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London Journals Press

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ISBN: 978-1-7340132-1-4

LONDON JOURNAL OF MEDICAL AND HEALTH RESEARCH

Volume 21 | Issue 2 | Compilation 1.0

COMPILED IN UNITED KINGDOM

ENGLISH

London
Journals Press

LONDON JOURNAL OF MEDICAL AND HEALTH RESEARCH

Volume 21 | Issue 2 | Compilation 1.0

PUBLISHER

London Journals Press
1210th, Waterside Dr,Opposite Arlington Building, Theale, Reading
Phone:+444 0118 965 4033 Pin: RG7-4TY United Kingdom

SUBSCRIPTION

Frequency: Quarterly

Print subscription
$280USD for 1 year
$500USD for 2 year
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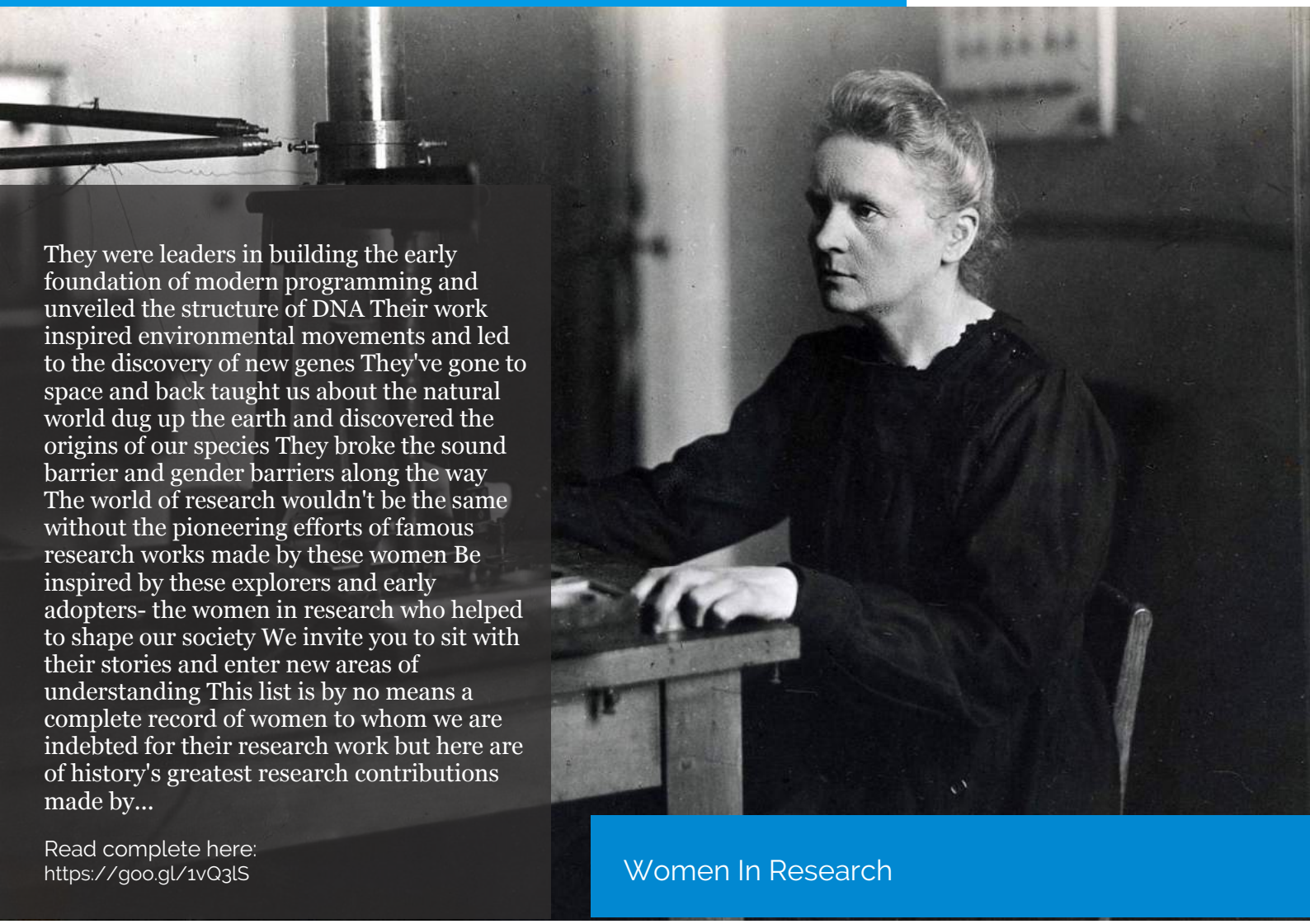

They were leaders in building the early foundation of modern programming and unveiled the structure of DNA Their work inspired environmental movements and led to the discovery of new genes They've gone to space and back taught us about the natural world dug up the earth and discovered the origins of our species They broke the sound barrier and gender barriers along the way The world of research wouldn't be the same without the pioneering efforts of famous research works made by these women Be inspired by these explorers and early adopters- the women in research who helped to shape our society We invite you to sit with their stories and enter new areas of understanding This list is by no means a complete record of women to whom we are indebted for their research work but here are of history's greatest research contributions made by...

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Molecular Classification of Breast Cancer in Malaysia

Nor Akmar Tak, Maalini Krishnasamy, Subasri Armon, Fazilah Hassan, Noorjehan Omar, Nurhidayah Hassan, Nik Raihan & Nurakmal Baharum

Negeri Sembilan

ABSTRACT

Objectives: This study is conducted to determine the distribution of various breast cancer molecular subtypes using immunohistochemical (IHC) analysis in a cohort of the Malaysian population, and their association with clinicopathologic parameters.

Methods: It is a retrospective study between June 2017 and December 2017 at 18 tertiary hospitals under the Ministry of Health Malaysia (MOH). A total of 368 cases of primary breast cancer in females are classified into six major molecular subtypes according to the IHC surrogate of molecular classification proposed by the 11th St. Gallen International Breast Cancer Conference Expert Panel: Luminal A (ER+/PR+/HER2-/Ki-67<14%); Luminal B HER2-Negative (ER+/PR+ /HER2-/Ki-67≥14%); Luminal B HER2-Positive (ER+/PR+/HER2+/Any Ki-67); HER2-Enriched (ER-/PR-/HER2+), Triple-negative basal-like (ER-/PR-/HER2-/CK5/6+) and Triple-negative non-basal-like (ER-/PR-/HER2-/CK5/6-). Chi- squared test is performed to evaluate the relationship between these subtypes and clinicopathological features.

Keywords: malaysia, breast cancer, molecular classification, histological subtype.

Classification: NLMC CODE: WP 840, QZ 20.5

Language: English

London Journals Press

LJP Copyright ID: 392851

London Journal of Medical and Health Research

Volume 21 | Issue 2 | Compilation 1.0

Molecular Classification of Breast Cancer in Malaysia

Nor Akmar Tak[1], Maalini Krishnasamy[2], Subasri Armon[3], Fazilah Hassan[4], Noorjehan Omar[5], Nurhidayah Hassan[6], Nik Raihan[7] & Nurakmal Baharum[8]

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Objectives: This study is conducted to determine the distribution of various breast cancer molecular subtypes using immunohistochemical (IHC) analysis in a cohort of the Malaysian population, and their association with clinicopathologic parameters.

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Results: The mean age at the time of diagnosis was 55.5 years and with patients being predominantly Malay ethnic (63.0%), followed by Chinese (23.1%), Indian (10.9%) ,and natives (2.2%). Luminal A (58.4%) was the most prevalent tumor subtype, followed by Triple -negative basal-like (13.3%), HER2-Enriched (12%), Luminal B HER2-Negative (9.5%), Triple-negative non-basal -like (5.5%) and Luminal B HER2-Positive (1.1%). Eighty percent of the patients presented with a tumor larger than 2cm in size ,and about 60% had lymph node involvement. Out of all cases, 85% were Grade 2 and Grade 3 tumors. 82.8% of Luminal A tumors were presented as Grade 1 tumor. Histological subtypes also show a statistically significant correlation with molecular subtypes. More than half of invasive carcinoma of no special type (NST), invasive lobular carcinoma, solid papillary carcinoma, cribriform carcinoma, invasive papillary carcinoma and most of mucinous carcinoma were of Luminal A subtype. Majority of metaplastic carcinoma and carcinoma with medullary features on the other hand, belonged to HER2- Enriched and Triple-negative subtypes. Triple-negative and HER2 Enriched tumours were significantly associated with women of Malay ethnicity seen in (n=34/232, 14.7%), as well as higher grade (n=33.141, 23.4%) and histologically more aggressive subtypes (carcinoma with medullary features 46.1% and metaplastic carcinoma 66.7%, respectively). Conclusions: Luminal A tumor was the most prevalent molecular subtype while Luminal B HER2-positive was the least. Most of the luminal A tumors were grade 1 tumors with less aggressive tumor morphology. Triple-negative and HER2 Enriched tumors were significantly associated with women of Malay ethnicity, as well as higher grade and histologically more aggressive subtypes.

Keywords: malaysia, breast cancer, molecular classification, histological subtype.

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I. INTRODUCTION

Breast cancer is one of the most common cancers and is the leading cause of cancer-related mortality among women worldwide. In Malaysia, a total of 21,634 cases of female breast cancer were diagnosed for the period of 2012-2016 compared with 18,206 cases in 2007-2011, accounting for nearly 19% increment of new cases [1]. Data from the National Cancer Registry of Malaysia 2012-2016 show that an age-standardised incidence rate (ASR) had increased from 31.1 in the previous reports to 34.1 per 100,000 population. The incidence was highest among the Chinese, followed by Indians and Malays [1]. Furthermore, deaths due to breast cancer in Malaysia showed an increase of 0.6% from 3.8% in 2016 to 4.4% in 2017 [2]. National Cancer Registry 2012-2016 reported that the incidence increased after 25 years old and the peak age was 60 to 64 years, and reduced after 65 years of age [1].

Breast cancer represents a heterogeneous group of tumors, consisting of various morphological features, clinical behaviors, and systemic therapy [3]. Currently used traditional classification systems based on histomorphological features, tumor-grade and stage alone are insufficient to reflect the clinical diversity of breast cancers [4]. In recent years, newer molecular methods have shown that histomorphologically similar breast carcinoma may show molecular heterogeneity with different patterns of gene expression, leading to different clinical outcomes and their responses to cancer treatment [5]. Since then, many investigations are conducted to characterize and revise the classification of breast cancer at the molecular level to customize treatment according to the current standard practice of targeted therapy.

In the year 2000, global gene expression profiling (GEP) using complementary DNA microarrays, pioneered by Perou and colleagues, had categorized breast cancer based on intrinsic genes into five major molecular subtypes: Luminal A, Luminal B, normal breast-like, HER2-Enriched and Basal-like, with various clinical outcome and responses to neoadjuvant therapy [6] [7]. Nevertheless, the use of GEP techniques for the purpose of clinical classification of breast cancers is not readily available in most diagnostic centers due to the cost and technical difficulties involved [8]. Although immunohistochemistry (IHC)-based molecular classification is not equivalent to intrinsic subtypes as defined by GEP; several studies have shown that this method, which is more feasible and widely available in clinical practice, can be used to sub classify breast cancer comparable to those defined by GEP [9].

According to the IHC surrogate of molecular classification proposed by 11th St. Gallen International Breast Cancer Conference Expert Panel, breast cancer can be divided into five molecular subtypes based on presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2)/neu on tumor cells, as well as and Ki-67 proliferative index. The five subtypes are as follows; i)Luminal A tumors are ER-positive and/or PR-positive, HER2-negative and with Ki-67<14%, ii) Luminal B tumors are also ER-positive and/or PR-positive but are further sub classified into Luminal B HER2-negative with Ki - 67≥14% and Luminal B HER2-positive with any Ki-67 value [4][5], iii) HER2 over - expression of HER2 - Enriched are those that are ER-negative, PR-negative, and HER2-positive, iv) Triple - negative basal - like subtype is characterized by negativity for ER, PR, and HER2 but positive for CK5/6 or EGFR, and v) Triple- negative non-basal-like subtype tumors are negative for ER, PR and HER2, as well as CK5/6 and EGFR [10] [11].

To our knowledge, there is limited data available on the molecular classification of breast cancer in Malaysia. In the present study, we aimed to determine the frequency of the molecular subtypes of breast carcinoma in a cohort of the Malaysian population and to evaluate their association with various clinicopathological features, which include age, ethnicity, tumor size, tumor grade, lymph node status, and histological subtypes.

II. MATERIAL AND METHODS

2.1 Data collection

This is a cross-sectional retrospective study involving Malaysian women with newly diagnosed primary breast carcinoma and had undergone either mastectomy or wide local excision with axillary resection, within the period of 1st June 2017 to 31st December 2017 in 18 tertiary hospitals under the Ministry of Health, Malaysia (MOH). All specimens are from the respective in-house histopathology laboratories. This study is conducted with prior approval from the Malaysian Research Ethics Committee (MREC). The inclusion criteria for this study were as follows: (a) Malaysian women with primary breast carcinoma; (b) Availability of data on patient demography and relevant histopathologic parameters from histopathology reports (i.e., tumor size, histological type, tumor grade, lymph node status, and IHC profile for ER, PR and HER2; (c) Availability of formalin-fixed paraffin-embedded (FFPE) tumor tissue that was in good quality. Male patients, cases that had undergone neoadjuvant therapy before surgery, as well as recurrent tumor cases are excluded from this study.

