Int J Clin Exp Pathol 2022;15(6):247-252
www.ijcep.com /ISSN:1936-2625/IJCEP0133072

Original Article

Serum testosterone level and semen quality in male patients with sickle cell disease in outpatient department of Chhattisgarh: a case-control study

Vinita Singh[4], Sabah Siddiqui[1], Nighat Hussain[2], Rachita Nanda[3], Amit Bugalia[2], Sarita Agrawal[4]

[1]Department of Medicine, AiimsRaipur, Chhattisgarh, India; [2]Department of Pathology, AiimsRaipur, Chhattisgarh, India; [3]Department of Biochemistry, AiimsRaipur, Chhattisgarh, India; [4]Department of Obstetrics and Gynecology, AiimsRaipur, Chhattisgarh, India

Received March 12, 2021; Accepted May 31, 2022; Epub June 15, 2022; Published June 30, 2022

Abstract: Introduction: Infertility issues in men with sickle cell disease (SCD) have been studied more frequently than those in women. Semen analysis of men with SCD often shows sperm abnormalities in up to 91%. No such study has been conducted in India so far, and Chhattisgarh being a state with a high incidence of male infertility as well as SCD, this study holds significance. Objectives: 1. To identify whether male patients attending All India Institute of Medical Sciences Outpatient Department with SCD have abnormal testosterone and/or poor semen quality. 2. Counseling of infertile male patients with SCD regarding future childbearing, prognosis, fertility preservation, and management options. Methods: This study was an age-matched case-control study; 58 participants of age between 18-45 years were assigned in each group. Results: The sperm count was higher in HbSS, while volume and pH were greater in HbAA. However, no significant difference ($P>0.05$) was found in total motility or progressive motility. A highly significant difference ($P<0.001$) was observed in pH, sperm count, total motility, and normal morphology. There was a significant difference ($P=0.005$) in volume. The values of the HbSS subjects were higher than the standard reference values. The values of the HbAA subjects were higher than the standard reference value. However, no significant difference ($P>0.05$) was found in sperm count or vitality. Conclusion: Men in Chhattisgarh with SCD do not suffer from any reproductive disorders such as delayed sexual maturity, low serum testosterone, poor semen quality,or hypogonadism.

Keywords: Infertility, anemia, sickle cell, semen analysis, androgen, hypogonadism

Introduction

Sickle cell disease (SCD) is an autosomal recessive genetic disorder marked by defective hemoglobin (Hb) synthesis, which leads to the production of abnormal sickle hemoglobin (HbS). The combination of a sickle β globin gene and a normal (HbAS) gene results in sickle cell trait, with no resultant anemia [1]. Infertility issues in men with SCD have been studied more frequently than those in women. Semen analysis of men with SCD often shows sperm abnormalities in as many as 91% [2]. Infertility in men with SCD can be due to hypogonadism, erectile dysfunction (ED), priapism, sperm abnormalities, or impaired sexual development [3, 4]. Sexual maturation is usually normal in men with SCD, but 24% of such men may develop hypogonadism, which can lead to insufficient testosterone production, infertility, ED, and poor libido [5, 6]. A study on children with SCD reported that gonadal function was depressed in the first decade of life, leading to delayed sexual maturation [6]. Another study also observed delayed sexual maturation in children with SCD [7]. Sperm abnormalities can be due to primary testicular failure marked by low levels of testosterone [8]. Gonadal dysfunction may result from the vaso-occlusion of hypothalamic-pituitary blood vessels, which leads to low serum gonadotropin (follicle stimulating hormone [FSH], luteinizing hormone [LH], and prolactin) and, consequently, low testosterone (secondary hypogonadism) as observed by Dada & Nduka. [9]. Vaso-occlusion of the testicular vessels may cause target organ failure

