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Assessment of Functional Ovarian Reserve in Homozygous Sickle Cell Patients in Kisangani

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Abstract

Introduction: Sickle cell disease is the most common autosomal recessive genetic haemoglobinopathy in the world. Progress in its management has significantly increased the life expectancy of patients with sickle cell disease, exposing them to age-related complications, including fertility problems. Assessment of ovarian reserve is a key test for estimating fertility potential in women with sickle cell disease. The aim of this study was to determine the ovarian reserve of women with sickle cell disease by measuring antimüllerian hormone and counting antral follicles and identify the associated factors. **Material and Methods:** This was a cross-sectional analytical study conducted from 5 April to 31 October 2024 in Kisangani, Democratic Republic of the Congo. It enrolled 268 women, categorised into four groups according to their haemoglobin status and the absence or presence of sickle cell crises: HbAA, asymptomatic HbAS (HbASa), symptomatic HbAS (HbASs) and HbSS. Participants with HbAA were grouped with HbASa to form the control group and those with HbSS were grouped with HbASs to form the patient groups. **Results:** The mean age at menarche was significantly later in the patients in the control group (P-value < 0.001), 15.5 years vs. 13 years. 5.1% of participants in the control group had low AMH levels compared with 22.5% of patients. 2.8% in the control group had

a low NFA compared with 53.9% among patients. 1.7% in the control group had a DOR, compared with 20.2% among patients. Ovarian reserve was associated with BMI (P-value = 0.008), number of vaso-occlusive crises (P-value = 0.013), number of haemolytic crises (P-value = 0.006) and Hb level (P-value = 0.009). **Conclusion:** Sickle cell disease leads to a decrease in ovarian reserve (ORR). This DOR is associated with low BMI, multiple vaso-occlusive and haemolytic crises, low haemoglobin levels and the use of non-steroidal anti-inflammatory drugs.

Keywords

Assessment, Ovarian Reserve, Homozygous Sickle Cell Disease

1. Introduction

Sickle cell disease (SCD) is an autosomal recessive genetic haemoglobinopathy caused by a point mutation in the β-globin gene, which results in the production of an abnormal haemoglobin (Hb), haemoglobin S (HbS). It includes haemoglobin SS (HbSS) syndromes and haemoglobin variants, such as haemoglobin SC (HbSC) and haemoglobin SE (HbSE) [1]. Sickle cell disease is a crucial public health problem worldwide; more than 300,000 children are born each year with the sickle cell trait, and around 75% of births take place in Sub-Saharan Africa [2].

The prevalence of sickle cell disease varies between 10% and 45% in various regions of Sub-Saharan Africa [3]. In Nigeria, an estimated 24% of the population has sickle cell anaemia, making it the second most affected country in the world after India, and the first in Africa [1]. The Democratic Republic of the Congo (DRC) is heavily affected, with a prevalence that ranks it second in Africa after Nigeria and third in the world [4]. Studies report around 1.5% of homozygotes and 20% - 40% of heterozygotes in the Congolese population [5]. In Kisangani, the neonatal prevalence of sickle cell trait varies between 18.3% and 23.3%, while that of the homozygous form is around 1% [4].

The symptoms of this condition are debilitating and polymorphous, characterised by chronic anaemia, increased susceptibility to infection, painful attacks due to poor tissue perfusion and irreversible complications associated with an infant mortality rate of 50% - 90% before the fifth birthday [6]. Progress in the management of sickle cell disease, with new therapies and the discovery of hydroxyurea, has significantly improved the daily lives of people with sickle cell disease and increased their expectancy. Worldwide, survival estimates have continued to improve, rising from a median survival of 42 to 48 years in 1994 to 58 years in 2014 [1]. As a result, the prevalence of sickle cell disease is increasing among adult women who are fit to give birth.

According to the World Database on the Epidemiology of Haemoglobinopathies, around 7% of pregnant women are carriers of haemoglobinopathies; more than 9 million adults are carriers of the sickle cell trait. Women with HbAS con-

ceive every year, and there are around 1.7 million pregnancies in carrier couples [7].

The increased life expectancy of homozygous sickle cell patients means that they are at risk of developing complications associated with advanced age, including fertility problems.

Studies carried out on women with sickle cell disease have shown that sickle cell disease is associated with a reduction in DOR compared with non-sickle cell women of the same age. In countries with limited resources, chronic inflammation, oxidative stress, haemochromatosis (iron overload) due to multiple transfusions, HU treatment, vaso-occlusive crises in the ovarian hilum and chronic anaemia causing ovarian ischaemia are the main factors affecting ovarian reserve in sickle cell women [8].