The tumor size is grouped into three categories: ≤2cm, >2 but ≤5cm, and >5cm. The tumor histological grade is based on the Modified Bloom and Richardson grading system, in which it is generally characterized by percentage of tubular differentiation, degree of nuclear pleomorphism and number of mitosis [12]. The status of lymph node metastasis is determined by evaluating axillary lymph nodes that are submitted together with the mastectomy or wide local excision specimens. Histological-types are based on WHO Classification of Tumor of the Breast, 2011 [12]. The ER, PR, and HER2 tests are scored according to The Guidelines of the College of American Pathologists. ER and PR immunostains are considered positive when ≥ 1% of the tumor cells showed nuclear staining [13]. HER2 test is scored from 0 to 3 i.e., 0 or 1+ (negative), 2+ (equivocal), and 3+(positive). A 3+ score is given only when there is intense full circumferential cytoplasmic membrane staining in more than 10% of invasive malignant cells [14]. All cases with equivocal HER2 immunohistochemical results are subjected to dual-color dual-hapten in-situ hybridization (DDISH) confirmatory test. Additional IHC staining for Ki-67 and CK5/6 is performed on FFPE tissue from primary tumor based on the ER, PR, and HER2 status. The Ki-67 index is determined from the percentage of positivity in 500 tumor cells in a hot-spot area (i.e., area with a dense concentration of positive tumor nuclei [15]). CK5/6 positivity is interpreted as any cytoplasmic or membranous staining with perinuclear enhancement of tumor cells [16].

The histological diagnosis, tumor grading, and hormonal receptor and HER2 status, assessments were independently done by at least 2 MOH-credentialed histopathologists. We then classified the breast cancer cases into six molecular subtypes, i.e., Luminal A, Luminal B HER2- negative, Luminal B HER2-positive, HER2-Enriched, Triple-negative basal-like, and Triple-negative non-basal-like.

2.2 Statistical analysis

The analyses are performed using the IBM SPSS Statistics for Windows Version 21.0. In this study, descriptive statistics were used where categorical variables were presented in frequency and percentage, while normally distributed numerical data is presented in mean and standard deviation. Otherwise, data can be shown in median and interquartile range. Fisher's exact test is used to study the association between molecular subtypes and clinico-pathological parameters. A one-way ANOVA test is performed to study the comparison between age and molecular subtypes. All probability values are two-sided, and a level of

significance of less than 0.05 (p-value < 0.05) is considered statistically significant.

2.3 Ethical consideration

Patients' data remained anonymous, and each subject are coded accordingly. These data were kept in a password-protected database and linked only with a study identification number for this research. Moreover, only the Principal Investigator had access to patients' records. The study protocol had been reviewed and approved by the Medical Research Ethics Committee (MREC; Reference Number NMRR-18-3326-449 08).

III. RESULTS

Included in this study are a total of 368 cases of primary breast cancer cases in Malaysian women from 18 MOH hospitals within the study periods fit into the inclusion criteria. Among these surgically treated cases, 311 (84.5%) underwent a mastectomy, and the remaining 57 cases (15.5%) had wide local excision.

ER and PR were positive in 68.2% and 60% of the cases, respectively. HER2 was positive in 15.8% and equivocal in 10.9% of the cases. Among the equivocal cases, 27.5% confirmed to be HER2-positive by DDISH test. The distribution of clinical and pathological characteristics among the molecular subtypes of breast cancer are presented in Table 1.

The mean age at diagnosis was 55.5 ± 11.42 years. No significant association is found between age and molecular subtypes. About 63.0% of the cases were of ethnic Malay, 23.1% were Chinese, 10.9% were Indian, while Sabah and Sarawak Natives represented 2.2% of the cases. We identified a strong association between ethnicity and molecular subtype (p=0.013). Triple-negative and HER2 Enriched tumours were significantly associated with women of Malay ethnicity seen in (n=34/232, 14.7%), as well as higher grade (n=33.141, 23.4%) and histologically more aggressive subtypes (carcinoma with medullary features 46.1% and metaplastic carcinoma 66.7%, respectively).

Approximately half of these patients had a tumor size between 2-5 cm (n=204, 55.4%). Patients with tumor size of > 5 cm and those with < 2 cm comprised about 25.8% (n=95) and 18.8% (n=69), respectively. There was no statistically significant association between tumor size and the molecular subtypes of breast cancer (p=0.191). Although more than half of the cases (n=218, 59.2%) had lymph node metastases, the association between this parameter and the molecular subtypes of breast cancer was not significant (p=0.301).

Majority of the tumors (85.1%; n=313) were Invasive carcinoma of no special type (NST), followed by Invasive lobular carcinoma (4.35%; n=16), Carcinoma with medullary features (3.2%; n=12) and Mucinous carcinoma (2.99%; n=11). The remaining cases comprised of Metaplastic (1.6%; n=6), Invasive papillary (1.36%, n=5), Mixed Invasive carcinoma (0.5%, n=2), Solid papillary (0.3%, n=1), Carcinoma with neuroendocrine features (0.3%, n=1).

In this study, the most prevalent molecular subtype was Luminal A (n=215, 58.4%) followed by, in descending order of frequency, Triple-negative (n=70, 19%), HER2-Enriched (n=44, 12%), Luminal B HER2 - negative (n=35, 9.5%) and Luminal B HER2 - positive (n=4, 1.1%). Among the 70 triple-negative cases, 49 (13.3%) were basal-like subtype, which showed positive staining for CK5/6. There is a statistically significant correlation between histological and molecular subtypes observed (p=<0.001). Most of the Invasive carcinoma (NST) were of the Luminal A (n=178, 57.1%), Luminal B HER2- negative (n=34, 10.9%) and HER2-Enriched (n=42, 13.5%) subtypes. Solid papillary, cribriform, invasive lobular and invasive papillary belonged to Luminal A subtype. The majority of Mucinous carcinoma (n=10, 91%) were in Luminal A subtype. Most of metaplastic carcinoma (n=4/6, 66.7%) and carcinoma with medullary features (n= 5/12, 46.1%), as well as a small number of invasive carcinoma NST (n=20/313, 12.8%) constituted the Triple-negative basal-like tumor subtype. One case of carcinoma with neuroendocrine features fell into the category of the HER2-Enriched subtype.

We found that most of the cases in our study were Grade 2 (n=169, 45.9%) and Grade 3 tumors (n=141, 38.3%). There was a significant statistical association seen between the molecular subgroups and tumor grade (p<0.001). Majority of Grade 1 tumors were Luminal A and Luminal B HER2-negative, which accounted for 48 (82.8%) and 8 (13.8%) cases, respectively. Triple-negative basal-like, HER2-Enriched and Triple-negative non-basal-like subtypes had higher frequencies of Grade 3 tumors as compared to Grade 1 and 2, seen in 33 (23.4%), 27 (19.1%), and 19 (13.5%) patients, respectively. None of the tumors with Triple-negative basal-like, Luminal B HER2 -positive, and Triple-negative non-basal-like molecular subtypes had Grade 1 histomorphology.

Table 1: Clinicopathological Features and Molecular Subtypes of Breast Cancer a Malaysian Cohort

Variable	All cases n=368	Luminal A n=215 (58.4%)	Luminal B HER2 Negative n=35 (9.5%)	Luminal B HER2 Positive n=4(1.1%)	HER2-Enriched n=44(12%)	Triple-negative Basal-like n=49(13.3%)	Triple-negative Non-Basal-like n=21(5.7%)	*P* value
Age, Mean (SD)	55.5 (11.42)	56.9 (11.77)	51.9 (11.95)	54.5 (13.77)	54.4 (9.82)	53.2 (10.34)	55.5 (10.81)	0.104[b]
Ethnic, n (%)								
Malay	232 (63.0)	132 (56.9)	28 (12.1)	0	28 (12.1)	34 (14.7)	10 (4.3)	
Chinese	85 (23.1)	53 (62.4)	4 (4.7)	3 (3.5)	10 (11.8)	11 (12.9)	4 (4.7)	
Indian	40 (10.9)	27 (67.5)	2 (5.0)	1 (2.5)	4 (10.0)	3 (7.5)	3 (7.5)	0.013[a]
Native Sabah & Sarawak	8(2.2)	2 (25.0)	1 (12.5)	0	1(12.5)	1 (12.5)	3 (37.5)	
Siamese	3 (0.8)	1 (33.3)	0	0	1 (33.3)	0	1 (33.3)	
Tumor Size, n (%)								
≤2 cm	69 (18.8)	48 (69.6)	5 (7.2)	0	5 (7.2)	9 (13.0)	2 (2.9)	
>2 to ≤5 cm	204 (55.4)	124 (60.8)	17 (8.3)	2 (1.0)	23 (11.3)	25 (12.3)	13 (6.4)	0.191[a]
>5 cm	95 (25.8)	43 (45.3)	13 (13.7)	2 (2.1)	16 (16.8)	15 (15.8)	6 (6.3)	
Histology Grade, n (%)								
Grade 1	58 (15.8)	48 (82.8)	8 (13.8)	0	2 (3.4)	0	0	<0.001[a]
Grade 2	169 (45.9)	114 (67.5)	19 (11.2)	3 (1.8)	15 (8.9)	16 (9.5)	2 (1.2)	
Grade 3	141 (38.3)	53 (37.6)	8 (5.7)	1 (0.7)	27 (19.1)	33 (23.4)	19 (13.5)	
Lymph Node Metastasis, n (%)								
Yes	218 (59.2)	130 (59.6)	25 (11.5)	2 (0.9)	27 (12.4)	24 (11.0)	10 (4.6)	0.306[a]
No	150 (40.8)	85 (56.7)	10 (6.7)	2 (1.3)	17 (11.3)	25 (16.7)	11 (7.3)	
Histology Subtype, n (%)								
Invasive carcinoma of no special type	313 (85.1)	178 (57.1)	34 (10.9)	4 (1.3)	42 (13.5)	40 (12.8)	15 (4.8)	<0.001[a]

Solid Papillary carcinoma	1 (0.27)	1 (100.0)	0	0	0	0	0	
Cribriform carcinoma	1 (0.27)	1 (100.0)	0	0	0	0	0	
Invasive Lobular Carcinoma	16 (4.35)	16 (100.0)	0	0	0	0	0	
Invasive papillary carcinoma	5 (1.36)	5 (100.0)	0	0	0	0	0	
Carcinoma with Medullary features	12 (3.26)	1(7.8)	0	0	0	5 (46.1)	6 (46.1)	
Metaplastic Carcinoma	6 (1.63)	1 (16.7)	0	0	1 (16.7)	4 (66.7)	0	
Mixed Invasive Carcinoma	2 (0.54)	2 (100.0)	0	0	0	0	0	
Mucinous carcinoma	11 (2.99)	10 (90.9)	1 (9.1)	0	0	0	0	
Carcinoma with Neuroendocrine features	1 (0.27)	0	0	0	1 (100.0)	0	0	

[a] Fisher's exact test [b] One-way ANOVA test

IV. DISCUSSION

We had conducted a retrospective study at 18 tertiary hospitals under the Ministry of Health, Malaysia between June 2017 and December 2017. A total of 368 cases of primary breast cancer in females were enrolled and were classified into six major molecular subtypes in accordance with IHC surrogate of molecular classification proposed by 11th St. Gallen International Breast Cancer Conference Expert Panel. We had also evaluated the clinicopathological features that are associated with these molecular subtypes.

Our study found that the average age of this cohort of patients was 55.5 years. This referenced age is in concordance with the observation in developed countries such as in the USA, where 65.1% of the reported cases were found in women older than 55 years of age, as evident from the Surveillance, Epidemiology, and End Results (SEER) Cancer Statistics Review [17]. In our studied population, breast cancer was seen more in women of ethnic Malay, followed by Chinese and Indian. This is in accordance with the ethnic ratio in Malaysian population which is predominantly Malays [35]. This result contrasted with that reported by Malaysia National Cancer Registry 2012-2016, which showed ethnic Chinese.

predominance, followed by Malay and Indian. However, since this study is conducted in only 18 selected MOH tertiary hospitals, it is possible that our finding did not reflect the overall prevalence of breast cancer in Malaysia. Most of our cases (81.2%) had tumor size > 2 cm at presentation. The number of patients with tumor size of 2 cm or smaller were considerably lower (18.8%) as compared to those reported in the USA (58.4%) [17].

Lack of awareness on breast cancer and non-comprehensive screening programs could contribute to their late presentation in seeking treatment.

This study revealed that the frequency of Triple-negative and HER2-Enriched tumors were relatively higher in the Malays as compared to women of another ethnicity, implying aggressive tumor presentation in this group of women. A large cohort study conducted in Southeast Asian women with breast cancer showed that Malays were more likely to have axillary lymph node

metastasis at similar tumor sizes, negative hormone receptors, poorly differentiated tumors, and shortest overall 5-year survival as compared to Chinese and Indian women [18].

Lymph node status serves as an important prognostic and predictive factor. Although the lymph node status is not significantly associated with molecular subtypes in our study, we found that Luminal B HER2-negative subtype showed more tendency for lymphatic spread as compared to Luminal A subtype. Also, Luminal B HER2-negative cases had a higher frequency of positive lymph nodes compared to Triple-negative subtypes. The relatively low frequency of lymphatic spread in Triple-negative tumors could probably be due to the aggressive nature of the tumor, in which the malignant cells may metastasize through pathways other than the lymphatic channel, most likely hematogenous spread [19][20]. Many studies have also shown no statistically significant correlation between molecular tumor subtypes and lymph node status, while several others had identified a high frequency of lymph node metastasis with HER2-positive tumors and a low frequency with Triple-negative basal-like tumors [20][21]. These inconsistent results show that lymph node status cannot be utilised as an independent prognostic factor for breast cancer. A high rate of lymph node metastasis and larger tumor size at the time of diagnosis observed in our study is in concordance with late-stage disease (III and IV) reported by the Malaysian National Cancer Registry 2012-2016, accounting for almost half (47.9%) of the breast cancer patients in Malaysia, thus explaining the high mortality rate of breast cancer in our population.

The distribution of molecular subtypes of breast cancer in Malaysian population observed in this study appears to concur with results from a similar work by Munira et al. [22]. Luminal A was also reported to be the most prevalent subtype in various Asian and Western countries, including China (46.5%), India (28%), Pakistan (45.8%), Saudi Arabia (58.5%) and the USA (47%) [23][24][25][26][27]. Luminal A tumors, which account for most of our cases, were separated from other non-HER2 expressing Luminal B subtype by Ki-67 score of 14% or less, conforming to the 11th St. Gallen International Breast Cancer Conference Expert Panel for IHC surrogates. A new cut-off value greater than 20% had been proposed by the expert panels in St Gallen 2013 [10][11]. In our cohort, the Ki-67 index ranged from 0 to 80% (mean 17%). Although there is currently no standardized cut-off value for the Ki-67 index, we are in the opinion that the cut-off value of 14% to 20% is appropriate to discriminate Luminal A and Luminal B tumors [28]. Besides having low proliferative index, breast cancers in the Luminal A subtype do not over-express HER2 and they exhibit a low percentage of p53 mutation, resulting in a more favorable outcome as compared to the Luminal B subtype [29].