owing to low testosterone and high gonadotrophin (primary hypogonadism), resulting in a normal semen analysis report with normal testosterone, FSH, and LH levels [10, 11]. Vaso-occlusion may affect all levels of the hypothalamic-pituitary-testicular axis, producing a complex of pituitary-testicular failure. Low sperm density, low sperm count, poor motility, low ejaculate volume, and increased abnormal morphology occurred more frequently in men with SCD than in the controls [12, 13]. India has nearly 20 million people with SCD, which is highly prevalent in Central India, with predicted frequencies of up to 10% [14]. The highest frequency of sickle cell gene in India has been reported in Orissa, followed by Assam, MP, UP, Tamil Nadu, and Gujarat [15]. Central India is a focus of SCD, with average sickle cell gene frequency in the form of sickle cell trait found in 9.30% and SS phenotype (SCD) in 0.21% of the population of Chhattisgarh (Central India) [16]. No such study has been conducted in India so far, and those performed outside the country have come up with varied results. While medical advances are improving the quality of life of affected individuals, enhancing their rate of survival, and reducing the disease-related morbidity, reproduction-related issues among patients with SCD warrant attention. Although men with SCD can have multifactorial etiologies for their impaired fertility, such as sperm abnormalities, ED, hypogonadism, and the effect of therapy on sperm function, the focus of this research is to identify the correlation, if any, among SCD, serum testosterone level, and semen quality. This knowledge is necessary for better counseling, guidance, management, treatment, and monitoring of infertile men with SCD. Moreover, no such study has been performed in India so far, and Chhattisgarh being a state with a high incidence of male infertility as well as SCD, this study holds significance.

Objective

Primary Objective: To identify whether male patients attending All India Institute of Medical Sciences Outpatient Department with SCD have abnormal testosterone and/or poor semen quality.

Secondary Objective: Counseling of infertile male patients with SCD regarding future childbearing, prognosis, fertility preservation, and management options.

Methods

This study was an age-matched case-control study.

Fifty-eight participants in the age group of 18-45 years were recruited in each group using the following formula: sample size n = [Np (1-p)]/[(d2/Z21-α/2*(N-1) + p*(1-p)] (approx.) = 58 in each group.

Z1-α/2 = 1.96 at 95% confidence interval.

Population size (No. of sickle cell positive male patients reporting to the hospital in 1 year) *N (approximately)* = 100.

Prevalence of sickle cell gene in Central India [17] p=9.5%.

Confidence limits (*d*) = 5%.

The subjects were apparently healthy married or unmarried men with positive homozygous/heterozygous sickling test using sodium metabisulphite, who were in a stable state.

Controls werehealthy married or unmarried HbAA control groups.

Exclusion criteria for both cases and controls

- Participants with homozygous SCD who were not in a steady-state.

- Participants on particular drugs such as chemotherapeutic agents, anabolic steroids, cimetidine, spironolactone, phenytoin, sulfasalazine, or nitrofurantoin, which might affect the serum testosterone assay or semen quality.

- Participants with any apparent medical disorders such as diabetes mellitus, hypertension, and rheumatoid arthritis and those who had undergone surgical procedures that might directly or indirectly hamper the semen quality.

- Participants with a history of recent or chronic alcohol consumption and smoking.

- Participants with azoospermia.

Table 1. Comparison of semen characteristics between HbSS and HbAA

	Semen characteristics	HbSS subjects		HbAA subjects		t value	*P*-value
		Mean	SD	Mean	SD		
Physical examination	Volume, ml	1.90	1.05	2.68	1.13	-3.81	0.01*
	Reaction (pH)	8.06	0.45	8.26	0.46	-2.33	0.01*
Microscopic examination	Total sperm count	57.60	38.23	39.75	28.13	2.86	0.01*
	Total motility	58.73	25.99	62.12	26.47	-0.69	0.54
	Progressive motility	35.82	27.18	52.62	25.55	-3.42	0.35
	Vitality	59.32	28.40	62.12	26.47	-0.50	0.53
Morphology	Normal morphology	78.27	20.25	79.82	10.55	-0.45	0.07
	Abnormal morphology	16.55	9.23	20.17	10.55	-2.23	0.74

Independent t test. *P<0.05: significant difference.

