 years and 32.53 in 19 year's age.

Table No. 5: Shows mean serum progesterone level in study group (SS)

Age group (yrs.)	13-14	15-16	17-18	19
No. Of cases	0	13	13	6
Serum progesterone level (ng/ml)		1.22	0.245	0.217

Mean serum progesterone level was 1.22 ng/ml in 15-16 years, 0.245 ng/ml in 17-18 years and 0.217 ng/ml in 19 years age.

Table No. 6: Shows mean serum estradiol level in controls (AA)

Age group (yrs.)	13-14	15-16	17-18	19
No. Of cases	16	13	9	12
Serum estradiol level (pg/ml)	47.51	50.52	53.74	48.69

Mean serum estradiol level was 47.51 pg/ml in 13-14 years, 50.52 pg/ml in 15-16 years, 53.74 pg/nl in 17-18 years and 48.69 pg/ml in 19 years age.

Table No. 7: Shows mean serum estradiol level in study groups (AS)

Age group (yrs.)	13-14	15-16	17-18	19
No. Of cases	23	8	14	5
Serum estradiol level (pg/ml)	35.11	33.92	42.27	32.87

Mean serum estradiol level was 35.11 pg/ml in 13-14 years, 33.92 pg/ml in 15-16 years, 42.27 pg/ml in 17-18 years and 32.87 pg/ml in 19 years age.

Table No. 8: Shows mean serum Estradiol level in study groups (SS)

Age group (yrs.)	13-14	15-16	17-18	19
No. Of cases	0	13	13	6
Serum estradiol level (pg/ml)		11.51	22.16	33.20

Mean serum estradiol level was 11.51 pg/ml in 15-16 years, 22.16 pg/ml in 17-18 years, 33.20 pg/ml in 19 years age.

Table No. 9: Shows Serum progesterone level in control Vs study group.

Group	No. Of cases	Serum progesterone level			
		Mean (ng/ml)	S.D.	P	Significance
Control group (AA)	50	28.9	2.178	<0.01	Significant
Study group (AS)	50	23.88	2.084		
Study group (SS)	30	0.66	1.579		

Above are Statistical results of serum progesterone level whose levels are ($p<0.01$) shown statistically significant.

Table No. 10: shows serum estradiol level in control Vs. Study group

Group	No. Of cases	Serum estradiol level			
		Mean (pg/ml)	S.D.	P	Significance
Control group (AA)	50	49.70	2.308	P<0.01	Significant
Study group (AS)	50	37.49	1.940		
Study group (SS)	30	24.30	3.100		

Above are statistical results of serum estradiol level whose levels are ($p<0.01$) shown statistically significant.

Table No.11: shows statistical data of serum estradiol and progesterone level.

Hormones	No. Of cases	Mean	S.D.	F value	P	Significance
Estradiol	130	39.14 (pg/ml)	7.348	10.79	<0.01	Significant
Progesterone	130	12.52 (ng/ml)	5.359	20.71	<0.01	Significant

Above are statistical results of serum estradiol and serum progesterone level whose levels are ($p<0.01$) which shows that the levels are statistically significant.

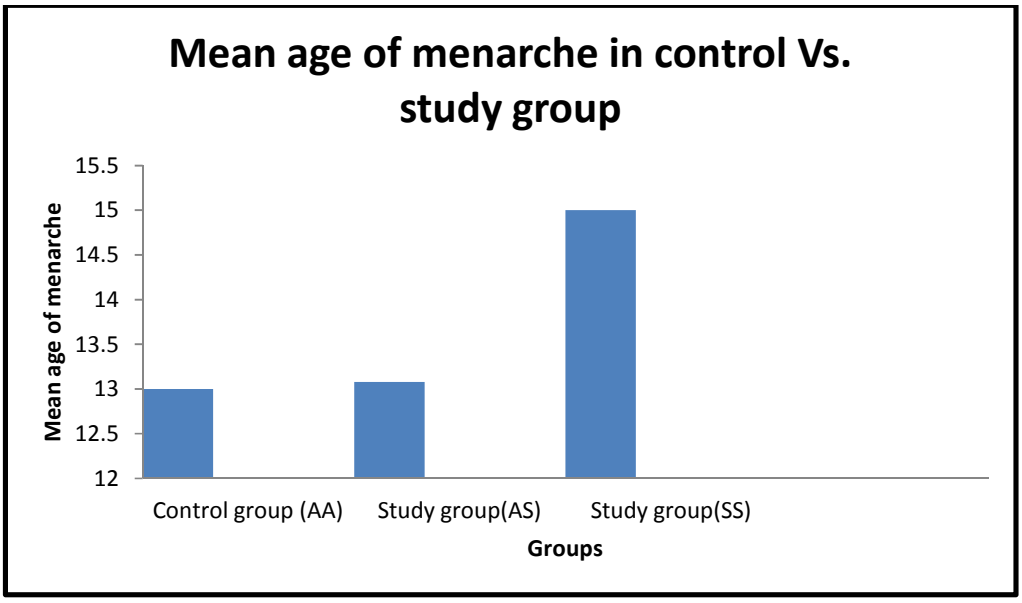

Figure 2: Mean age of menarche in control Vs. Study group.