In our study, two-thirds of HER2 positive breast carcinomas fell into the category of HER2-Enriched subtype, while Luminal B HER2-positive subtype constituted less than 10% of cases. Fountzilas et al. showed that Luminal B HER2-positive and HER2-Enriched subtypes were clinically distinct, with the former having a shorter disease-free survival curve and more frequent nodal metastasis [30]. We also found that most of the Luminal HER2-positive tumors in our studied population were ER+/PR+/HER2+ (triple positive cancer) rather than ER+/PR-/HER2+. A recent study showed that the survival of patients with ER+/PR+/HER2+ tumors was superior to those with ER+/PR-/HER2+ across all stages, supporting that loss of PR is an unfavourable event [31][32]. This finding highlighted the clinical importance of separating triple positive tumors (ER+/PR+/HER2+) from ER+/PR-/HER2+ ones.

We utilized CK5/6 IHC to differentiate triple - negative tumors into basal-like and non-basal-like subtypes. This discrimination is clinically important as each subtype has a specific gene expression pattern and different clinical behavior [9]. Rakha et al. demonstrated that non-basal-like tumors are less likely to be associated with BRCA 1 mutation and have better breast cancer-specific survival and disease-free survival compared to basal-like tumors [33].

Our study validated the strong association between histological subtypes and molecular subtypes as shown by a significant p-value. Most invasive carcinoma of no special type (57.1%), most of mucinous carcinoma (90.9%), and all cases of invasive lobular carcinoma, cribriform carcinoma and solid papillary carcinoma in our study belonged to the Luminal A subtype. Similar pattern was observed with Luminal B HER-2 negative subtype in which almost all tumors comprised of invasive carcinoma of no special type.

On the other hand, metaplastic carcinoma, and carcinoma with medullary features, which are histologically characterized by poor-differentiation, tumor necrosis and high mitotic index, are found to be of basal-like subtype.

The association of the tumor grade with different molecular subtypes of breast cancer was found to be statistically significant. The majority of grade 3 tumors belonged to Triple-negative basal-like, HER2-Enriched and Triple-negative non-basal-like subtypes, while grade 1 tumors are dominated by Luminal A and Luminal B HER2-negative subtypes. It is interesting to note that Triple-negative basal-like and non-basal-like subtypes, and a high proportion of HER2-Enriched cancers, did not present as Grade 1 tumors. More than half of HER2- Enriched breast cancers were Grade 3 tumors. The aggressive behavior of HER2-Enriched subtype is notably explained by high expression of ERBB2 gene, high incidence of p53 mutation and the activation of receptors in the tyrosine kinase pathway such as EGFR and HER2 [34].

V. LIMITATION

There were limitations to this study. One of them was because immunohistochemical stains for ER, PR, HER2, and Ki-67 were performed at multiple different centers using different types of antibody clones, which technically may lead to non-standardization. Another limitation was the identification of Triple-negative basal-like tumors was based on CK5/6 positivity alone due to the unavailability of EGFR test.

VI. CONCLUSION

Our study on Malaysian female breast carcinoma from 18 MOH tertiary hospitals revealed that the most prevalent molecular subtypes was Luminal A, followed by Triple-negative basal-like and HER2-Enriched tumors. All invasive lobular carcinoma, solid papillary carcinoma, cribriform carcinoma, invasive papillary carcinoma, and a majority of Mucinous carcinoma are found in Luminal A. Also, most Luminal A cases presented as Grade 1 tumors. On the other hand, carcinoma with medullary features and metaplastic carcinoma, both with high-grade histomorphology belonged to Triple-negative subtypes. Furthermore, Malay women were more likely to have unfavourable tumor subtypes such as Triple-negative and HER2-Enriched. Many studies, including ours, have demonstrated the relevance of identifying molecular subtypes of breast cancer. Thus, we recommend this should be included in routine histopathology assessment and reporting. Our data also highlighted the problem of late presentation among our cohort, as depicted by the high frequency of lymph node metastasis and large tumor size at the time of diagnosis. Hence, this issue needs to be addressed, including ensuring comprehensive screening programs that aim for early detection of breast cancer.

ACKNOWLEDGMENTS

I would like to express our appreciation to all pathologists and medical laboratory technicians in 18 tertiary hospitals of the Ministry of Health Malaysia for helping us with data collection.

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Serum Electrolytes, Creatinine and Urea Concentrations, Reticulocyte and Thrombocyte Counts in Pediatric and Adult Sickle Cell Disease Patients Placed on Immunozin™ Therapy in Northern Nigeria

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ABSTRACT

Introduction: Therapeutic management of sickle cell disease (SCD) has proven to be a major task to both patients and clinicians in Africa. Investigation of blood and serum parameters are essential tools for assessing efficacy of medical interventions and eventual outcome of the disease. There is paucity of studies on modern African medicinal treatment and resulting post-intervention hematological parameters of SCD in Nigeria. This study aimed at determining serum electrolytes and urea and some hematological parameters among SCD patients who were treated with a study agent compared to SCD patients who were not treated with the study agent but given normal hospital care. Objective: The objective of this study was to assess the differences in reticulocytes, thrombocytes, and serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and in control patients.

Keywords: creatinine, electrolytes, nigeria, reticulocytes, sickle cell disease, thrombocytes, urea.

Classification: NLMC CODE: WH 170

Language: English

London Journals Press

LJP Copyright ID: 392852

London Journal of Medical and Health Research

Volume 21 | Issue 2 | Compilation 1.0

Serum Electrolytes, Creatinine and Urea Concentrations, Reticulocyte and Thrombocyte Counts in Pediatric and Adult Sickle Cell Disease Patients Placed on Immunozin™ Therapy in Northern Nigeria

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ABSTRACT

Introduction: Therapeutic management of sickle cell disease (SCD) has proven to be a major task to both patients and clinicians in Africa. Investigation of blood and serum parameters are essential tools for assessing efficacy of medical interventions and eventual outcome of the disease. There is paucity of studies on modern African medicinal treatment and resulting post-intervention hematological parameters of SCD in Nigeria. This study aimed at determining serum electrolytes and urea and some hematological parameters among SCD patients who were treated with a study agent compared to SCD patients who were not treated with the study agent but given normal hospital care. Objective: The objective of this study was to assess the differences in reticulocytes, thrombocytes, and serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and in control patients.

Materials and Method: This was a double-blind, two-arm, randomized control pilot study involving a total of 62 subjects, including 33 cases with SCD who were given the study agent and 29 controls with SCD who were not given the study agent. After preliminary evaluation, the study drug was administered at enrollment into the study on Day 1 and each study participant was re-evaluated at each monthly administration of the test drug for 6 consecutive visits conducted monthly. Study drug was administered one month after enrolment on each subsequent month for 5 months. Venous blood sample was collected and all other variables were investigated at each visit. A full blood count (hemoglobin (Hb) concentration, packed cell volume (PCV), white blood cells (WBC), reticulocytes (RTC), platelets (PLT) counts were done within 2 hours of collection, and were recorded. Serum electrolytes and urea, liver enzymes were also investigated. NCSS statistical software was used for analysis.

Results: At the end of study, mean (±sd) reticulocyte count of pediatric cases (1.54 [1.00]) was significantly lower (t-test=4.19, P-value = 0.0002) than the enrolment value (2.44 [0.77]) and greater drop in reticulocyte count occurred among pediatric cases than among control subjects. A significant decrease (t-test=2.07, P-value=0.02) in the mean (±sd) thrombocyte count of adult controls at enrolment (515.0 [77.9]) compared to the value at the end of the study (432.3 [29.4]) was observed. The mean (±sd) creatinine blood level of pediatric cases at enrollment was significantly lower (t-test= -3.12, P-value=0.002) than that at end of study (49.5 [11.2]). Serum potassium levels were elevated in all cases and controls at the end of the study. Simple linear regression analysis showed that the estimated change in total thrombocyte count per unit change in reticulocyte count varied

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between pediatric and adult case and control subjects.

Conclusion: The significant reduction in mean reticulocyte count of pediatric SCD patients on test drug and the difference in the slope of the equation of straight line relating thrombocyte count and reticulocyte count may reflect the therapeutic effect of the test drug among pediatric patients. Clinicians should monitor serum potassium level when managing sickle cell disease patients for cardiac response to hyperkalemia. Further studies are needed to confirm these findings.

Keywords: creatinine, electrolytes, nigeria, reticulocytes, sickle cell disease, thrombocytes, urea.

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I. INTRODUCTION

Sickle Cell Disease (SCD) results from a point mutation where glutamic acid is replaced by valine at position 6 on the β globin. [1,2] The abnormal β^s chains combine with normal α chains to form the sickle hemoglobin (HbS), a less soluble complex compared to the fetal or adult hemoglobin. SCD is a condition consequent to the inheritance of abnormal allelomorphic genes controlling the formation of the beta (β) chains of hemoglobin (Hb). [3] About 5% of the world's population carry at least one of the two alleles responsible for sickle hemoglobinopathies. [4] The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Sahara -n Africa, India, and the Middle East. [5] Migration of substantial populations from these high - prevalence areas to low - prevalence countries in Europe has dramatically increased in recent decades and in some European countries, sickle cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis. [6] In 2015, it resulted in about 114,800 deaths. [7] Organizations such as the World Health Organization (WHO) and United Nations (UN) have recognized SCD as a global health issue. In 2006, the World Health Assembly passed a resolution recognizing SCD as a public health priority and called on countries to tackle the disease. This resolution was also adopted by the United Nations in 2009. [8] Nigeria has the highest burden of the disease in the world with over 150,000 children born every year with SCD. [9]

Clinical studies evaluating modern approaches of managing of sickle cell disease in the African populace are scarce. Although developed countries have access to novel and innovative therapies such as monoclonal antibodies and small molecule hemoglobin S polymerization inhibitors, crizanlizumab and voxelotor respectively, therapies available in developing African countries are stunted at the use of hydroxyurea (HU), an agent with "anti-sickling" effect by inducing fetal hemoglobin (HbF, $\alpha_2\gamma_2$). [10] The gap resulting from the lack of innovative therapies is filled by local complementary and herbal remedies available in respective African countries. Despite the consequent encroachment, data on the use of local complementary and herbal remedies in patients with SCD is also scarce, leading to the objective of this study to evaluate the effect of an indigenous therapeutic agent on metabolic and hematological parameters in pediatric and adult patients with SCD in Nigeria.

II. MATERIALS AND METHODS

This has already been described in a previous publication [11]. Briefly, the initial study, of which this was extracted, was conducted between January and May of 2018, as a double-blind, two-arm, randomized control pilot study. The

study agent in this trial is a commercially available herbal nutritional capsule supplement with a mixture of Allium sativum, Balanites aegyptiaca, Guiera senegalensis and Azadirachta indica.

Sample size calculation

A universal formula for selecting the sample size for a clinical trial or research problem based on a level of significance and a chosen margin of error was proposed by Cochran [12] and Levy and Lemeshow [13]. Cochran's formula for sample size determination used for determining the sample size has been reported earlier [11].

Study area

The study was conducted at Kaduna City, (10.52° North latitude, 7.44° East longitude and 614 meters elevation above sea level) in Northern Nigeria with a projected population of 1,582,102, based on the 2006 national census figures [14]

Study population

These were patients diagnosed with Sickle Cell Disease attending the pediatric and adult hematology clinics at Barau Dikko Teaching Hospital, Kaduna City in Nigeria.

Recruitment, inclusion and exclusion criteria

Recruitment of participants was carried out at Barau Dikko Teaching Hospital, Kaduna. Participants aged 5 to 45 years were included if they had been diagnosed with SCD, provided informed consent or assent for minors and stated willingness to comply with all study procedures and availability for the duration of the study. Participants were included if they exhibited any clinical signs and symptoms of sickle cell disorder including at list an episode of crisis monthly and were able to take oral medication and compliant with the medication regimen. Individuals with concomitant use of any other medication or medical devices not part of the study were excluded. Other exclusion criteria included those with known allergic reactions to any of the components of study drug, pregnant or lactating women or women who were planning to get pregnant within five months after commencement of the study, patients who had cardiac, hepatic or kidney disease, patients who had one or more episodes of febrile illness within 1 month preceding the study (to exclude patients potentially with malaria, tuberculosis, measles) or those who were alcohol or tobacco users 4 months prior to the start of the study.

Study design and protocol

Following a screening period, patients were enrolled into the study protocol after completing baseline blood and urine sample collection and baseline clinical examination. Participants were then initiated on the intervention, receiving a monthly dose of 500 mg (pediatric patients 5-18 years) or 1000 mg (adult patients>18 years) every 12 hours for a minimum of 120 days, in addition to standard of care practices. After preliminary evaluation, the study drug was administered at enrollment into the study on Day 1 (first visit) and each study participant was re-evaluated at each monthly administration of the test drug for 6 consecutive visits conducted monthly (approximately 30 days apart).

Screening and baseline data at recruitment into study

At the initial visit, inclusion/exclusion criteria and informed consent form for study subjects were verified and urine pregnancy test was conducted for females in the reproductive age group. Other relevant information was recorded, blood was aseptically collected for various analyses and clean-catch mid - stream urine was collected for urinalysis. At the next visit (Visit 2) case subjects were given appropriate dosage of the test drug as specified above.

Hypotheses

There is no difference in mean counts of reticulocytes and thrombocytes of pediatric and adult SCD patients on the study agent and those not on the study agents. There is no difference in mean serum concentrations of serum electrolytes, urea and creatinine of pediatric and adult SCD patients on the study agent and those not on the study agents.

