Imbalance of Serum Estrogen and Progesterone concentrations in puberty age girls suffering from sickle cell anemia in tribal population in India

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 steroids (Estradiol and progesterone) alters in puberty age girls suffering from sickle cell anemia which causes complications such as hypogonadism which includes amenorrhea or delayed menarche etc. Enzyme linked immunosorbent Assay (ELISA) method was used to estimate the level of hormones . From the result the level of hormones showed a significant difference ($P<0.01$) in the three human haemoglobin electrophoretic patterns. Estradiol and progesterone level were found to be markedly decreased in sickle cell diseased 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 and hormonal level in sickle cell trait (AS) and normal individuals (AA), supporting the fact that the sickle cell trait lead normal life and seldom require treatment for their genetic condition By determining the level of these hormones in sickle cell patients we can avoid many complications associated with the Sickle cell disease (SCD) thus present study will help in prognosis of the disease in tribal individuals.

Key words:- Amenorrhea, SCD, Electrophoresis, Estradiol,Progesterone,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 to high frequency of 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 is 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 are 15.6 years .

Reasons for decreased growth are multi-factorial with contributions to abnormal endocrine function. Estradiol is a steroid hormone (molecular weight of 272.3daltons), which are secret principally by ovarian follicles and also by adrenals, corpus luteum and placenta and in males by testes estrogenic hormones are secret at varying rates during the menstrual cycle throughout period of ovarian activity[5,6,7].Progesterone is a steroid hormone, which plays an important role in the preparation and maintenance of pregnancy. it is synthesized from cholesterol via pregnenolone then rapidly rapidly metabolized to pregnanediol primarily in the liver the ovary and placenta is major sites of production but small amount is also produced by adrenal cortex in both men and women. Circulating progesterone levels, which are characteristically low in follicular phase and sharply increase in

luteal phase of menstrual cycle,reaching a maximum approximately 5 to 10 days after midcycle LH peak[8].

The level of these ovarian steroids 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 aims to 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 chemicals procured by standard analytical and preparative grade.

Study Population-

Blood samples were collected from subjects and volunteers (sickle cell patients) as puberty age girls 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 of 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 groups to 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 was sickle cell disease (SS) and rest 50 was sickle cell traits (AS).

Biochemical tests

Collection of sample:

All possible aseptic techniques were 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) was 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 to sickle cell haemoglobins deoxygenated (This precipitated to deform 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 minutes. 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 line.

Hb Electrophoresis:

Each of the major haemoglobin types have 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 to an electrical field. The components then move away from each other at different rates, and when separated, form a series of distinctly pigment bands. The bands are then compared with the other samples on the same membrane strip called as control[12].

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 to the biotin attached to the antibody and the streptavidin immobilized on the micro well 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 different serum references from 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 with 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. 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 at sickle cell disease (SS) girls, in sickle cell trait (AS) girls and control individuals (AA) And mean age of menarche of study group was 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, [4], 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 between 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 secret principally by ovary. Its levels have been found to be markedly decreased to 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.[4] reported that Hgb concentration was associated with height and weight in prepubertal Jamaican males, but not females. The finding of this study is that the

estradiol and progesterone levels were found to be markedly decreased to 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 to 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 between 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 normal life and seldom require treatment for their genetic condition.