In well-equipped countries, apart from the above-mentioned factors which affect ovarian reserve in sickle cell patients, gene therapy and haematopoietic cell transplantation have a cure rate of around 95% [9]. However, preconditioning for haematopoietic cell transplantation requires extensive chemotherapy and radiotherapy, which destroys almost all the follicular cells. In China, a study of women who had undergone haematopoietic stem cell transplantation (HSCT) revealed a drop in pregnancy rates of less than 5% after HSCT. And among them, 70% to 100% of women who had undergone HSCT had premature ovarian failure [10]. A study conducted in the USA evaluating ovarian reserve in sickle cell patients undergoing HU revealed that all patients had DOR [8].

Another American study comparing serum levels of antimüllerian hormone (AMH) in sickle cell women, one group on HU and the other not on HU, showed a significant difference between the two groups, with a median AMH level of 0.87 ng/mL in the first group compared with 1.23 ng/mL in the second group [11]. A similar study in France found the same trend with a significantly lower median AMH level in the hydroxyurea (HC) group than in the non-HC group (1.31 vs. 2.47 ng/mL, respectively) [12]. In the UK, in a study on the assessment of RO in women with sickle cell disease, the mean AMH level in the SCD case group was 7.6 pmol/l compared with 13.4 pmol/l in the control group [13].

In Africa, few studies have been carried out on this subject. A study carried out in Nigeria concluded that homozygous sickle cell women had an ORD compared with others, with a mean ± standard deviation of the serum AMH level in women with HbSS of 3.64 ± 0.65 ng/mL compared with that in women with HbAA of 7.35 ± 1.19 ng/mL [1]. In the DRC, we did not have access to studies on the reproductive health of patients with sickle cell disease. The available data have generally focused on epidemiology [2] [14], neonatal screening [4]-[6], screening and complications of sickle cell disease in pregnancy [7] [15], complications and management of sickle cell disease [2] [4] [16]. We therefore initiated this study with the aim of assessing ovarian reserve in Congolese women with sickle cell disease, in order to plan maternity in the context of a country with limited resources.

An assessment of ovarian reserve would be useful to predict lifespan and fertil-

ity potential to plan motherhood in a sickle cell patient, in the context of a country with limited resources. Serum AMH and the number of antral follicles are considered the most reliable markers for assessing ovarian reserve [1] and were used in this study. We therefore initiated this study with the aim of assessing ovarian reserve in Congolese women with sickle cell disease to plan maternity in the context of a country with limited resources.

2. Materials and Methods

2.1. Study Framework

We conducted this study in two medical facilities specialising in gynaecology and obstetrics in the city of Kisangani, in the Democratic Republic of the Congo. These were the Cliniques Universitaires de Kisangani (CUKIS) and the Clinique des Anges de Kisangani (CAKIS).

2.2. Study Population and Sample

1) ***Study population***: Our study population consisted of women aged 18 to 35 who consented to participate in this study.

2) ***Sample***: Sampling in our study was voluntary non-probability, having recorded women aged 18 to 35 who had met the inclusion criteria. The sample size was calculated using Epi Info© 7.2.2.6 in its Stat Cal function. In order to compare the study factor (ovarian reserve) between female subjects with and without sickle cell disease, we allowed for a first order error of 5%, with an accuracy of 95%. The study sample consisted of 268 women. These women were categorised according to their haemoglobin status and according to the absence or presence of sickle cell crises, failing determination of the proportion of haemoglobin S in heterozygotes, into HbAA, asymptomatic HbAS (HbASa), symptomatic HbAS (HbASs) and HbSS, and then compared with each other.

In view of the common and comparable characteristics between the groups taken in pairs, the participants with HbAA were mixed with those with HbASa to form the control group. Those with HbSS were mixed with those with HbASs to form the patient group. Finally, the ovarian reserve was compared between two groups: a control group and a patient group.

- **Inclusion criteria**

For symptomatic homozygous and heterozygous sickle cell women:

- Any homozygous woman between 18 and 35 who presented to one of the health facilities selected during our study.
- Any heterozygous woman who was being monitored for sickle cell crises, with at least 3 vaso-occlusive and/or haemolytic crises in the last 12 months, and with a haemoglobin level of less than 10 g%.
- Any woman who has given written consent to take part in the study.

For the comparison group:

- Any asymptomatic non-sickle cell or heterozygous sickle cell woman aged 18 to 35 who presented to one of the health facilities during our study.