Ethical approval

Each study subject (or caregiver/guardian) signed a consent form to participate in the study and was assured that his/her data will be discreet, coded, and unnamed. The study was approved by the Human Research Ethics Committee (HREC) with a reference number 17-0025 and protocol number 17-0027-1.

Data management and statistical analysis

The coded data was transferred from Excel spreadsheet into NCSS (LLC, Kaysville, Utah, USA) software which was used for further analysis. For the purpose of this study, age (years) was categorized into<10, 10-19.9 and ≥20. Multivariate regression analysis was performed to determine the association between thrombocytes (independent variables) and reticulocyte counts at 1st and 6th visits (dependent variable). Student's t-test was used to evaluate significant differences in means between two continuous variables. Data were presented as numbers and percentages for categorical variables, as mean with standard deviations for continuous variables and as Tables and Figures for all variables. A P-value <0.05 was regarded as statistically substantial.

III. RESULTS

3.1 Demographic characteristics of the study participants. Table 1

Of the 62 SCD subjects included in the study, 33 (53.2%) received the intervention (23 [69.7%] pediatric and 10 [30.3%] adult patients), while 29 (46.8%) were in the control group (22 [75.9%] pediatrics and 7 [24.1%]) adult patients) respectively. There was no significant difference in the means of age and body mass index among the pediatric or adult cases and control (Table 1).

3.2 Reticulocyte count: Table 2, Figures 1a-d, Figures 2a-d

At enrolment, the mean ± standard deviation (SD) reticulocyte count of pediatric cases of 2.44% ±0.77 was similar to controls of 2.56% ±0.81 (t-test = -0.51, P-value = 0.31) and the similarity persisted at end of study. In adult participants, the baseline reticulocyte count in the intervention group was marginally varied from that of the control group, 2.54% ±0.90 compared to 1.97% ±0.40 respectively (t-test =-1.77, P-value =0.05) but trended towards non-significance difference at the end of the study. However, the reticulocyte count of pediatric patients in the intervention group decreased significantly at the end of the study 1.54% ±1.00 (t-test = 4.19, P-value = 0.0002) indicating a 36.9% reduction. A significant reduction was also reflected in the mean reticulocyte count of pediatric patients in the control group at the end of the study 1.70% ±0.69 (t-test=3.34, P-value=0.001), albeit to a less degree (33.6% reduction). At baseline, 60.9% of pediatric patients that had reticulocyte count >2.0%, which decreased to 21.7% at the end of the study, reflecting a decrease of 38.4% compared to 32.8% in the control group. A significant difference was also noticed in the mean reticulocyte count of adult control subjects at enrollment 1.97% ±0.40 and at the end of the study 1.24% ±0.63 (t-test = 2.37, P-value = 0.02). Figure 1a and 1b illustrate the histogram and normal probability plot of reticulocyte count of pediatric cases at enrolment and the end of the study, indicating percent of values equal to, below or greater than 2%; Figure 1c and 1d show the histogram and normal probability plot of the reticulocyte count of pediatric controls at enrolment and at end of study also indicating percent of values equal to, below or greater than 2%. Figures 2a-d illustrate the histogram and normal probability plot of reticulocyte count of adult cases and controls at enrolment and at end of the study, indicating values equal to, below or greater than 2%.

3.3 Thrombocyte count: Table 2. Figures 3a-d, Figures 4a-d

Although there were no observable significant differences in the mean thrombocyte count of pediatric cases and control at enrolment 444.7± 168.8 and 442.8 ±165.4 respectively and the end of the study 414.1 ±153.7 and 428.2 ±119.2 respectively, there was a 7% reduction (444.7-414 .1) in mean thrombocyte count of patients in the intervention group compared to 3% reduction (442.8-428.2) among the controls. While there

were no significant differences in the mean thrombocyte count of adults in the intervention and control groups at enrolment (547.0 ±271.6 versus 515.0 ±77.9 respectively; t-test = -0.35 (0.3 7) and at the end of the study (intervention group 401.4 ±179.1 versus control group 432.2 ±29.4), the mean thrombocyte count of adults in the intervention group at enrolment 515. ±77.9 was significantly higher than the end of the study (t-test ==2.63, P-value =0.02]). Figures 3a and 3b illustrate the histogram and normal probability plot of thrombocytes of pediatric cases at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L and Figures 3c and 3d elaborate on the histogram and normal probability plot of thrombocytes of pediatric controls at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L. Figures 4a-d show the histogram and normal probability plot of thrombocytes of adult cases and adult controls at enrolment and at the end of the study respectively, showing values equal to, below or greater than 400 x 10^9/L.

3.4 Hemoglobin concentration: Table 2

Astonishingly, there were no observable significant alterations in hemoglobin and in urea values of pediatric or adult cases and control throughout the study. While there was a slight insignificant reduction in the mean hemoglobin concentration of pediatric cases from 76.7 ±9.6 g/dl at enrolment to 76.2 ±9.6 at the end of the study, there were increases in the values among pediatric controls 74.6 ±10.1 g/dl at enrolment to 75.3 ±13.0 at the end of the study, among adults cases 80.2 ±15.3 g/dl at enrolment to 83.2 ±11.5 at end of the study and controls 79.1 ±11.9 at enrolment to 91.3 ±2.9 at the end of the study.

3.5 Serum Urea and Creatinine

There were no observable differences at enrolment in the mean creatinine blood level (µmol/L) of pediatric patients in the intervention 38.3 ±10.8 and control 41.5 ±10.3 (t-test =-1.01, P -value =0.16), at end of study 58.2 ±37.2 and 54.8 ±9.9 respectively (t-test =0.41, P - value =0.34) at end of study and in the adult case and control subjects. Yet, the mean creatinine blood level of pediatric patients in the intervention group at the end of the study 58.2 ±37.2 was significantly higher than levels at enrollment 38.3 ±10.8; (t-test = -2.46, P-value = 0.01). The mean creatinine level of control pediatric subjects at the end of the study 54.8 ±9.93 was even more significantly higher than levels obtained at enrollment 41.5 ±10.3 (t-test = -3.78, P-value = 0.0004).

3.6 Serum electrolytes, Sodium, Potassium, Chloride and Bicarbonate: Table 3

At the end of the study, the mean sodium concentration value of pediatric patients in the intervention group of 137.1 ±3.9 mmol/l and 136.8 ±3.3 mmol/l in the control were significantly reduced compared to their values at enrolment 140.3 ±3.8 mmol/l and 140.1 ±3.6 mmol/l respectively (t-test =2.82, P-value =0.00 4 and t-test =2.76, P-value =0.005 respectively), though all values were within normal range. The end of study mean values of serum potassium among pediatric patients in the intervention group was significantly higher 6.2 ±0.8 mmol/l compared to levels at enrolment 5.5 ±0.9 mmol/l (t-test =-2.79; P-value=0.004) and this difference was wider in pediatric patients in the control group from enrolment level of 4.9 ±0.9 mmol/l to end of study level of 6.4 ± 0.8 mmol/l (t-test =- 5. 11; P-value=0.0000001). There was no noticeable change in adult potassium levels throughout the study.

The mean serum chloride level of pediatric patients in the control group at enrolment of 103.3 ±4.9 mmol/l was notably higher than that at end of study 100.5 ±2.1 mmol/l (t-test = 2.34, P-value =0.01) and the value of adult controls at enrolment 104.7 ±3.3 mmol/l was also significantly higher than the end of study value 102.1 ±1.6 mmol/l (t-test = 2.34, P-value = 0.01). There was no momentous disparity in the values among pediatric cases or controls at enrolment or at the end of the study.

The mean serum bicarbonate levels were significantly elevated from 17.7 ±5.5 mmol/l enrolment value to 23.2 ±3.3 mmol/l end of the

study value among pediatric cases (t-test =-4.26, P-value =0.0001), and less so from 19.5 ±5.0 mmol/l enrolment level to 22.5 ±3.4 mmol/l end of study level among pediatric control subjects (t-test =-2.11, P-value =0.02). The increase in mean serum bicarbonate concentration (mmol/l) among adult cases and among control subjects were, to a lesser extent, significantly elevated from 20.8 ±4.6 mmol/l enrolment level to 26.1 ± 2.2 mmol/l end of study level and from 23.0 ±2.9 mmol/l enrolment level to 25.7 ±2.4 mmol/l end of study level (t-test =-3.29, P-value =0.003 and t-test -1.90, P-value =0.04 respectively).

3.7 Pearson's correlation tests between thrombocyte count and reticulocyte count. Figures 5a-d, Figures 6a-d

As depicted in Figure 5a, the equation of the straight - line relating thrombocyte count (TC) and reticulocyte count (RC) of pediatric cases at enrolment was estimated as: TC = (368.4) + (31.2) RC using the 23 observations in this data set. The y-intercept, the estimated value of TC when RC was zero, was 368.4 with a standard error of 120.5. The slope, the estimated change in TC per unit change in RC, was 31.2 with a standard error (SE) of 47.1. The value of R^2, the proportion of the variation in TC that can be accounted for by variation in RC, was 0.0205. Pearson's correlation between TC and RC was 0.14 with a P-value of 0.51. As shown in Figure 5b, the equation of the straight-line relating TC and RC of pediatric cases at the end of the study was estimated as: TC = (426.7) + (-8.1) RC using the 23 observations in this data set. The y-intercept was 426.7 with an SE of 61.3, the slope was -8.1 with an SE of 33.6 and the value of R^2 was 0.0028. The correlation between TC and RC was - 0.05 with a P - value of 0.81. In Figure 5c.,the equation of the straight - line relating TC and RC of pediatric controls at enrollment was estimated as: TC = (451.4) + (-3.4) RC using the 22 observations in this data set. The y-intercept was 451.4 with an SE of 122.0. The slope was - 3.4 with an SE of 45.4. The value of R^2 was 0.0003 and the correlation between TC and RC was insignificant (P-value = 0.94) at -0.02. Figure 5d illustrates that the equation of the straight-line relating TC and RC of pediatric controls at end of the study was estimated as: TC =(511.06) +(-48.8) RC using the 13 observations in this data set. The y-intercept was 511.06 with an SE of 90.8, the slope was -48.8 with an SE of 49.7, R^2 was 0.0804 and Pearson's correlation was insignificant (P-value = 0.35) at -0.28.

In Figure 6a, the equation of the straight-line relating TC and RC of adult cases at enrolment is estimated as: thrombocytes = (316.1) + (90.9) reticulocytes using the 10 observations in this data set. The y-intercept was 316.1 with an SE of 271.6. The slope (SE) was 90.9 (101.3), R^2, was 0.0914 and the correlation between TC and RC was insignificant (P-value = 0.40) at 0.30. In Figure 6b, the equation of the straight - line relating TC and RC of adult cases at the end of the study was estimated as: TC = (244.1) + (81.9) RC using the 10 observations in this data set. The y-intercept (SE) was 244.1 (99.4), the slope (SE) was 81.9 (44.6) and C was 0.30. The correlation (P-value) between TC and RC was 0.5446 (0.10). In Figure 6c, the equation of the straight - line relating TC and RC of adult controls at enrolment was estimated as: TC = (478.8) + (18.4) RC using the 7 observations in this data set. The y - intercept (SE) was 478.8 (172.7), the slope (SE) was 18.4 (86.1) and R^2 was 0.009. The correlation (P-value) was 0.10 (0.84). Finally, in Figure 6d, the equation of the straight-line relating TC and RC of adult controls at end of the study was estimated as: TC =(407.1) + (20.3) RC using the 7 observations in this data set. The y-intercept (SE) was 407.1 (201.9), with a slope (SE) of 20.3 (143.5). The value of R^2 was 0.0040 and Pearson's correlation between TC and RC was 0.0631 (0.89).

IV. DISCUSSION

This unique study evaluated the effects of an African pharmaceutical agent (Immunozin®) on hematological and metabolic parameters in pediatric and adult patients with SCD. The study agent is approved by the National Agency for Food and Drugs Administration and Control (NAFDAC) in Nigeria and has been in production and sold as a nutritional supplement and immune and vitality booster for eight years. When taken by sickle cell sufferers, their frequency of

vaso-occlusive crisis drastically reduces. No adverse drug effect (ADR) from the study agent has been reported, even among those who have been using it for more than seven years, though information on its mechanism of action is unavailable as no study has been done on this aspect of the study agent. Analyses suggest significant changes to hematological parameters including a global decrease in reticulocyte count with pronounced change in the intervention group and decreased thrombocyte levels specific to the pediatric cohort, whereas the adult cohort saw an increasing trend over the duration of the study. Use of this novel African pharmaceutical agent has not been previously reported with the exception of our previous study (11) in which we sought out to identify optimal management of patients with sickle cell disease in the context of affordable and available medicine to realize shorter hospital stay, lower out of pocket expenses and improved quality of life. Our previous study revealed overall pre- and post-hoc leucocytosis, thrombocytosis, hyperkalemia and significant variations in reticulocytes, monocytes, eosinophils, and some liver enzymes which may be due to the administration of the test drug. This led to the purpose of this paper to evaluate the safety and efficacy of Immunozin ® in pediatric and adult patients with SCD using hematological and metabolic surrogate markers. Hemogram or complete blood count is probably the most routinely conducted laboratory investigation at any time a SCD patient visits the hospital. However, scanty information is usually presented on serial reticulocyte count, platelet count, hemoglobin concentration and least of all serum electrolyte, urea and creatinine. The prognostic significance of these is consequent upon the therapeutic efficacy or otherwise of a drug, especially in a clinical trial. This is the case with the study drug under investigation and is the primary reason for conducting this clinical trial. This paper endeavors to originate or improve upon case-control studies of African therapeutic agents for positive impact on hematological parameters of SCD patients.