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

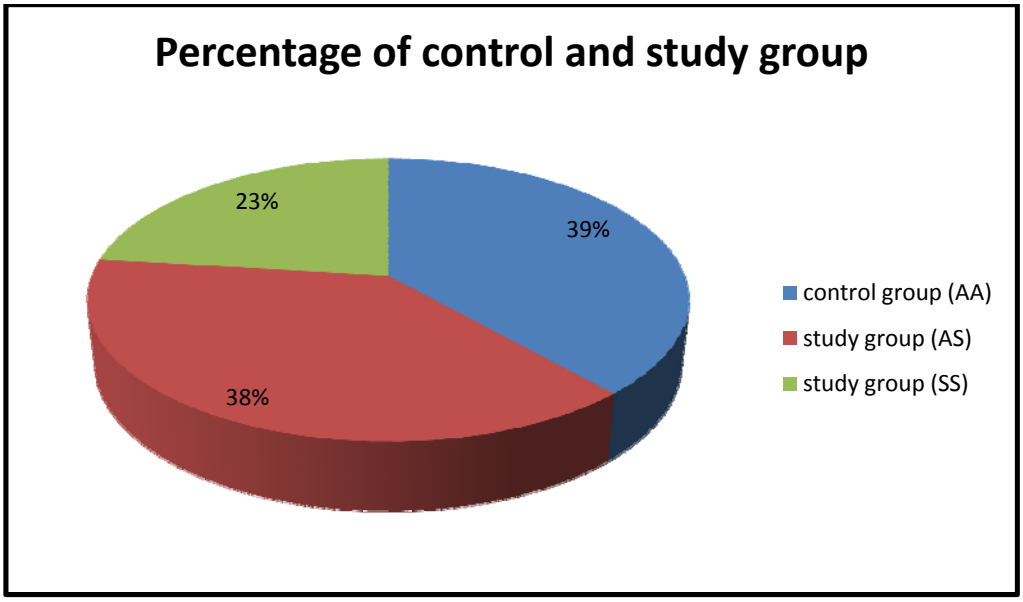

Figure 1: shows percentage of Control and Study group.

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

Group	No. Of cases	Mean age of menarche (years.)	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 was summarised by using mean and standard deviation. Age of menarche in control group was found to be 13 years. 13.08 years. In sickle cell trait patients (AS) and 15 years. In sickle cell diseased patients.

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

Age groups (years.)	13-14	15-16	17-18	19
No.of cases	16	13	9	12
Mean 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 (years.)	13-14	15-16	17-18	19
No. of cases	23	8	14	5
Mean 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 (years.)	13-14	15-16	17-18	19
No. Of cases	0	13	13	6
Mean 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 (years.)	13-14	15-16	17-18	19
No. Of cases	16	13	9	12
Mean 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 (years.)	13-14	15-16	17-18	19
No. Of cases	23	8	14	5
Mean 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 (years.)	13-14	15-16	17-18	19
No. Of cases	0	13	13	6
Mean 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		
Study group (AS)	50	23.88	2.084	<0.01	Significant
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		
Study group (AS)	50	37.49	1.940		
Study group (SS)	30	24.30	3.100	P<0.01	Significant

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.

The result and observation recorded in this study was done by using statistical model ANOVA (Analysis of Variance)

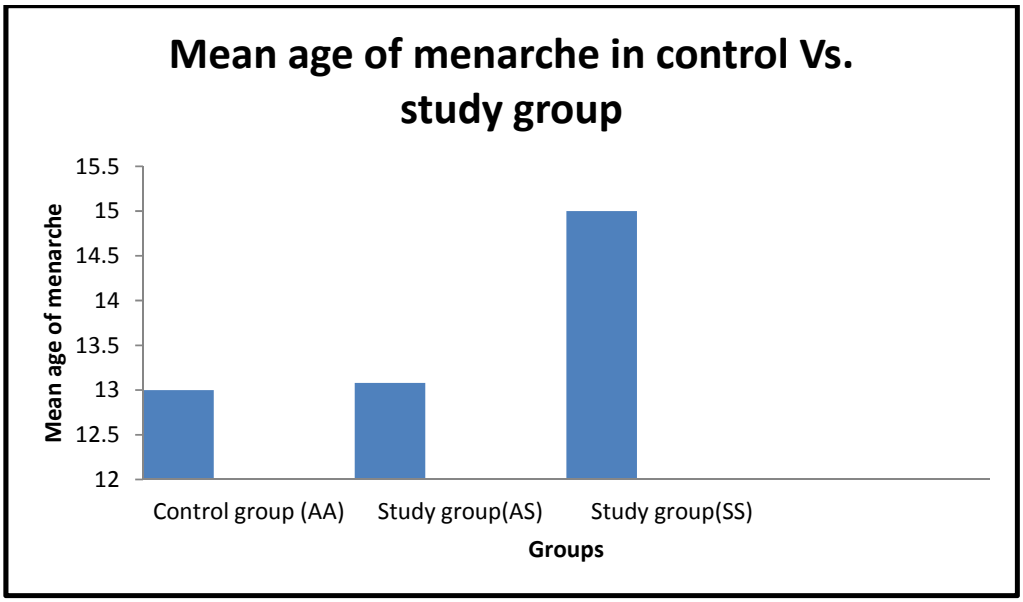

Figure 2: Mean age of menarche in control Vs. Study group.