- Any woman who has given written consent to take part in the study。

- **Exclusion criteria**

All women in both groups were excluded from the study:

- Any woman under the age of 18 or over the age of 35.
- With a history of ovarian surgery.
- Never had sexual intercourse.
- With notion of taking combined oral contraceptives currently or in the previous 6 months or with notion of ovarian stimulation (induction ovulation) currently or in the 6 months preceding the study.
- With goitre or other thyroid disease.
- With menstrual and/or menstrual cycle disorders (ovulation disorders, cyclical pelvic pain, amenorrhoea, hypomenorrhoea, oligomenorrhoea, polymenorrhoea, spaniomenorrhoea, menorrhagia, metrorrhagia, etc.).
- With clinical hyperprolactinemia outside lactation.
- With ovarian pathology (PCOS).
- Pregnant or breast-feeding.
- Having refused to consent to the study.

2.3. Type and Period of Study

This was an analytical cross-sectional study conducted from 5 April to 31 October 2024.

2.4. Data Collection

2.4.1. Data Collection and Analysis

Data collection was prospective. Data were collected using a previously prepared data collection form, then entered and encoded on the Excel spreadsheet program (Microsoft, CDC, 2010) before being analysed using R software version 4.4.2 (2024-10-31 ucrt). The sample was described in terms of frequencies and proportions. Proportions were compared using the chi-square test of independence, Fischer's exact test at the $P < 0.05$ significance level. Quantitative variables were presented as means and standard deviations. Given the number of study arms, the means were compared by Student's t test at the $P < 0.05$ significance level.

2.4.2. Study Variables

1) ***Dependent variable***: Ovarian reserve AMH level and number antral follicles).

2) ***Independent variables***:

Socio-demographic and clinical characteristics: Age, physical address, level of education, occupation, marital status, parity and gestational age, history of abortions, age at menarche and characteristics of the menstrual cycle (length, duration, quantity and frequency of menses), medical, surgical and alcoholic-smoking history, history of hormonal contraception, desire to conceive, use of ovulation inducers and pelvic X-ray, weight and height. In addition, we determined the frequency of

vaso-occlusive crises and transfusions, haemoglobin levels, and treatment received or in progress in women with sickle cell disease.

3) ***Haemoglobin status***

2.4.3. Operational Definitions

- HbAA: Homozygous participant with adult haemoglobin (Hb) (A).
- HbASa: Heterozygous participant with adult haemoglobin (A) and sickle cell disease (S = sickle) and who has never presented with an acute or chronic complication linked to sickle cell disease (a = asymptomatic).
- HbASs: Heterozygous participants with adult (A) and sickle cell haemoglobin (S = sickle) who were being monitored for sickle cell crises, with at least 3 vaso-occlusive and/or haemolytic crises in the last 12 months, and with a haemoglobin level of less than 10 g% (s = symptomatic).
- HbSS: Homozygous participant with sickle-cell haemoglobin (Hb) (S = Sickle).
- Number of vaso-occlusive crises: Number hospitalisations for painful crises.
- Number of haemolytic crises: Number of hospital admissions for decompensated anaemia requiring transfusion.

Reserve marker values [17]**:**

- Low AMH: Less than 1.5 ng/ml.
- Normal AMH: Between 1.5 and 5 ng/ml.
- High AMH: Greater than 5 ng/ml.
- Low number of antral follicles (NFA): Less than 8 follicles for both ovaries.
- Normal AFN: 8 to 15 follicles in both ovaries.
- High NFA: More than follicles in both ovaries.
- Decreased ovarian reserve: This was defined for a participant with low AMH and NFA.
- Alcoholism: More than 14 glasses of beer (at 5%) per week or more than one glass per day.
- Smoking: More than 3 cigarettes a day.

2.4.4. Laboratory Investigations and Ultrasound Examination

We compared markers of ovarian reserve between the different subgroups. To do this, serum levels antimüllerian hormone (AMH) and endovaginal ultrasound for counting antral follicles (CFA) were measured. The ultrasound was performed between the third and fifth day of the menstrual cycle. In addition to the above-mentioned ovarian reserve tests, the haemoglobin status of the participants was determined. In homozygous and heterozygous sickle cell patients, we also measured haemoglobin levels. AMH was measured by immunofluorescence using a Fluocare® quantitative immunofluorescence analyser, model MF-T 1000, SN: MF-T 1000-21120200. Haemoglobin status was determined using a hemotypeSC rapid test kit, designed to determine the presence of haemoglobin A, S and C in whole blood. Haemoglobin levels were determined using an automatic digital haemoglobimeter, BioAid HB (serial number 12000045526), after taking a drop of capillary blood from the pulp of the index finger. Endovaginal ultrasound was performed

using an EDAN D3 ultrasound scanner, SN 317156-M 1661 1380005, 2016-06. Prior to folliculometry a pelvic ultrasound was performed to rule out ovarian pathology.