This study has some key points, one of which needs further clarification. First, there was a decrease in enrolment value of reticulocyte count among both pediatric cases (2.68 -1.44/2.68 x 100% or 46%) and control (2.56 -1.76/2.56x100% or 31%) at the end of the study, which reflects the findings of Borba et al [15], but the decrease was greater among cases than among the control subjects. There was also decrease in the enrolment value of reticulocyte count of both adult cases (2.28-1.86/2.28 x 100% or 18%) and controls (2.15-1.26/2.15 x 100% or 41%) at the end of study, though the decrease was greater in control subjects than among cases, as evidenced by the percentage of pediatrics cases with reticulocytosis (>2.0%) at enrolment (75.0%) and at end of study (18.8%), compared with controls with reticulo- cytosis at enrolment (63.2%) and at end of study (36.4%). This observation implies that the efficacy of study drug appears more pronounced among pediatric cases than among adult cases. The mechanism for this change is uncertain. Elevated reticulocyte count (>2.0%) at enrolment, as observed in many subjects in this study, is an indication of some degree of hemolysis and thus anemia. [16] In this case, a decrease in reticulocyte count may be used as an index of therapeutic effectiveness of the study drug. However, further studies are definitely needed on this point.

Surprisingly, the thrombocyte counts of pediatric cases and controls at enrollment were lower than the values at end of study while that of adult cases and controls were higher. Thrombocytosis appears to be a common phenomenon in steady-state SCD [17-20] though the prognostic implication of thrombocytosis at enrolment into study is controversial regarding its association with disease severity or complication. The prognostic implication of elevated baseline platelet count is debatable with no definitive information of its associations with disease severity or complications [18], though some authors [21, 22] associate thrombocytosis to background auto-splenectomy and hemolytic anemia.

An anticipated result was the increase in serum potassium level in both pediatric and adult cases and controls. The end of study potassium concentrations recorded in this study resonates with the findings in other studies. [23-26]

According to Dunlop and Bennett [27], potassium fluctuation in sickle erythrocytes is related to cell dehydration and sickling. Potassium leakage into the extracellular fluid, and the consequent serum hyperkalaemia, might have resulted from cell dehydration and hypoxia often observed among sickle cell patients. One intriguing novel finding is the y-intercept interpretation of the relationship between reticulocytes and thrombocytes at enrolment and at end of study in both pediatric and adult cases and control, especially in the pediatric cases. That, prior to administration of the test drug, thrombocytes turnover per unit change in reticulocyte count was 31.2 and that at the end of the study, this value was -8.1 is a topic that needs further in-depth investigation.

V. CONCLUSION

This study evaluates the effect of the agent on hematological parameters potentially informing on safety and to an extent efficacy in patients with SCD. Efficacy studies will indicate if patients still have SCD crisis or pain, exploring need for higher levels of care, oxygen levels, fraction of sickled blood in serum etc. The evidential reduction in mean reticulocyte count of pediatric SCD patients on test drug and the difference in the slope of the equation of straight line relating thrombocyte count and reticulocyte count may reflect the therapeutic effect of the test drug among pediatric patients. It will be an advantage to the scientific world if this study is carried further in respect to the mechanism of action of the study pharmaceutical agent.

Conflict of interest statement

Competing Interests: The authors have no conflicts of interest to disclose.

Funding

This study was sponsored by Rahma Integrated Concepts Limited, National Board for Technology Incubation (TIC Kaduna).

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Table 1: Demographic characteristics of study participants

Variable		Statistics	Case (n=33, 53.2%)		Control (n=29, 46.8%)		Pediatric (Case-Control)		Adult (Case-Control)	
			Pediatric	Adults	Pediatric	Adults	t-test	P-value	t-test	P-value
Age		Freq. (%)	23 (69.7)	10 (30.3)	22 (75.9)	7 (24.1)	0.77	0.22	0.60	0.28
		Mean (±sd)	10.2 (3.2)	21.2 (5.0)	9.5 (2.9)	20.0 (3.2)				
		Std. Err.	0.7	1.6	0.6	1.2				
		95% CL Mean	8.9 - 11.6	17.6 - 24.8	8.2 - 10.8	17.1 - 22.9				
		Median	11.2	21.5	10.0	21.0				
		Min. – Max.	5.2 - 15.0	15.0 - 27.0	5.0 - 15.0	16.0 - 25.0				
BMI		Freq. (%)	23	10	22	7	0.00	1.00	-0.35	0.36
		Mean (±sd)	15.3 (1.7)	18.4 (3.2)	15.3 (1.7)	18.9 (2.6)				
		Std. Err.	0.4	1.0	0.4	1.0				
		95% CL Mean	14.5 - 16.0	16.1 - 20.7	14.6 - 16.1	16.5 - 21.3				
		Median	15.1	17.8	15.2	18.5				
		Min. – Max.	12.6 - 19.6	16.0 - 26.9	11.7 - 18.1	15.2 - 22.0				
Sex	Male	Freq. (%)	10 (43.5)	5 (50.0)	10 (45.5)	2 (28.6)	-		-	
	Female		13 (56.5)	5 (50.0)	12 (54.5)	5 (71.4)				

Table 2: Mean distribution of some hematological parameters among case and control study subjects at enrolment (1st visit) and at end of study (6th visit) post administration of test medication

Hematological variable	Statistics	At enrolment (1st visit)				ttest (Pvalue)		Post administration of Study drug (6th visit; 6th month)			
		Case		Control				Case			
		Pediatric	Adult	Pediatric	Adult	Ped	Adult	Pediatric	Adult	Pediatric	
Reticulocytes (%)	n	23	10	22	7	- 0.51 (0.31)	1.77 (0.05)	23	10	13	
	Mean (±sd)	2.44 (0.77)!	2.54 (0.90) *	2.56 (0.81) ^	1.97 (0.40) #			1.54 (1.00)!	1.92 (1.19)*	1.70 (0.69)^	
	Median	2.10	2.50	2.50	2.0			1.30	1.65	1.6	
	Min. – Max.	1.7 – 4.1	1.2 – 4.1	1.6 – 4.3	1.4-2.4			0.4-3.9	0.4 – 4.0	0.6 – 3.0	
	No. (%) >2.0%	14 (60.9%)	7 (70.0%)	14 (63.6%)	3 (42.9)			5 (21.7)	3 (30.0)	4 (30.8)	
	Normality test@	0.82 (0.00) R	0.99 (0.99) CR	0.91 (0.04) R	0.90 (0.35) CR			0.89 (0.01) R	0.90 (0.23) CR	0.99 (1.00) CR	
Thrombocytes ($x10^9$/L)	Mean (±sd)	444.7 (168.8)!!	547.0 (271.6)**	442.8 (165.4)^^^	515.0 (77.9)##	0.28 (0.39)	- 0.35 (0.37)	414.1 (153.7)!!	401.4 (179.1)**	428.2 (119.2)^^^	0.17 (0.447)
	Median	429.0	510.0	447.5	490.0			407.0	399.5	415.0	
	Min. – Max.	149.0-924.0	158.0-928.0	193.0-916.0	428.0-637.0			141.0-702.0	127.0-720.0	230.0- 666.0	
	No. (%) >400	13 (56.5%)	6 (60.0)	14 (63.6)	7 (100.0)			12 (52.2)	5 (50.0)	8 (61.5)	
	Normality test@	0.96 (0.406.) CR	0.94 (0.56) CR	0.92 (0.06) CR	0.94 (0.61) CR			0.98 (0.82) CR	0.97 (0.91) CR	0.97 (0.91) CR	
Hemoglobin (g/dl)	Mean (±sd)	76.7 (9.6)!!!	80.2 (15.3)***	74.6 (10.1)^^^	79.1 (11.9)###	0.71 (0.24)	0.17 (0.447)	76.2 (9.6)!!!	83.2 (11.5)***	75.3 (13.0)^^^	
	Median	77.0	78.0	74.5	83.0			74.0	82.0	71.0	
	Min. – Max.	59.0-96.0	60.0-103.0	59.0-105.0	57.0-92.0			60.0-94.0	61.0-103.0	59.0-110.0	
	Normality test@	0.97 (0.73) CR	0.94 (0.56) CR	0.93 (0.10) CR	0.91 (0.41) CR			0.97 (0.61) CR	0.97 (0.87) CR	0.86 (0.04) R	
Urea (mmol/L)	Mean (±sd)	2.1 (0.9)$	2.2 (1.07)$$	2.2 (0.8)&	2.3 (0.6)&&	- 0.39 (0.35)	- 0.25 (0.40)	3.04 (2.9)$	2.05 (0.50)$$	2.3 (0.8)&	0.86 (0.04) R
	Median	1.9	1.9	2.1	2.3			2.3	1.95	2.2	
	Min. – Max.	1.1 – 4.6	1.0-4.7	1.1-4.4	1.5-3.1			1.5-16.2	1.4-2.9	1.2-4.3	
	No. (%) >9.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (4.3)	0 (0.0)	0 (0.0)	
	Normality test@	0.80 (0.0004) R	0.85 (0.05) CR	0.92 (0.08) CR	0.91 (0.39) CR			0.41 (0.000000001) R	0.93 (0.45) CR	0.86 (0.04) R	
Creatinine (µmol/L)	Mean (±sd)	38.3 (10.8)€	46.1 (11.3) €€	41.5 (10.3)£	48.0 (9.6) ££	- 1.01 (0.16)	- 0.37 (0.36)	58.2 (37.2) €	55.0 (8.2) €€	54.8 (9.9) £	0.94 (0.50) CR
	Median	39.0	46.5	44.0	47.0			50.0	55.5	51.0	
	Min. – Max.	14.0-56.0	29.0-65.0	16.0-61.0	33.0-63.0			33.0-221.0	45.0 - 72.0	42.0-73.0	
	No. (%) > 133	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (4.3)	0 (0.0)	0 (0.0)	
	Normality test@	0.96 (0.41) CR	0.92 (0.40) CR	0.92 (0.07) CR	0.98 (0.95) CR			0.49 (0.000000001) R	0.94 (0.50) CR	0.90 (0.13) CR	

	Control		t-test (P-value)	
	Adult		Ped.	Adult
	7		- 0.57 (0.29)	1.47 (0.08)
	1.24 (0.71)#			
	1.4			
	0.3 – 2.1			
	1(14.3%)			
	0.92 (0.47) CR			
	432.3 (29.4)##		- 0.31 (0.38)	- 0.54 (0.30)
	360.0			
	211.0-876.0			
	3 (42.9)			
	0.88 (0.24) CR			
	81.3 (2.9)###		0.22 (0.41)	0.50 (0.31)
	82.0			
	78.0-84.0			
	0.79 (0.04) R			
	2.8 (1.1)&&		1.15 (0.13)	- 10.69 (0.07)
	2.4			
	1.6-4.5			
	0 (0.0)			
	0.91 (0.41) CR			
	53.4 (14.9) ££		0.41 (0.34)	0.26 (0.40)
	54.0			
	24.0-72.0			
	0 (0.0)			
	0.86 (0.16) CR			

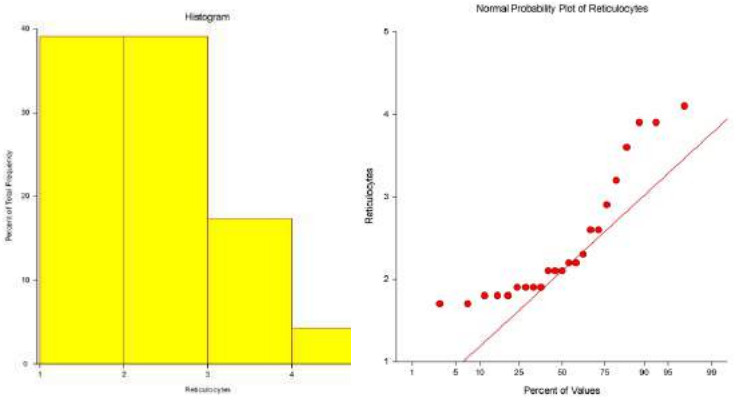
Reticulocyte t-test (P-value): !=4.19 (0.0002); = 1.13 (0.10); 3.34 (0.001); #= 2.37 (0.02): *Thrombocyte t-test (P-value):* !!= 0.64 (0.26); **= 1.42 (0.09); ^^- 0.30 (0.38); ##= 2.63 (0.02): *Hemoglobin t-test (P-value):* !!!= - 0.18 (0.43); ***= -0.50 (0.31); ^^^= - 0.17 (0.43); ###= - 0.48 (0.32); *Urea t-test (P-value):* $= - 1.48 (0.07); $$= 0.40 (0.35); &= -0.36 (0.36); &&= - 1.06 (0.16); *Creatinine t-test (P-value):* €= - 2.46 (0.01); €€= -2.01 (0.03); ££= - 3.78 (0.0004); @=Shapiro Wilk W for normality test (P-value); R=reject normality; CR=Cannot reject normality; Ped.=Pediatric.