Values tested and method used for testing

Sickling: Blood sample of 3 ml, collected in ethylenediaminetetraacetic acidvacutainer tube, was analyzed for a routine blood count using Sysmex XN 1000 Hematology analyzer. The same sample was examined for hemoglobin variant on a Bio-Rad D10 HPLC instrument as per the standard conditions specified by the manufacturer.

Semen analysis: The semen samples were collected in a private room near the pathology laboratory after a minimum of 2 days and a maximum of 7 days of sexual abstinence. The collected samples were analysed for volume, concentration, motility, vitality, and morphology and were reported as per the World Health Organization 2010 guidelines [18].

Serum testosterone: Serum testosterone analysis was performed by chemiluminescence two-step sandwich immunoassay on Advia Centaur XP. This method involved a competitive immunoassay using direct chemiluminescent technology. Testosterone present in the serum sample competes with acridinium ester-labeled testosterone in the Lite Reagent for a limited amount of polyclonal rabbit anti-testosterone antibody bound to monoclonal mouse anti-rabbit antibody, which is coupled to paramagnetic particles in the solid phase. The assay employs a testosterone releasing agent to release the bound testosterone from the endogenous binding proteins in the sample.

Statistical analysis: Statistical analysis was carried out using statistical packages for SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The continuous variables were expressed as mean ± standard deviation. An independent samples t-test was applied to compare age, testosterone, and semen characteristics between the two groups, namely, HbSS subjects and HbAA subjects. One-sample t-test was applied to compare the semen characteristics of HbSS and HbAA with the standard reference value. Two-sided *p* values were considered significant at P<0.05.

Results

Table 1 shows the presence of a significant difference in volume (p=0.02), pH (p=0.01), and total sperm count (p=0.01) of the semen between the two groups. The sperm count was higher in HbSS, while volume and pH were greater in HbAA. However, no significant difference (P>0.05) was found in total motility, progressive motility, vitality, normal morphology, and abnormal morphology of the semen.

Table 2 compares the semen characteristics of HbSS to the standard reference value. A highly significant difference (P<0.001) was observed in pH, sperm count, total motility, and normal morphology. There was a significant difference (P=0.005) in volume. The values of the HbSS subjects were higher than the standard reference value. Nonetheless, no significant difference (P>0.05) was seen in progressive motility and vitality.

Table 3 compares the semen characteristics of HbAA with the standard reference value. A highly significant difference (P<0.001) was perceived in volume, pH, total motility, progressive motility, and normal morphology. The values of the HbAA subjects were higher than the standard reference value. However, no significant

Table 2. Comparison of semen characteristics of HbSS with normal reference value

Semen characteristics	HbSS subjects		Reference value	t value	*P*-value
	Mean	SD			
Volume, ml	1.90	1.05	1.5	2.91	0.005*
pH	8.06	0.41	7.2	16.02	<0.001**
Sperm count	57.6	38.23	39	3.706	<0.001**
Total motility	58.73	25.99	40	5.44	<0.001**
Progressive motility	35.82	27.18	32	1.07	0.28
Vitality	59.32	28.40	58	0.41	0.68
Normal morphology	78.27	20.25	4	27.51	<0.001**

* not significant; ** Highly significant.

Table 3. Comparison of semen characteristics of HbAA men with normal reference values

Semen characteristic	HbAA subjects		Reference value	t value	*P*-value
	Mean	SD			
Volume, ml	2.68	1.13	1.5	7.95	<0.001**
pH	8.26	0.46	7.2	17.63	<0.001**
Sperm count	39.75	28.13	39	0.20	0.83
Total motility	62.12	26.47	40	6.65	<0.001**
Progressive motility	52.62	25.55	32	6.14	<0.001**
Vitality	62.12	26.47	58	1.18	0.24
Normal morphology	79.82	10.55	4	54.72	<0.001**

** Highly significant.

difference (P>0.05) was found in sperm count and vitality.