2.5. Ethical Considerations

Before carrying out this study, we sought and obtained authorisation from the ethics committee at the University of Kisangani.

Each participant enrolled in the study received a clear and detailed explanation of the study. Those who agreed signed an informed consent form (in French or the local language) in which all the information relating to the study was recorded. All the information collected from the women was kept confidential. Security measures concerning confidentiality were guaranteed by anonymity during the collection, processing and analysis of the results, by limited access to the data, and by the impossibility of identifying the subjects when the results of the study were published.

3. Results

3.1. Distribution of Participants According to Haemoglobin Status and Symptoms

Figure 1 shows the distribution of participants according to haemoglobin status and the presence of symptoms in heterozygotes (AS).

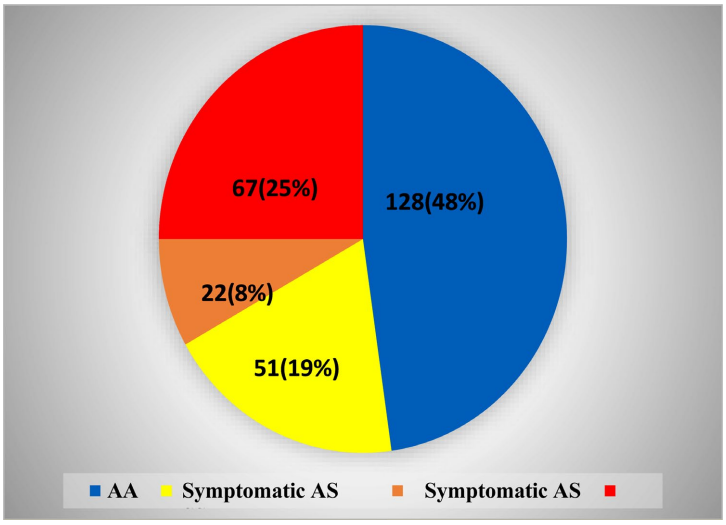

Figure 1. Distribution of participants by haemoglobin status and symptoms.

3.2. Comparison between Control Group Participants and Patients

The following table shows that there was a significant difference between the two groups in terms history of abortion, with a P-value of 0.022 (Table 1).

In the following table, there was a significant difference between the two groups in terms of age at menarche, family history of sickle cell anaemia, BMI and alcoholism (Table 2).

Table 1. Distribution of participants in the control group and patients according to socio-demographic characteristics and antecedents.

Variables	Control N = 179	Patients N = 89	P-value
Age (years)			
Average age	23.6 (3.7)	22.6 (4.2)	0.060
Age			0.5
18 - 24	132 (73.7%)	66 (74.2%)	
25 - 30	30 (16.8%)	18 (20.2%)	
31 - 35	17 (9.5%)	5 (5.6%)	
Profession			0.7
With employment	19 (10.6%)	11 (12.4%)	
Unemployed	160 (89.4%)	78 (87.6%)	
Parity			0.5
Nulliparous	158 (88.3%)	80 (89.9%)	
Paucipare	14 (7.8%)	8 (9.0%)	
Multipare	7 (3.9%)	1 (1.1%)	
Gestité			0.089
Nulligest	130 (72.6%)	76 (85.4%)	
Paucigeste	26 (14.5%)	9 (10.1%)	
Multigeste	22 (12.8%)	4 (4.5%)	
Abortion			**0.022**
No	144 (80.4%)	81 (91.0%)	
One to two	24 (13,4%)	8 (9.0%)	
Three to four	11 (6,2%)	0 (0%)	

Table 2. Distribution of participants in the control group and patients according to history, BMI and smoking habits.

Variables	Control N = 179	Patients N = 89	P-value
Age at menarche (years)			**<0.001**
10 - 16	176 (98.3%)	65 (73.0%)	
17 - 20	3 (1.7%)	24 (27.0%)	
Average age of menarche	**13**	**15.5**	**0.00**
Family history of sickle cell disease			**0.003**
No	158 (88.3%)	66 (74.2%)	
Yes	21 (11.7%)	23 (25.8%)	

Continued

BMI			**<0.001**
≤18.5	23 (12.8%)	61 (68.5%)	
18.6 - 25	118 (65.9%)	28 (31.5%)	
25.1 - 30	23 (12.8%)	0 (0%)	
30.1 - 40.1	15 (8.4%)	0 (0%)	
Average	22.8	17.7	
Alcoholism			**<0.001**
No	136 (76.0%)	87 (97.8%)	
Yes	43 (24.0%)	2 (2.2%)	
Smoking			0.2
No	174 (97.2%)	89 (100.0%)	
Yes	5 (2.8%)	0 (0%)	