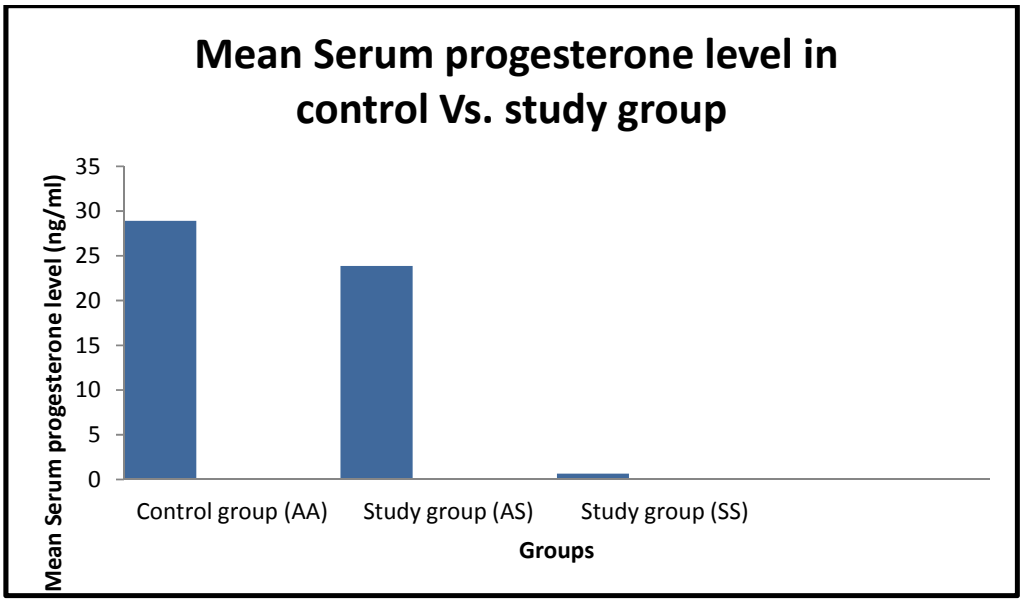

Figure 3: Mean Serum progesterone level in control vs. study group

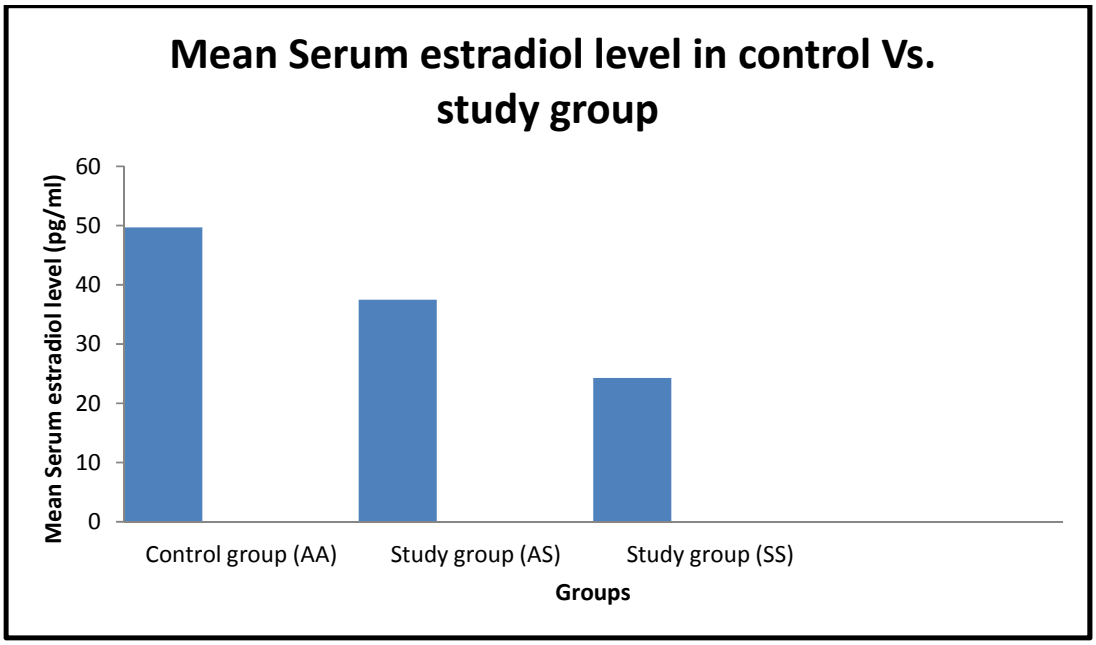

Figure 4: Mean Serum estradiol level in control Vs. study group

References:

1. Serjeant GR and Serjeant BE. Sickle Cell Disease New York(2001). Oxford University Press.

2.Bhatia HM and Rao VR. Genetic Atlas of the Indian Tribes(1987). Publish by Institute of Immunohaematology, (ICMR), Bombay, India.

3.Shamsuddeen, U., Mukhtar, M. D. and Maimuna, A. S. Prevalence of Sickle Cell Anaemia among patients attending Malam Aminu Kano Teaching Hospital (A.K.T.H.), Kano Nigeria. Department of biological sciences, Bayero university, Kango, Nigeria.

4. Zemel BS, Kawchak DA and Ohene-Frempong K. Effects of Delayed Pubertal Development, Nutritional Status, and Disease Severity on Longitudinal Patterns of Growth Failure in Children With Sickle Cell Disease. Pediatric Research. 61:607–613, 2007.

5. Balgir RS Age at menarche and first conception in sickle cell hemoglobinopathy. Indian Pediatr 31: 827–832, 1994.

6. Soliman AT. Growth and pubertal development in transfusion-dependent children and adolescents with thalassaemia major and sickle cell disease: a comparative study. J Trop Pediatr 45: 23–30, 1999.

7. Thomas PW. Height and weight reference curves for homozygous sickle cell disease. Arch Dis Child 82: 204–208, 2000.

8. Chatterjee R and Katz M. Reversible hypogonadotrophic hypogonadism in sexually infantile male thalassaemic patients with transfusional iron overload. Clin Endocrinol (Oxf) 53: 33–42, 2000.