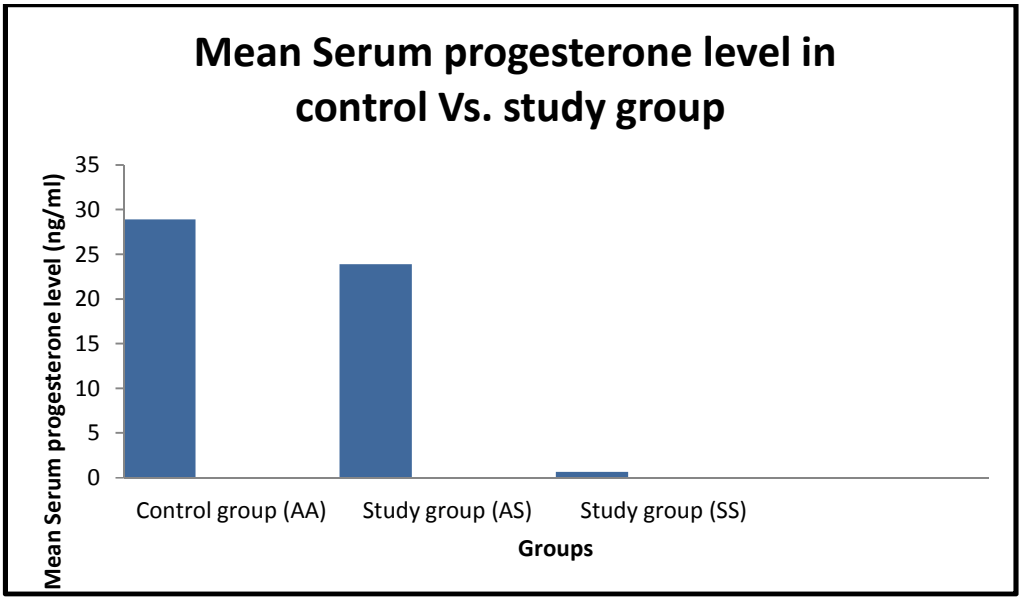

Figure 3: Mean Serum progesterone level in control vs. study group

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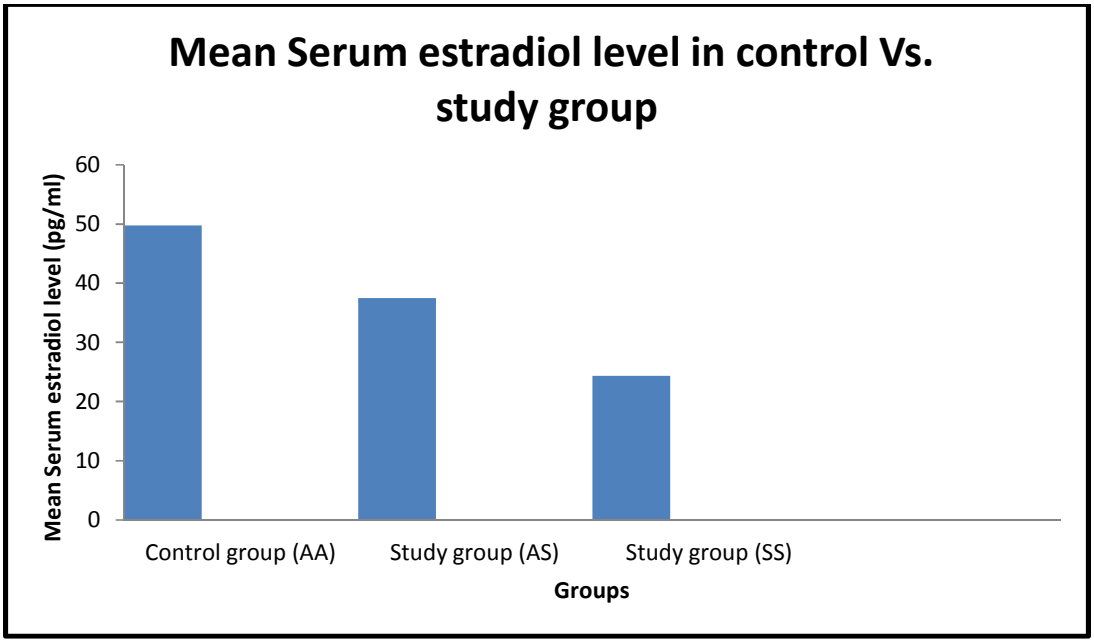

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360 **Figure 4: Mean Serum estradiol level in control Vs. study group**