Discussion

The prevalence of sickle cell disease (SCD) in Chhattisgarh is about 9.5%-10.5%, affecting approximately 2.5 million individuals in the state. SCD is an inherited hematologic disorder; approximately 99.5% of the affected individuals have the sickle cell trait and 0.5% has SCD [19, 20]. The rate of childlessness in India is around 2.5% as per the National Family Health Survey-3 data [21]. Chhattisgarh is one of the most impoverished states in the country, with limited medical facilities and a high prevalence of illiteracy, ignorance, and superstition. A sound understanding of the effects of SCD is essential for better counseling and management of male infertility in this region [22].

Many studies have been performed on men with SCD, which have shown the presence of delayed sexual development, poor semen quality, hypogonadism, low serum testosterone level, and ED. These issues could be attributed to various proven and unproven causes [23, 24]. Less is known about the etiology and pathophysiologic mechanism of infertility in men with SCD. All studies conducted to date, though limited in number, have observed that men with SCD exhibit impaired fertility as their semen analysis values, including sperm count, ejaculate volume, sperm motility, sperm density, and normal sperm morphology were significantly impaired when compared with those of the controls. Besides, reduced serum testosterone concentration has been observed in patients seeking infertility treatment. Still, much less is known about the etiology and pathophysiologic mechanism of infertility in males with sickle cell disorder.

All studies to date, though limited in number, observed that male patients with SCD had impaired fertility as their semen analysis report comprising sperm count, ejaculate volume, sperm motility, sperm density, and normal sperm morphology showed impairment when compared to the controls along with reduced serum testosterone concentration in patients seeking infertility treatment [25].

In contrast to the results of the above-mentioned studies, there was no significant difference in mean age and testosterone level bet-

ween the two groups. Instead, the sperm count was higher in HbSS and volume and pH were greater in HbAA. No significant difference (P>0.05) was found in total motility, progressive motility, vitality, normal morphology, or abnormal morphology of the semen. When compared to the standard reference range, the values were significantly higher in both the groups, suggesting that SCD has not affected the semen quality of the men in Chhattisgarh. Testosterone level was also unaffected, and none of the men complained of ED or loss of libido. Furthermore, no patient with SCD exhibited delayed sexual maturity. The patients selected for this study were not receiving any long-term treatment for SCD. Hence, they could possibly have been spared from the cytotoxicity and other adverse effects of the drugs used for therapy.

Conclusion

Our study clearly shows that men in Chhattisgarh with SCD did not suffer from any reproductive disorders such as delayed sexual maturity, low serum testosterone, poor semen quality, or hypogonadism. However, further large-scale prospective studies are needed to identify the pathophysiologic mechanism of fertility impairment in men with SCD.

Acknowledgements

Sincere thanks to the patients who consented participated cooperated well to complete this project. Also sincere thanks to my colleagues and juniors who helped me in recruiting patients and record maintenance of data.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Vinita Singh, Department of Obstetrics and Gynecology, AIIMS, RAIPUR, TATIBANDH, G.E. ROAD, RAIPUR 492099, Chhattisgarh, India. Tel: 0771 4004317; +9185-18881708; E-mail: ddvinitasingh@gmail.com

References

[1] Ejindu VC, Hine AL, Mashayekhi M, Shorvon PJ and Misra RR. Musculoskeletal manifestations of sickle cell disease. Radiographics 2007; 27: 1005-1021.

[2] Berthaut I, Guignedoux G, Kirsch-Noir F, de Larouziere V, Ravel C, Bachir D, Galactéros F, Ancel PY, Kunstmann JM, Levy L, Jouannet P, Girot R and Mandelbaum J. Influence of sickle cell disease and treatment with hydroxyurea on sperm parameters and fertility of human males. Haematologica 2008; 93: 988-993.

[3] Luban NL, Leikin SL and August GA. Growth and development in sickle cell anemia. Preliminary report. Am J Pediatr Hematol Oncol 1982; 4: 61-65.

[4] Zemel BS, Kawchak DA, Ohene-Frempong K, Schall JI and Stallings VA. Effects of delayed pubertal development, nutritional status, and disease severity on longitudinal patterns of growth failure in children with sickle cell disease. Pediatr Res 2007; 61: 607-613.