3.3. Markers of Ovarian Reserve in Patients: Anti-Mullerian Hormone (AMH) Levels and Number of Antral Follicles (NFA)

3.3.1. AMH Levels and Associated Factors

The table below shows that AMH levels were significantly associated BMI (P-value = 0.005), the number of vaso-occlusive attacks (P-value = 0.041) and haemoglobin levels (P-value = 0.011) (Table 3).

Table 3. Distribution of ill participants according to AMH and associated factors.

Variables	AMH levels in sick participants			P-value
	Low N = 20	Normal N = 54	High N = 15	
Average age (years)	22.1 (4.0)	23.0 (4.6)	21.8 (2.6)	0.5
Age				0.2
18 - 24	17 (85.0%)	35(64.8%)	14 (93.3%)	
25 - 30	2 (10.0%)	15 (27.8%)	1 (6.7%)	
31 - 35	1 (5.0%)	4 (7.4%)	0 (0%)	
BMI				**0.005**
≤ 18.5	19 (95.0%)	23 (42.6%)	4 (26.7%)	
18.6 - 25	1 (5.0%)	31 (57.4%)	11 (73.3%)	
Age of menarche				0.10
10 - 16	11 (55.0%)	43 (79.6%)	11 (73.3%)	
17 - 20	9 (45.0%)	11 (20.4%)	4 (26.7%)	
Number of vaso-occlusive attacks/in the last 12 months				**0.041**
≤5	2 (10.0%)	21 (38.9%)	8 (53.3%)	

Continued

6 - 10	3 (15.0%)	15 (27.8%)	2 (13.3%)	
>10	15 (75.0%)	18 (33.4%)	5 (33.7%)	
Hb level (g%)				**0.011**
<7	14 (70.0%)	23 (42.6%)	3 (20.0%)	
7 - 9.6	6 (30.0%)	31 (57.4%)	12 (80.0%)	
Number of haemolytic attacks in the last 12 months				**0.008**
≤5	7 (38.9%)	32 (80.0%)	6 (60.0%)	
6 - 10	11 (61.1%)	8 (20.0%)	4 (40.0%)	
Daily folic acid treatment				**0.042**
No	11 (55.0%)	15 (27.8%)	7 (46.7%)	
Yes	9 (45.0%)	39 (72.2%)	8 (53.3%)	
Daily treatment with NSAIDs				**0.009**
No	4 (20.0%)	32 (59.3%)	6 (40.0%)	
Yes	16 (80.0%)	22 (40.7%)	9 (60.0%)	
Treatment with tramadol				0.7
No	12 (60.0%)	33 (61.1%)	11 (73.3%)	
Yes	8 (40.0%)	21 (38.9%)	4 (26.7%)	

Table 3 shows that AMH levels were significantly associated with the number of haemolytic attacks (P-value = 0.008), treatment with folic acid (P-value = 0.042) and non-steroidal anti-inflammatory drugs (P-value = 0.009).

3.3.2. Number of Antral Follicles (NFA) and Associated Factors

The number of antral follicles was significantly associated with BMI (P-value = 0.006), the number of vaso-occlusive attacks (P-value = 0.003) and Hb level (P-value = 0.006) (Table 4).

The number of antral follicles was significantly associated with the number of haemolytic attacks (P-value = 0.011) and folic acid treatment (P-value = 0.041) (Table 5).

3.3.3. Breakdown of Participants by AMH Rate and NFA

There was a statistically significant difference between the control group and the patients with to AMH level (P-value = 0.001) and NFA (P-value = 0.001) (Table 6).

3.4. Ovarian Reserve

3.4.1. Prevalence of Reduced Ovarian Reserve in Participants

Ovarian reserve in the patient group was significantly reduced compared with the control group (Table 7).

Table 4. Distribution of ill participants according to number antral follicles and associated factors.