Table 3: Mean distribution of serum electrolytes among case and control study subjects at enrolment (1st visit) and at end of study (6th visit)

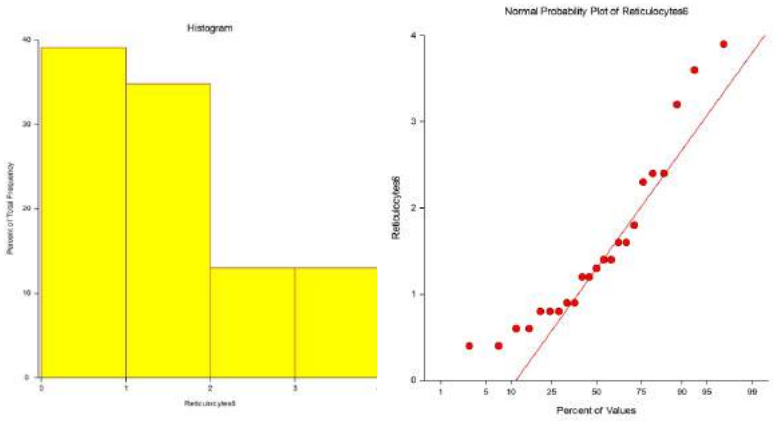
		At enrolment (1st visit)						t-test (P-value)	
		Case		Control					
		Pediatric	Adult	Pediatric (Ped.)	Adult			Ped.	Adult
Sodium (mmol/l)	n	23	10	22	7			0.18 (0.43)	- 0.05 (0.48)
	Mean (±sd)	140.3 (3.8)!	137.6 (5.6) *	140.1(3.6) ^	136.7 (1.50) #				
	Median	140.0	138.0	139.0	137.04				
	Min. – Max.	130.0-146.0	122.0-145.0	134.0-148.0	135.0-139.0				
	No. (%) > 145.0	1(4.3)	0 (0.0)	1(4.5)	0 (0.0)				
	Normality test@	0.94 (0.19) CR	0.89 (0.04) R	0.96 (0.51) CR	0.93 (0.59) CR				
Potassium (mmol/l)	Mean (±sd)	5.5 (0.9)!!	5.5 (1.0) **	4.9 (0.9) ^^	6.5 (3.6) ##			2.24 (0.015)	- 1.23 (0.11)
	Median	5.2	5.2	5.1	5.3				
	Min. – Max.	3.9-7.4	4.0-7.0	2.9-6.2	4.5-14.7				
	No. (%) > 5.2	11 (47.8)	4 (40.0)	9 (40.9)	4 (57.1)				
	Normality test@	0.93 (0.13) CR	0.91 (0.10) CR	0.93 (0.22) 3CR	0.55 (0.000006) R				
Chloride (mmol/l)	Mean (±sd)	101.6 (3.7)!!!	99.1(6.1) ***	103.3 (4.9) ^^^	104.7 (3.3) ###			-1.31 (0.10)	- 0.32 (0.38)
	Median	103.0	100.0	102.0	106.0				
	Min. – Max.	93.0-108.0	82.0-108.0	97.0-115.0	99.0-108.0				
	No. (%) > 108.0	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)				
	Normality test@	0.91 (0.04) R	0.90 (0.08) CR	0.91 (0.09) CR	0.90 (0.32) CR				
Bicarbonate (mmol/l)	Mean (±sd)	17.7 (5.5) $	20.8 (4.6) $$	19.5 (5.0) &	23.0 (2.9) &&			- 1.15 (0.13)	- 1.37 (0.09)
	Median	17.0	19.0	19.0	23.0				
	Min. – Max.	8.0-30.0	15.0-29.0	13.0-29.0	19.0-27.0				
	No. (%) > 32.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
	Normality test@	0.98 (0.93) CR	0.92 (0.14) CR	0.94 (0.23) CR	0.95 (0.81) CR				

Post administration of Study drug (6th visit; 6th month)						
Case		Control		t-test (P-value)	
Pediatric	Adult	Pediatric (Ped.)	Adult	Ped	Adult
23	10	13	7		
137.1 (3.9)!	139.9 (2.7) *	136.8 (3.3) ^	138.9 (2.4) #		
137.0	139.5	137.0	139.0		
127.0-144.0	136.0-144.0	130.0-142.0	135.0-142.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.93 (0.12) CR	0.94 (0.52) CR	0.95 (0.53) CR	0.98 (0.98) CR	0..25 (0.40)	0.80 (0.22)
6.2 (0.8)!!	5.1 (0.9) **	6.4 (0.8) ^^	6.4 (0.9) ##		
6.2	5.0	6.4	6.1		
4.4-7.6	4.0-7.2	4.4-7.4	5.3-8.0		
13 (56.5)	4 (40.0)	9 (69.2)	7 (100.0)		
0.95 (0.35) CR	0.91 (0.27) CR	0.88 (0.07) CR	0.94 (0.60) CR	- 0.72 (0.24)	- 2.93 (0.006)
100.7 (3.8)!!!	100.7 (2.0) ***	100.5 (2.1) ^^^	102.1 (1.6) ###		
102.0	101.0	101.0	102.0		
89.0-105.0	96.0-103.0	96.0-104.0	100.0-104.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.84 (0.002) R	0.85 (0.07) CR	0.97 (0.87) CR	0.91 (0.42) CR	- 0.20 (0.42)	- 1.60 (0.07)
23.2 (3.3) $	26.1 (2.2) $$	22.5 (3.4) &	25.7 (2.4) &&		
24.0	26.0	23.0	26.0		
14.0-27.0	22.0-29.0	15.0-27.0	22.0-29.0		
0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
0.88 (0.009) R	0.94 (0.53) CR	0.94 (0.50) CR	0.98 (0.4) CR	- 0.60 (0.28)	0.35 (0.37)

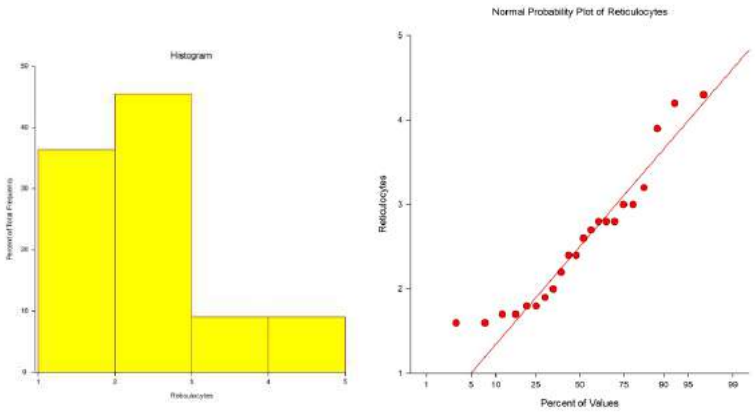
Sodium t-test (P-value): !=2.82 (0.004); *= -1.17 (0.13); ^2.76 (0.005); #= - 0.25 (0.40): *Potassium t-test (P-value):* !!= - 2.79 (0.004); **= 0.94 (0.18); ^^- 5.11 (0.0000001); ##= 0.07 (0.47): *Chloride t-test (P-value):* !!!= 0.81 (0.21); ***= -0.79 (0.22); ^^^= 2.34 (0.01); ###=1.88 (0.04); *Bicarbonate t-test (P-value):* $= - 4.26 (0.0001); $$= -3.29 (0.003); &= - 2.11 (0.02); &&= - 1.90 (0.04); @=Shapiro Wilk W (P-value) for normality test; R=reject normality; CR=Cannot reject normality.



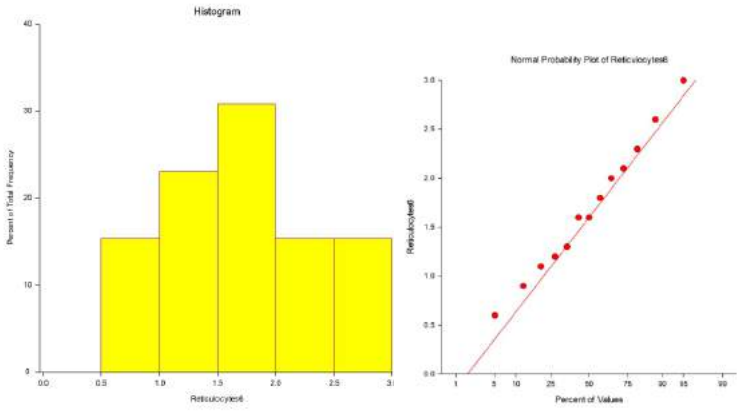
a. Pediatric Cases: Reticulocyte at enrolment (1st visit)



b. Pediatric cases: Reticulocytes count at end of study (6th visit)

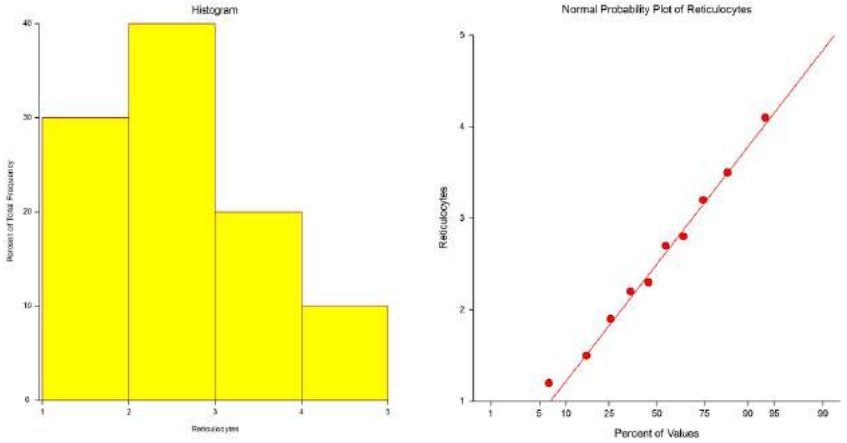


c. Pediatric Controls: Reticulocyte at enrolment (1st visit)

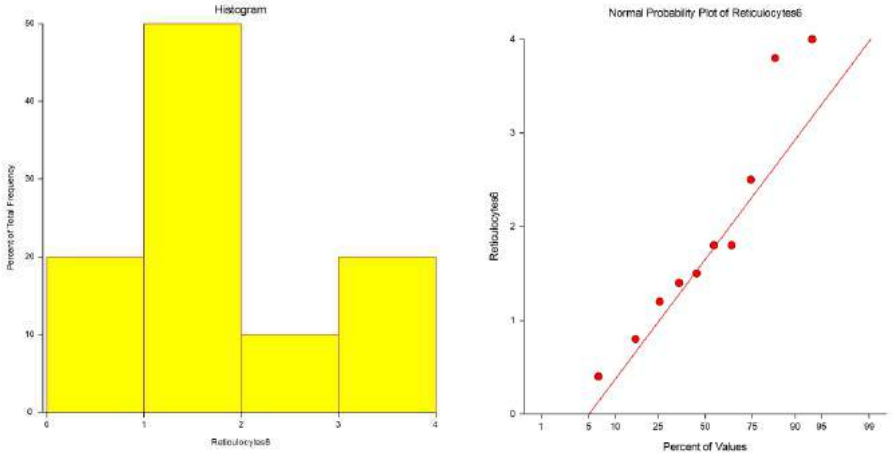


d. Pediatric control: Reticulocyte count at end of study (6th visit)

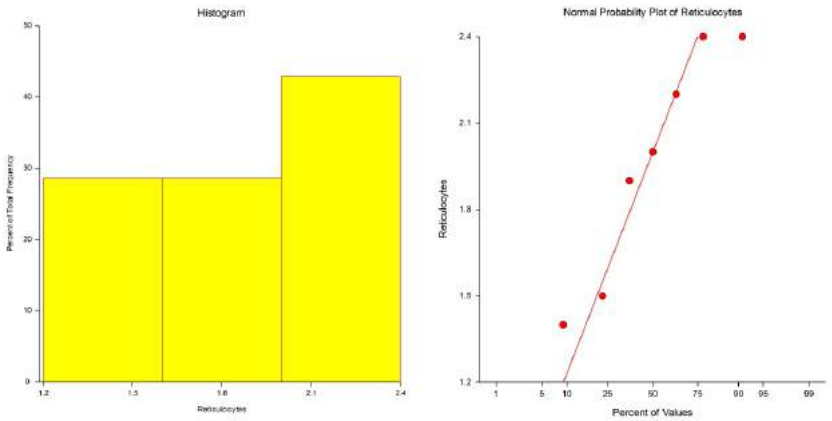
Figure 1 a-d: Reticulocyte count of pediatric cases at enrolment (a), at end of study (b) and pediatric controls at enrolment (c) and at end of study (d)



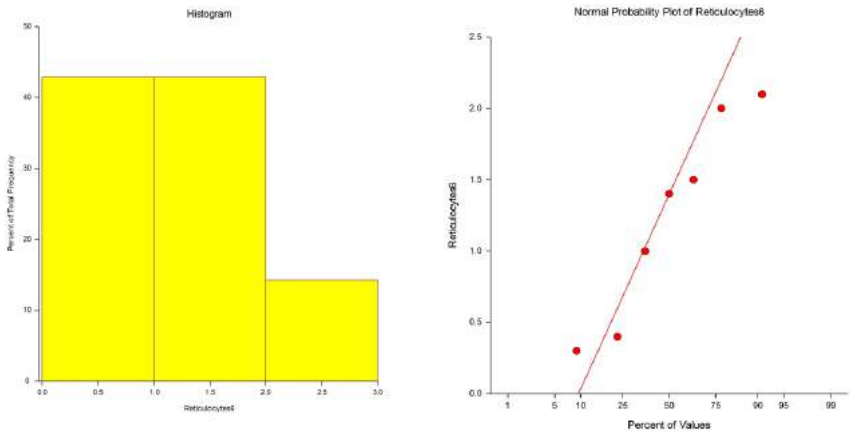
a. Adult Cases: Reticulocyte Count at enrolment (1st visit)



b. Adult Cases: Reticulocyte count at end of study (6th visit)



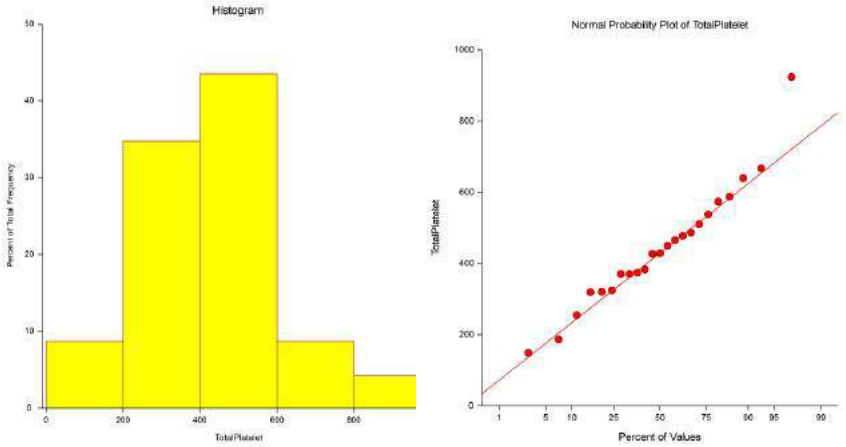
c. Adult Controls: Reticulocyte count at enrolment (1st visit)