[5] Taddesse A, Woldie IL, Khana P, Swerdlow PS, Chu JW, Abrams J and Abou-Samra AB. Hypogonadism in patients with sickle cell disease: central or peripheral? Acta Haematol 2012; 128: 65-68.

[6] Olambiwonnu NO, Penny R and Frasier SD. Sexual maturation in subjects with sickle cell anemia: studies of serum gonadotropin concentration, height, weight, and skeletal age. J Pediatr 1975; 87: 459-464.

[7] Ashcroft MT, Serjeant GR and Desai P. Height, weights and skeletal age of Jamaican adolescents with sickle cell anaemia. Arch Dis Child 1972; 47: 519-524.

[8] Friedman G, Freeman R, Bookchin R, Boyar R, Murthy G and Hellman L. Testicular function in sickle cell disease. Fertil Steril 1974; 25: 1018-1021.

[9] Dada OA and Nduka EU. Endocrine function and haemoglobinopathies: relation between the sickle cell gene and circulating plasma levels of testosterone luteinizing hormone (LH) and follicle stimulating hormone in adult males. Clin Chim Acta 1980; 105: 269-273.

[10] Abdulwaheed OO, Abdulrasaq AA, Sulaiman AK, Abdulgafar AJ and Munirdeen AI. The hormonal assessment of the infertile male in Ilorin, Nigeria. Afri J Endocrinol Metab 2002; 3: 62-64.

[11] Osegbe DN, Akinyanju O and Amaku EO. Fertility in males with sickle cell disease. Lancet 1981; 2: 275-276.

[12] Agbaraji VO, Scott RB, Leto S and Kingslow LW. Fertility studies in sickle cell disease: semen analysis in adult male patients. Int J Fertil 1988; 33: 347-352.

[13] Nahoum CR, Fontes EA and Freire FR. Semen analysis in sickle cell disease. Andrologia 1980; 12: 542-545.

[14] Hockham C, Bhatt S, Colah R, Mukherjee MB, Penman BS, Gupta S and Piel FB. The spatial epidemiology of sickle-cell anaemia in India. Sci Rep 2018; 8: 17685.

[15] Negi RS. Sickle cell trait in India. A review of known distribution, Bull. Anthropos Survey India 1972; 17: 439-449.

[16] Patra PK, Chauhan VS, Khodiar PK, Dalla AR and Serjeant GR. Screening for the sickle cell gene in Chhattisgarh state, India: an approach to a major public health problem. J Community Genet 2011; 2: 147-151.
[17] Colah RB, Mukherjee MB, Martin S and Ghosh K. Sickle cell disease in tribal populations in India. Indian J Med Res 2015; 141: 509-15.
[18] WHO laboratory manual for the Examination and processing of human semen, 5th edition.
[19] Panigrahi S, Patra PK and Khodiar PK. The screening and morbidity pattern of sickle cell anemia in chhattisgarh. Indian J Hematol Blood Transfus 2015; 31: 104-109.
[20] Mishra H and Neralwar A. Prevalence of sickle cell disease among school-age children in Chhattisgarh, India: predictions, implications and interventions. J Health Manage 2019; 21: 601-611.
[21] Ganguly S and Unisa S. Trends of infertility and childlessness in India: findings from NFHS data. Facts Views Vis Obgyn 2010; 2: 131-138.
[22] CENSUS 2011 Government of India Planning Commission July 2013.
[23] Modebe O and Ezeh UO. Effect of age on testicular function in adult males with sickle cell anemia. Fertil Steril 1995; 63: 907-912.
[24] Huang AW and Muneyyirci-Delale O. Reproductive endocrine issues in men with sickle cell anemia. Andrology 2017; 5: 679-690.
[25] Abudu EK, Akanmu SA, Soriyan OO, Akinbami AA, Adediran A, Adeyemo TA and Okany CC. Serum testosterone levels of HbSS (sickle cell disease) male subjects in Lagos, Nigeria. BMC Res Notes 2011; 4: 298.