Variables	Number of antral follicles		P-value
	Low N = 48	Normal N = 41	
Age groups (years)			0.3
18 - 24	38 (79.2%)	28 (68.3%)	
25 - 30	7 (14.6%)	11 (26.8%)	
31 - 35	3 (6.3%)	2 (4.9%)	
BMI (kg/m²)			**0.006**
≤18.5	39 (81.3%)	19 (46.3%)	
18.6 - 25	9 (18.8%)	22 (53.7%)	
Age at menarche (years)			0.7
10 - 16	36 (75.0%)	29 (70.7%)	
17 - 20	12 (25.0%)	12 (29.3%)	
Number of vaso-occlusive attacks in the last 12 months			**0.003**
≤5	11 (22.9%)	18 (43.9%)	
6 - 10	7 (14.6%)	13 (31.7%)	
>10	30 (62.5%)	10 (24.4%)	
Hb level (g%)			**0.006**
<7	28 (58.3%)	12 (29.3%)	
7 - 9.6	20 (41.7%)	29 (70.7%)	

Table 5. Distribution of diseased participants according to number of antral follicles and associated factors.

Variables	Number of antral follicles		P-value
	Low N = 48	Normal N = 41	
Number of haemolytic attacks in the last 12 months			**0.011**
≤5	23 (54.8%)	22 (84.6%)	
6 - 10	19 (45.2%)	4 (15.4%)	
Daily folic acid treatment			**0.041**
No	23 (47.9%)	11 (26.8%)	
Yes	25 (52.1%)	30 (73.2%)	
Daily treatment with NSAIDs			0.8
No	22 (45.8%)	20 (48.8%)	
Yes	26 (54.2%)	21 (51.2%)	

Continued

Daily treatment with tramadol			0.7
No	31 (64.6%)	25 (61.0%)	
Yes	17 (35.4%)	16 (39.0%)	

Table 6. Distribution of AMH and FA levels among participants.

Variables	Control N = 179	Patients N = 89	P-value
AMH			**<0.001**
Low	9 (5%)	20 (22.5%)	
Normal	71 (39.7%)	54 (60.7%)	
High	99 (55. 3%)	15 (16.9%)	
Mean (ng/ml)	4.4	2.72	**0.01**
NFA (for both eggs)			**<0.001**
Low	5 (2.8%)	48 (53.9%)	
High	7 (3.9%)	0 (0%)	
Normal	167 (93.3%)	41 (46.1%)	
Mean	9	5	**0.01**
Low AMH + low NFA (DOR)	3 (1.7 %)	18 (20.2%)	**<0.001**

Table 7. Prevalence of ovarian reserve among patients.

Ovarian reserve	Control N = 179 (%)	Patients N = 89 (%)	P-value
Decreased	3 (1.7)	18 (20.2)	**0.002**
Normal	176 (98.3)	71 (79.8)	

3.4.2. Distribution of Patients According to Ovarian Reserve and Associated Factors

The reduction in ovarian reserve was associated BMI (P-value = 0.008) and the number vaso-occlusive attacks (P-value = 0.013) (Table 8).

Table 8. Distribution of patients according to ovarian reserve and associated factors.

Variables	Reduction in ovarian reserves		P-value
	Yes N = 18	No N = 71	
Age bracket (years)			0.6
18 - 24	15 (83.3%)	51 (71.8%)	
25 - 30	2 (11.1%)	16 (22.5%)	

Continued

31 - 35	1 (5.6%)	4 (5.6%)	
BMI			**0.008**
≤18.5	17 (94.4%)	44 (62.0%)	
18.6 - 25	1 (5.6%)	27 (38.0%)	
Number of vaso-occlusive attacks in the last 12 months			**0.013**
≤5	2 (11.1%)	27 (38.0%)	
6 - 10	2 (11.1%)	18 (25.4%)	
>10	14 (77.8%)	26 (36.6%)	
Number of haemolytic attacks in the last 12 months			**0.006**
≤5	6 (37.5%)	39 (75.0%)	
6 - 10	10 (62.5%)	13 (25.0%)	
Missing	2	19	
Hb level			**0.009**
<7	13 (72.2%)	27 (38.0%)	
7 - 9.6	5 (27.8%)	44 (62.0%)	
Treatment folic acid			**0.090**
Yes	8 (44.4%)	47 (66.2%)	
No	10 (55.6%)	24 (33.8%)	
Treatment with ATBs			**0.7**
No	14 (77.8%)	58 (81.7%)	
Yes	4 (22.2%)	13 (18.3%)	

Table 8 shows that the decrease in ovarian reserve was associated with the number of haemolytic attacks (P-value = 0.006) and the Hb level (P-value = 0.009).

4. Discussion

4.1. Body Mass Index

In our study, most patients (HbSS and HbASs) had a low BMI (≤18.5 kg/m^2). Our results corroborate those of M'Pemba-Loufoua, who states that patients with sickle cell disease tend to have a low BMI because of the chronicity of the disease, which leads to delayed growth in height and weight [18].