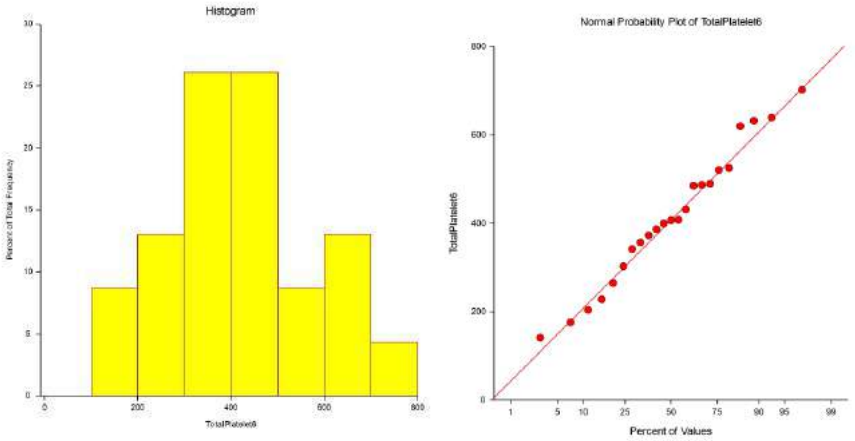
d. Adult Controls: Reticulocyte count at end of study (6th visit)

Figure 2 a-d: Reticulocyte count of adult cases at enrolment (a), at end of study (b) and of adult controls at enrolment (c) and end of study (d)

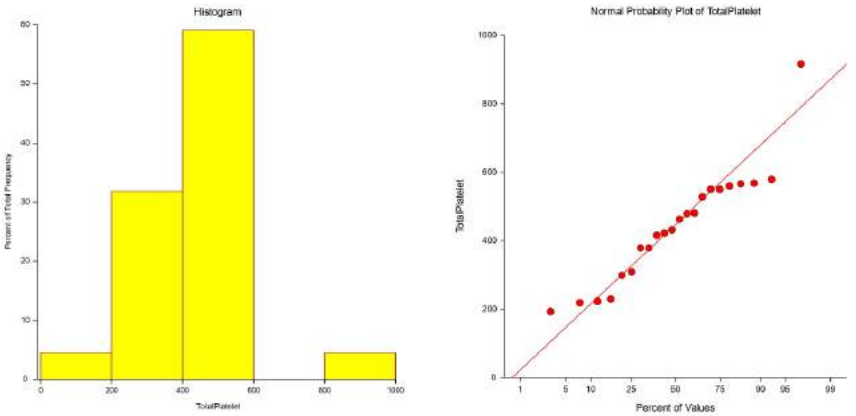
Figures 2a-d illustrate the histogram and normal probability plot of reticulocyte count of adult cases and controls at enrolment and at end of the study, indicating values equal to, below or greater than 2%



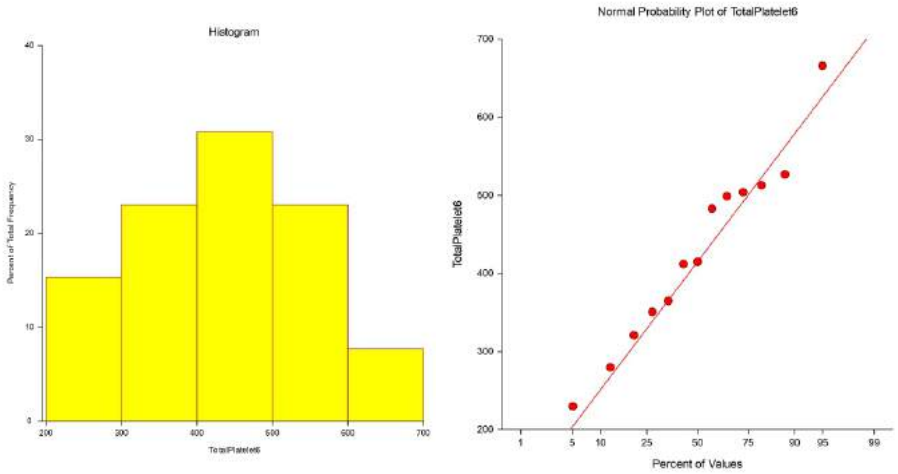
a. Pediatric Cases: Platelets count at enrolment (1st visit)



b. Pediatric Cases: Platelets count at end of study (6th visit)



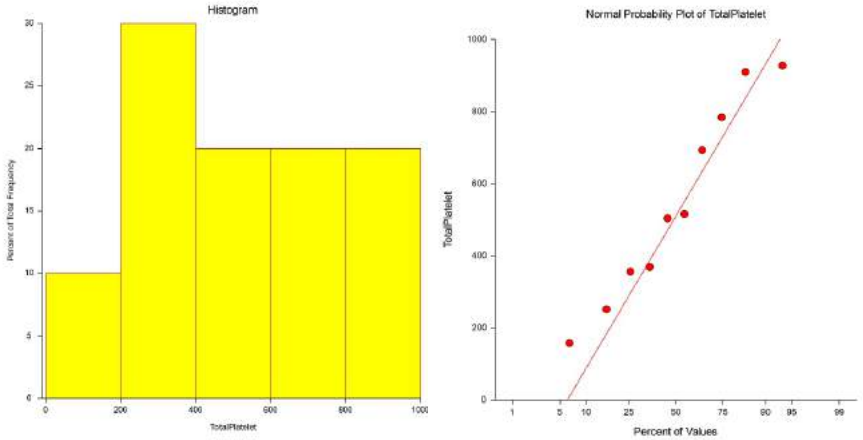
c. Pediatric Controls: Platelets count at enrolment (1st visit)



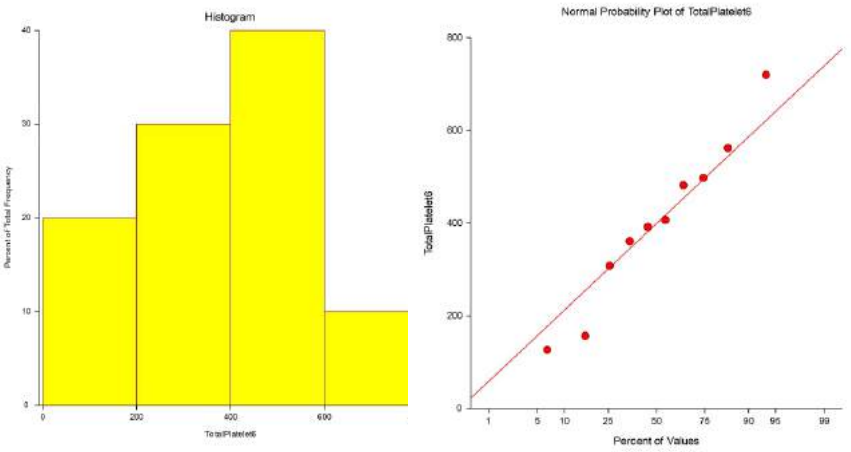
d. Pediatric Controls: Platelet count at end of study (6th visit)

Figure 3 a-d: Platelet count of pediatric cases at enrolment (a), at end of study (b) and pediatric controls at enrolment (c) and end of study (d)

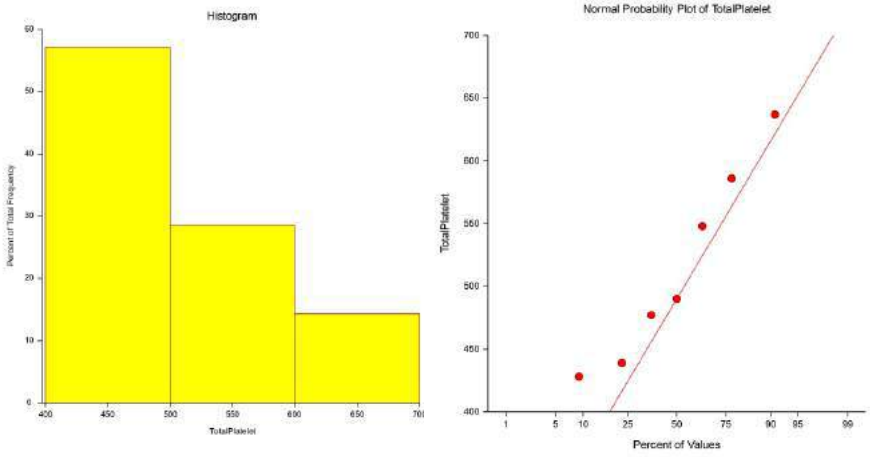
Figures 3a and 3b illustrate the histogram and normal probability plot of thrombocytes of pediatric cases at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L and Figures 3c and 3d elaborate on the histogram and normal probability plot of thrombocytes of pediatric controls at enrolment and at the end of the study showing values equal to, below or greater than 400 x 10^9/L.



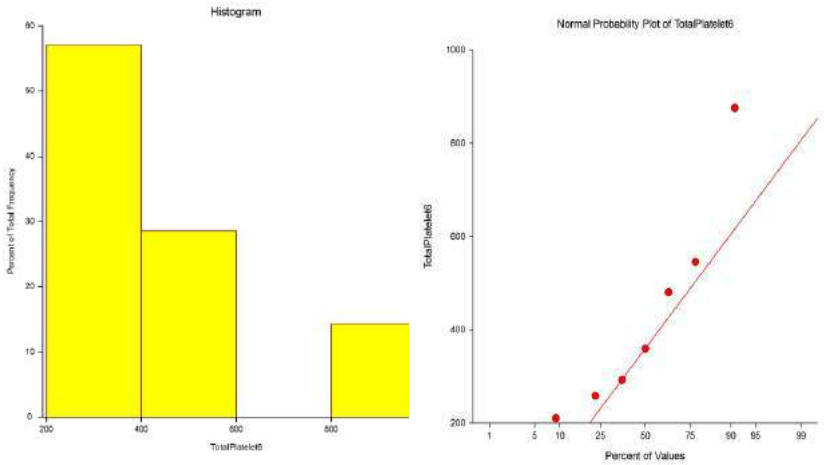
a. Adult Case: Platelets count at enrolment (1st visit)



b. Adult Cases: Platelets count at end of study (6th visit)

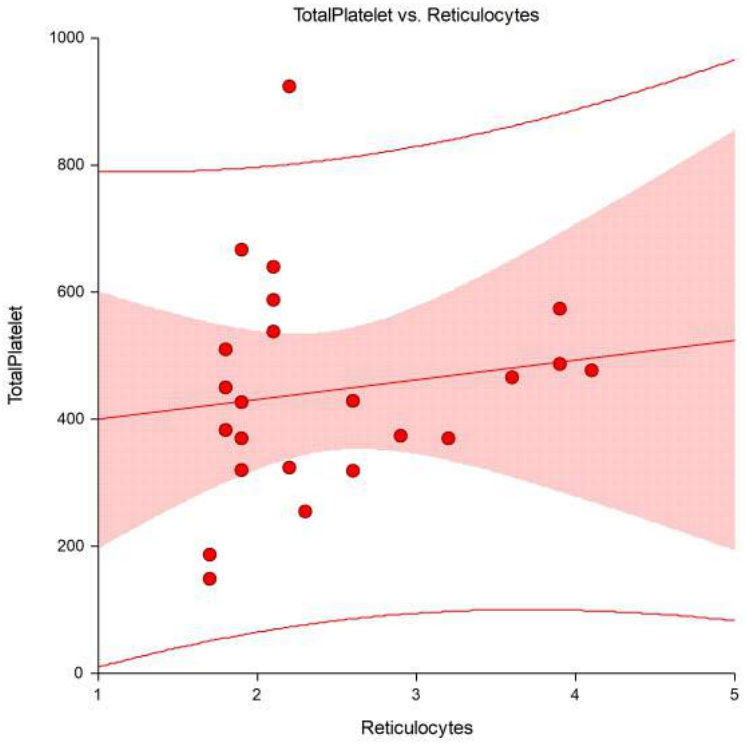


c. Adult Controls: Platelet count at enrolment (1st visit)

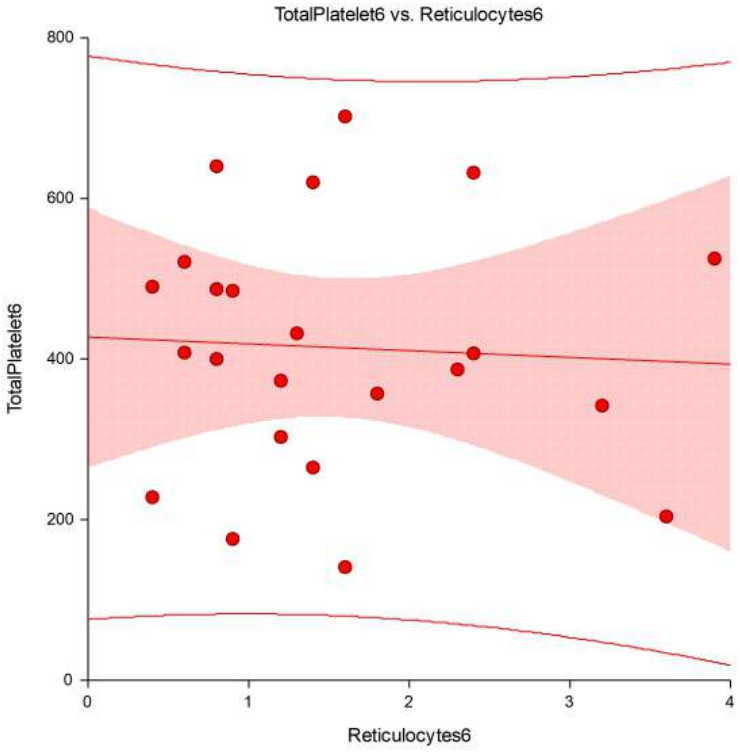


d. Adult Controls: Platelet count at end of study (6th visit)

Figure 4 a-d: Platelet count of adult cases at enrolment (a), at end of study (b) and adult controls at enrolment (c) and end of study (d)