9. El-Hazmi MA. Endocrine functions in sickle cell anaemia patients. J Trop Pediatr 38: 307–313, 1991.

10. Barden EM. Body composition in children with sickle cell disease. Am J Clin Nutr 76: 218–225, 2002.

11. Colah RB, Surve RR, Nadkarni AH, GorakshakarAC, Phanasgaonkar SP, Satoskar P, and Mohanty D. Prenatal diagnosis of sickle syndromes in India dilemmas in counseling. Prenat. Diag. Vol. 25.pp345-349, 2005.

12. Martin, H.and Steinberg. Disorders of hemoglobin: genetics, pathophysiology, and clinical management, The molecular cellular and genetic basis of hemoglobin disorders.25-30, 2001.

13. Nagel RL, Fabry ME and Pagnier J. Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type. N Engl J Med 312:880-4, 1985.

14. Thein SL, Lynch JR, Old JM and Weatherall DJ. Direct detection of haemoglobin E with Mnl I. J Med Genet, 24: 110-112, 1987.

15. Woodson RF. Statistical methods for the analysis of Biomedical data,Probability and Mathematical statistics. Wiley, Chichester. 1987;315 -318.

16. Winsor T and Burch GE. Habitus of patients with active sickle cell anemia of long duration. Arch Intern Med 76:47–53, 1945.

17. Jiminez CT, Scott RB and Henry WL. Studies in sickle cell anemia. XXVI. The effects of homozygous sickle cell disease on the onset of menarche, pregnancy, fertility, pubescent changes, and body growth in Negro subjects. Am J Dis Child 111:497–504, 1966.

18. Alleyne SI, D'Hereux Rauseo R and Serjeant GR. Sexual development and fertility of Jamaican female patients with homozygous sickle cell disease. Arch Intern Med 141:1295–1297, 1981.