4.2. Comparison between Participants

Our study showed that there was a statistically significant difference between the control group and the sickle cell group in terms of history of abortion, mean age at menarche and BMI.

A history abortion was more common in the control group than in the patients.

Although we did not determine the presence of a sexual partner or the frequency of sexual intercourse among the respondents, we think that the sickle-cell condition could confer a low sexual frequency on sickle-cell patients, which could explain the low rate of abortions among patients. In addition, given the average ages of the two groups, most of them being single and unemployed, we think that the majority of abortions could be induced, a risk that sickle cell sufferers would take less, compared with non-sickle cell sufferers.

The mean age of menarche in the sickle-cell group was 15.5 years, compared with 13 years in the control group, *i.e.* the sickle-cell group had an average delay of two and a half years. Our results were similar to those found by M'Pemba-Loufoua *et al.* in Brazzaville, who found that sickle cell patients had a delay in pubertal development of at least two years compared with non-sickle cell patients [18], those of Platt *et al.* in the United States [19], Oyedeji *et al.* in Nigeria [20] and Betoko *et al.* in Cameroon [21] who estimated a delay of 3 years.

The normal development of secondary sexual characteristics is the result of activation of the hypothalamo-pituitary-gonadal axis. However, this activation requires a sufficient supply of energy (leptin), determined by the abundance of adipose tissue and with an ideal weight (pondérostat) [22]. We think that the staturo-ponderal deficit in sickle-cell patients could therefore justify the delay in onset of menarche found in our study.

In our study, sickle cell patients had a lower average BMI than controls. The same trend was found by M'Pemba-Loufoua *et al.* in Brazzaville [18]. We believe that this could be due to chronic anaemia, resulting in increased diversion nutrients to active haematopoiesis, causing hypoproteinemia, which is detrimental to staturo-weight growth in sickle cell patients [23].

4.3. Markers of Ovarian Reserve in Patients: Anti-Mullerian Hormone (AMH) Levels and Number of Antral Follicles (NFA)

4.3.1. AMH Levels and Associated Factors

We observed that AMH levels significantly associated with BMI (P-value = 0.005), the number of vaso-occlusive (P-value = 0.041) and haemolytic (P-value = 0.008) attacks, haemoglobin levels (P-value = 0.011) and treatment with folic acid (P-value = 0.042) and non-steroidal anti-inflammatory drugs (P-value = 0.009). In terms of BMI, 95% of patients with low AMH levels compared with 42.6% with normal levels had a BMI less than or equal to 18.5 kg/m^2. Patients with more than 10 vaso-occlusive attacks accounted for 75% of patients with low AMH, compared with 33.7% with normal AMH; the same trend was observed for haemolytic attacks. 70% of patients with low AMH compared with 42.6% with normal AMH had Hb level of less than 7 g%. 72.2% of patients with normal AMH were on folic acid compared with 45% with low levels. For patients with NSAIDs, 80% had a low AMH level, compared with 40% with a normal level.

A normal BMI is an expression of good nutritional status, suggesting an energy reserve of lipids available for metabolism. At ovarian level, lipids drive follicular growth by making energy substrates available after β-oxidation, for meiosis and

cytoplasmic maturation of follicular cells. This could justify the relationship between BMI and AMH levels in our study [24].

Our study showed that patients who had more vaso-occlusive and haemolytic attacks had low AMH levels. Our results corroborate the argument put forward by Chase *et al.* in a clinical case report, proposing that frequent episodes intravascular sickle cell disease, vascular occlusion and infarction, as well as tissue hypoxia associated with chronic anaemia, could explain the ovarian dysgenesis, which could justify the low AMH levels in our series [25].

Haemosiderosis induced by multiple haemolytic crises can affect oocyte quality through the accumulation of reactive oxygen species (free radicals) generated by free iron, creating increased oxidative stress. As, the window of the iron concentration range in biological systems is narrow for optimal beneficial effects with minimised adverse consequences [26]. There is a delicate balance between reactive oxygen species and antioxidant enzymes in ovarian tissues. Antioxidant enzymes neutralise the production of reactive oxygen species and protect the follicles against ageing. The granulosa and luteal cells respond to hydrogen peroxide with a loss of gonadotropin action and inhibition of progesterone secretion [27]. We believe that this oxidative stress, associated with a chronic inflammatory state in sickle cell disease, could be the reason for impaired ovarian reserve in sickle cell disease. Chronic anaemia in sickle cell disease leads to hypoxaemia, resulting in ovarian hypoxia, which could justify premature ovarian dysgenesis and failure manifested by a decrease in AMH levels in sickle cell disease patients [25].