19. Ashcroft MT and Serjeant GR. Growth, morbidity, and mortality in a cohort of Jamaican adolescents with homozygous sickle cell disease. W Ind Med J 30:197–201, 1981.

20 Graham C, Maude GH and Serjeant GR. Delayed menarche in homozyous sickle cell disease. W Ind Med J 35:18–22, 1986.

21. Platt OS, Rosenstock W and Espeland MA. Influence of sickle hemoglobinopathies on growth and development. N Engl J Med 311:7–12, 1984

22. Colah RB, Surve RR, Nadkarni AH, GorakshakarAC, Phanasgaonkar SP, Satoskar P, and Mohanty D. Prenatal diagnosis of sickle syndromes in India dilemmas in counseling. Prenat. Diag. Vol. 25.pp345-349, 2005.

23. Balgir RS. Genetic epidemiology o the three predominant abnormal hemoglobins in India. JAPI Vol. 44.pp25-28, 1996.

24. Martin, H.and Steinberg. Disorders of hemoglobin: genetics, pathophysiology, and clinical management, The molecular cellular and genetic basis of hemoglobin disorders.25-30, 2001.

25. Ramesh M, Kali TM and Veerraju P. Presence of sickle gene in four endogamous populations of North coastal Andhra Pradesh, South India. Ind J Multi. Res. Vol.2.pp11-16, 2006.

26. Patra Pradeep Kumar, Tripathi sumeet, Khodiar Prafulla, Sablania Praveen, Keshari JR and Dalla A.R. Epidemiology of Sickle cell disease in State of Chhattisgarh.Indian journal of public health research and development. 1:64-68, 2010.

27. Koch A.A, yang Q. and Olney R.S. Sickle Hemoglobin (HbS) Allele and Sickle cell disease: A Huge Review, American journal of Epidemiology,151: 839-845, 2000.

28. Antonarakis SE, Boehm CD, Serjeant GR, Theisen CE, Dover GJ and Kazazian HH. Origin of the βS-globin gene in Blacks: the contribution of recurrent mutation or gene conversion or both. Proc Natl Acad Sci USA 81:853-6, 1984.

29. Pagnier J, Mears JG and Dunda-Belkhodja O. Evidencefor the multicentric origin of the sickle cell haemoglobin gene in Africa. Proc Natl Acad Sci USA 81:1771-3, 1984.

30. Nagel RL, Fabry ME and Pagnier J. Hematologically and genetically distinct forms of sickle cell anemia in Africa. The Senegal type and the Benin type. N Engl J Med 312:880-4, 1985.

405
406 31. Kulozik AE, Wainscoat JS and Serjeant GR. Geographic survey of βS-globin gene
407 haplotypes: evidence for the independent Asian origin of the sickle-cell mutation. Am J Hum
408 Genet 39:239-44, 1986.

409 32.Lapouméroulie C, Dunda O and Ducrocq R. A novel sickle cell mutation of yet another
410 origin in Africa: theCameroon type. Hum Genet 89:333-7, 1992.

411 33. Zago MA, Silva Jr WA and Dalle B. Atypical βS haplotypes are generated by diverse
412 genetic mechanisms. AmJ Hemat 63:79-84, 2000.
413
414 34. Herrick JB. Peculiar elongated, Sickle shaped red blood corpuscles in a case of severe
415 anemia. Arch Intern Med, 6: 517, 1910.
416
417 35. Pauling L, Itano HA, Singer SJ and Wells IC. Sickle cell anaemia, a molecular disease.
418 Science, of Tamilnadu, ISHG, Indian Anthropological Society, Calcutta, 1949.
419
420 36. Itano HA. A third abnormal haemoglobin associated with hereditary haemolytic anaemia.
421 Proc Natl Acad Sci Washington, 37: 775-784, 1951.
422
423 37. Beale D and Lehmann H. Abnormal haemoglobins and the genetic code. Nature, 207:
424 259, 1965.
425
426 38. Bird GWG, Lehmann H and Mourant AE. A third example of haemoglobin D. Trans R
427 Soc Trop Med Hyg, 49: 399-400, 1995.
428
429 39. Zeng YT, Huang SZ, Ren ZR and Li HJ. Identification of HbD Punjab gene: Application
430 of DNA amplification in the study of abnormal haemoglobins. Am J Hum Genet, 44: 886-
431 889, 1989.
432
433 40. Thein SL, Lynch JR, Old JM and Weatherall DJ. Direct detection of haemoglobin E with
434 Mnl I. J Med Genet, 24: 110-112, 1987.
435 41. Bunn HF. Pathogenesis and treatment of sickle cell disease. N Engl J Med. 337(11):762–
436 769, 1997.