Our study showed that most patients with low AMH levels were taking NSAIDs. Although the effects of NSAIDs on ovulation inhibition are well described in the literature [28] [29], we have not been able to access any literature determining the effects of NSAIDs on ovarian reserve. Nevertheless, as NSAIDs inhibit the production of prostaglandins, we believe that inhibition of the latter would deprive the ovary of the beneficial effect of prostaglandins on the ovarian vascular system, namely vasodilatation and inhibition of platelet aggregation [30]. This will result in a reduction in ovarian blood flow, which may justify ovarian dysgenesis.

In our study, we found that most women with normal AMH levels were taking folic acid. In fact, a Polish cohort study had revealed that women who had received folic acid supplementation prior to infertility treatment by medically assisted reproduction (MAP) had better quality follicles and a higher degree of mature follicles than women who had not folic acid [31]. We therefore believe that this improvement in follicle quality could be the reason for normal AMH levels in some women with sickle cell disease.

4.3.2. Number of Antral Follicles (NFA) and Associated Factors

We observed that the number of antral follicles was significantly associated with BMI (P-value = 0.006), the number of vaso-occlusive attacks (P-value = 0.003), the Hb level (P-value = 0.006), the number of haemolytic attacks (P-value = 0.011) and folic acid treatment (P-value = 0.041). The number of antral follicles (ANF) tended to improve as BMI and haemoglobin improved, with a reduction in vaso-occlusive

and haemolytic attacks. We believe that this would be justified in the same way as the AMH level.

4.3.3. Distribution of Participants According to AMH Level and NFA Ovarian Reserve

In our series, there was a statistically significant difference between the control group and the patients with regard to AMH level (P-value < 0.001) and NFA (P-value < 0.001). There were 5.1% in the control group with low AMH compared with 22.5% in the patients. 2.8% in the control group had a low NFA compared with 53.9% among patients.

Taking AMH and NFA together, thus defining ovarian reserve in our series, decreased ovarian reserve (DOR) was found in 1.7% of the control group, compared with 20.2% of patients. This difference was statistically significant (P-value < 0.001). In patients, ovarian reserve was associated with MC (P-value = 0.008), the number of vaso-occlusive crises (P-value = 0.013), the number of haemolytic crises (P-value = 0.006) and the Hb level (P-value = 0.009). 94.4% of patients with reduced ovarian reserve versus 62% of patients without reduced ovarian reserve had a BMI ≤ 18.5 kg/m^2 and the trend was the opposite with a BMI greater than 18.5 kg/m^2. 11.1% of patients with reduced ovarian reserve had fewer than 5 vaso-occlusive attacks, compared with 38% without reduced ovarian reserve, and with more than 10 vaso-occlusive attacks, 77.8% of patients with reduced ovarian reserve had more than 10, compared with 36.6% of patients without reduced ovarian reserve.

The same trend was observed with haemolytic crises. In terms of Hb levels, the majority of patients with a reduced ovarian reserve (72.2%) had Hb below 7, compared with 38% without a reduced ovarian reserve.

These results corroborate those found in the literature, indicating that there were more sickle cell women with low ovarian reserve compared with non-sickle cell women of the same age [1] [8] [11] [13]. This can be explained by the pathophysiology of sickle cell disease and its management as described above (vaso-occlusive and haemolytic crises, haemosiderosis, chronic anaemia and the use of non-steroidal anti-inflammatory drugs).

5. Conclusions

This study was carried out in a country with a high prevalence of sickle cell disease (third in the world after India and Nigeria), where the proportion of women with sickle cell disease is constantly increasing. The aim was to elucidate one aspect of the reproductive health of women with sickle cell disease, namely ovarian reserve, and determine the factors associated with impaired ovarian reserve in homozygous sickle cell patients.

This study showed that sickle cell disease leads to a reduction in ovarian reserve. There are more cases of reduced ovarian reserve among women with sickle cell disease of childbearing age compared with those in the control group. Several factors are linked to this reduction in ovarian reserve in sickle cell patients, including:

- Low body mass index.

- Multiple vaso-occlusive and haemolytic attacks.
- Low haemoglobin levels.
- Taking non-steroidal anti-inflammatory drugs.

Whereas folic acid has a positive effect on markers of ovarian reserve.

6. Outlook

- To carry out a study on the rate of conception and the progress of pregnancy among people with sickle cell disease in our community.
- To determine the relationship between the proportion of foetal haemoglobin (in homozygotes) and haemoglobin S (in heterozygotes) and ovarian reserve.
- To study the pubertal development in homozygous sickle cell patients in our environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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