42. Leikin SL. Mortality in children and adolescents with sickle cell disease. Cooperative Study of Sickle Cell Disease. Pediatrics. 84:500–508, 1989.

43. Serjeant GR, Higgs DR and Hambleton IR. Elderly survivors with homozygous sickle cell disease. N Engl J Med. 2007; 356:642–643, 1989.

44. Mary Lieh-Lai, Katherine A Ling-McGeorge, Maria C Asi-Bautista. Lippincott Williams and Wilkins. Complications in sickle cell disease. Pediatric acute care. 01; pp. 88, 2001.

45. Powars D, Wilson B and Imbus C. The natural history of stroke in sickle cell disease. Am J Med. 65(3):461–471, 1978.

46. Sims NR and Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochimica et Biophysica Acta. 1802 (1): 80–91, 2009.

47. Donnan GA, Fisher M, Macleod M and Davis SM. Stroke. *Lancet* 371 (9624): 1612–23, 2008.

48. El-Hazmi MA, Warsy AS and Addar MH. Fetal haemoglobin level--effect of gender, age and haemoglobin disorders. Mol Cell Biochem. 29; 135(2):181-6, 1994.

49. Dunlop, K.J. and Mazumder, U.K. The occurrence of sickle cell anemaia group of tea garden labourers of upper Assam, Indian Med Gaz. 387-39, 1952.

50. Goldstein, A.R., Anderson, M.J. and Sejeant G.R. Parvovirus associated aplastic crisis in homozygous sickle cell disease, Arch Dis Child. 62: 585-588, 1987.

51. Ingram V. M. Dimethyl-amino end group method: a micro end-group method for peptides. Nature 166: 1038, 1950.

52. Ingram V. M. Dimethyl-amino end group method: a micro end-group method for peptides. Nature 166: 1038, 1950.

53. DeSimone J., Heller P., Hall L. and Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. Proc. Natl. Acad. Sci. USA 79: 4428–4431, 1982.

54. DeSimone J., Heller P., Hall L. and Zwiers D. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. Proc. Natl. Acad. Sci. 79: 4428–4431, 1982.

55. Winsor T and Burch GE. The habitus of patients with sickle cell anemia. Hum Biol 16:99–114, 1944.

56. Dawn Smiley, Samuel Dagogo, Jack and Guillermo umpierrez. Therapy insight: metabolic and endocrine disorders in sickle cell disease. Nature Clinical practice endocrinology and metabolism. 4:102-109, 2008.

57. Winsor T and Burch GE. Habitus of patients with active sickle cell anemia of long duration. Arch Intern Med 76:47–53, 1945.

58. Jiminez CT, Scott RB and Henry WL. Studies in sickle cell anemia. XXVI. The effects of homozygous sickle cell disease on the onset of menarche, pregnancy, fertility, pubescent changes, and body growth in Negro subjects. Am J Dis Child 111:497–504, 1966.

59. Alleyne SI, D'Hereux Rauseo R and Serjeant GR. Sexual development and fertility of Jamaican female patients with homozygous sickle cell disease. Arch Intern Med 141:1295–1297, 1981.

60. Ashcroft MT and Serjeant GR. Growth, morbidity, and mortality in a cohort of Jamaican adolescents with homozygous sickle cell disease. W Ind Med J 30:197–201, 1981.

61 Graham C, Maude GH and Serjeant GR. Delayed menarche in homozyous sickle cell disease. W Ind Med J 35:18–22, 1986.

62. Platt OS, Rosenstock W and Espeland MA. Influence of sickle hemoglobinopathies on growth and development. N Engl J Med 311:7–12, 1984.