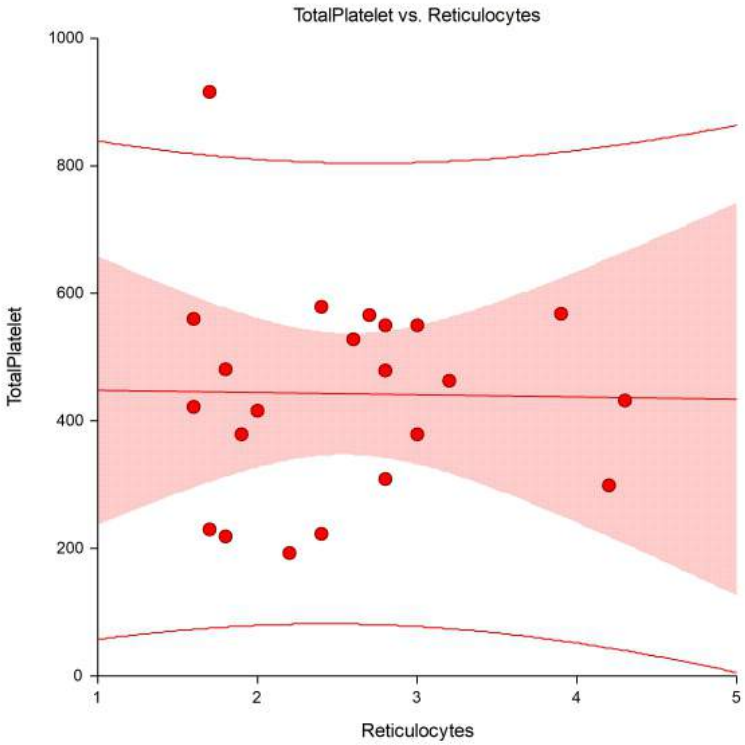


a

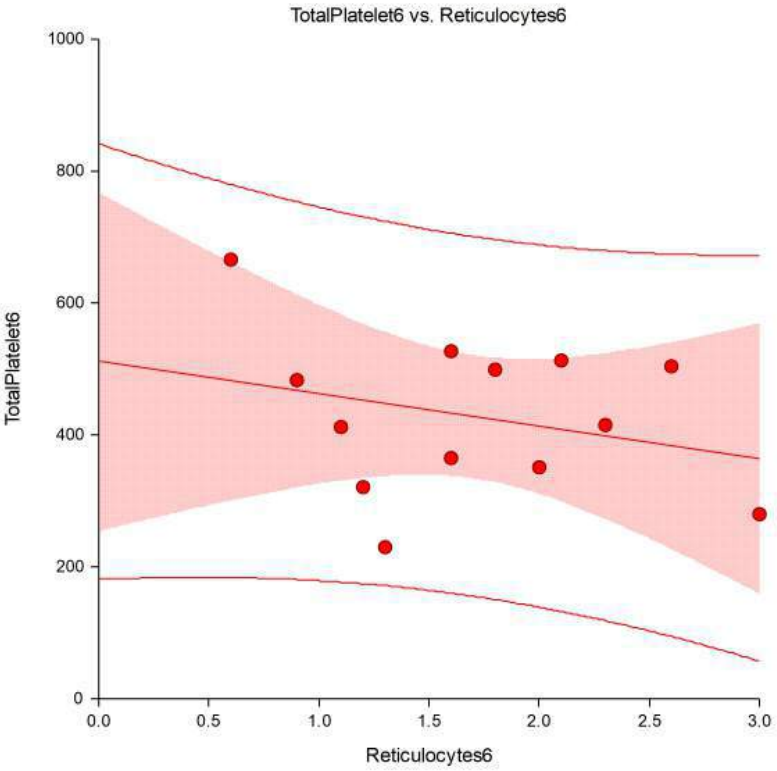


b

Figure 5 a,b: Simple linear regression (scatter plot) of pediatric cases reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

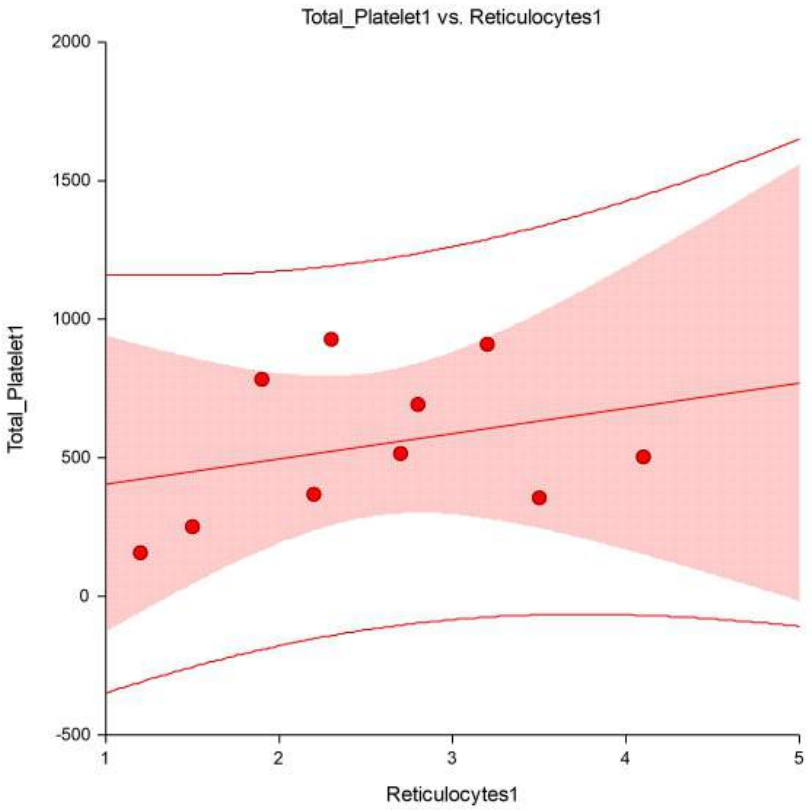


c

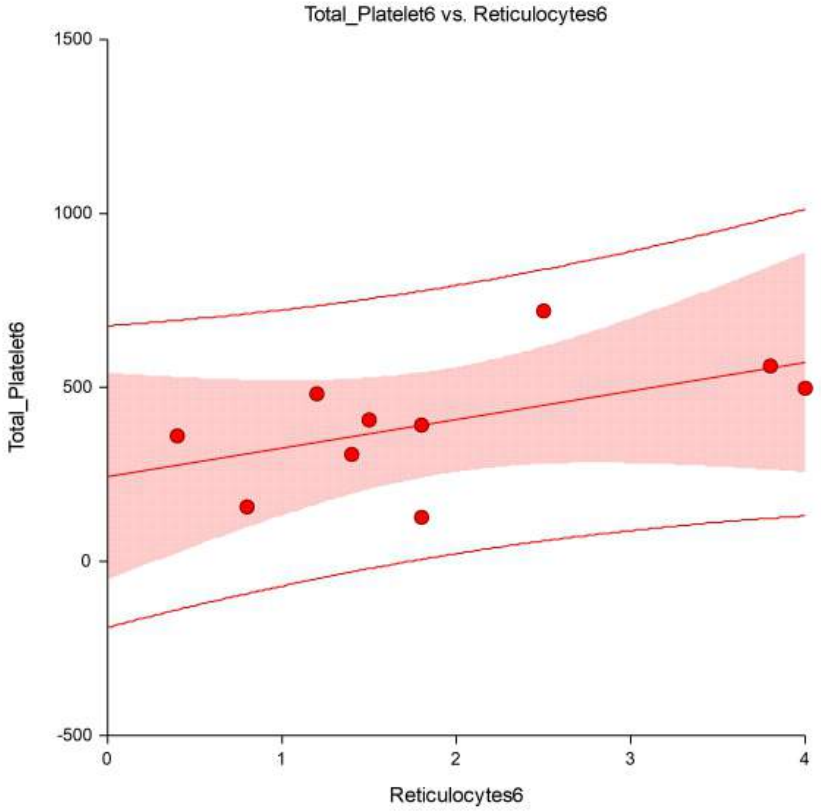


d

Figure 5 c,d: Simple linear regression between pediatric control reticulocyte and platelet counts at enrolment 1st visit (c) and at end of the study 6th visit (d)



a



b

Figure 6 a,b: Simple linear regression (scatter plot) of adult cases reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

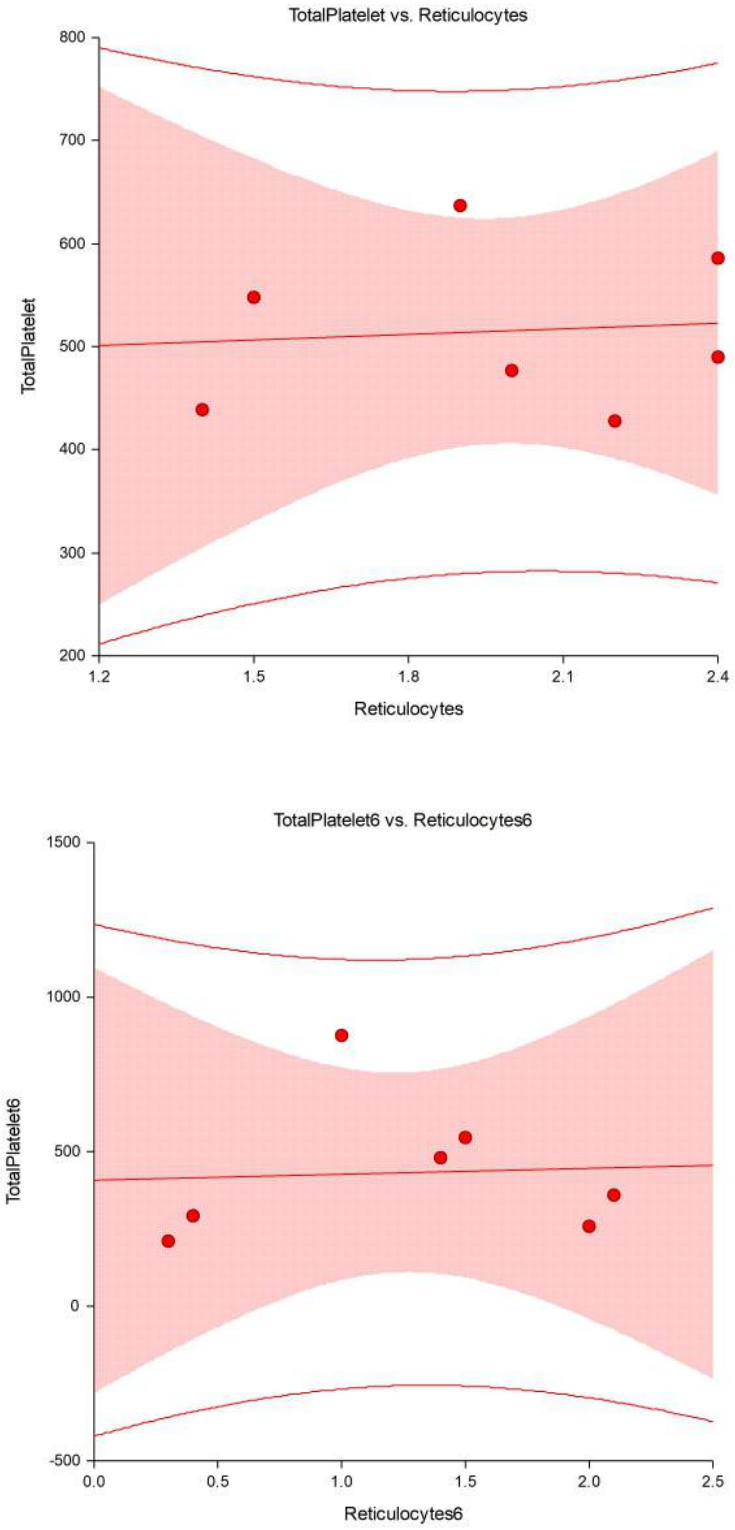


Figure 6 c,d: Simple linear regression (scatter plot) of adult control reticulocyte and platelet counts at enrolment 1st visit (a) and at end of the study 6th visit (b)

Vertebral Osteonecrosis in Childhood Sickle Cell Anaemia: A Case Report

Odunlade OC, Osho PO, Akinlosotu M, Osho ES, Joseph AA, Ojo M & Okunnuga AN

University of Medical Science

ABSTRACT

Bone infarction is a common presentation in sickle cell disease. Vertebral osteonecrosis is however not commonly reported, especially among children. We report a case of a 6 year old boy diagnosed with sickle cell anemia, presenting with vertebral osteonecrosis involving multiple lumbar vertebrae. This report highlights vertebral osteonecrosis in childhood sickle cell anemia which is an uncommon manifestation of sickle cell anemia in the pediatric age group.

Keywords: NA

Classification: NLMC CODE: WH 170

Language: English

London Journals Press

LJP Copyright ID: 392853

London Journal of Medical and Health Research

Volume 21 | Issue 2 | Compilation 1.0

Vertebral Osteonecrosis in Childhood Sickle Cell Anaemia: A Case Report

Odunlade OC[α], Osho PO[σ], Akinlosotu M[ρ], Osho ES[Ω], Joseph AA[¥], Ojo M[§] & Okunnuga AN[χ]

ABSTRACT

Bone infarction is a common presentation in sickle cell disease. Vertebral osteonecrosis is however not commonly reported, especially among children. We report a case of a 6 year old boy diagnosed with sickle cell anaemia, presenting with vertebral osteonecrosis involving multiple lumbar vertebrae. This report highlights vertebral osteonecrosis in childhood sickle cell anaemia which is an uncommon manifestation of sickle cell anaemia in the paediatric age group.

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I. INTRODUCTION

Sickle cell disease is a genetic disorder, and its mode of inheritance is autosomal recessive.[1] It is characterized by the inheritance of mutant haemoglobin genes from both parents. The disorder manifests with abnormally shaped red blood cells due to the distortion of the haemoglobin structure. The abnormally shaped red blood cells have reduced flexibility which results in occlusion within the microcirculation with resultant vaso-occlusion and bone ischaemia.

The bone is commonly involved in the manifestation of sickle cell disease.[2] Repetitive vessel occlusion involving most organ of the body including the skeleton is characteristic of the disease. Infarction, thrombosis, infection and marrow hyperplasia are some of the common bone manifestation of sickle cell disease.[3] There is usually an interplay between infarction resulting from repeated vaso-occlusion and increased susceptibility to infections, as the areas of necrosis serves as nidus for growth of organisms.[1] Avascular necrosis involving the femoral head is common in children and its prevalence increases with age.[4] Spinal necrosis is not common in children, especially in the younger age group. The clinical presentation of low back swelling and pain could connote tuberculosis infection in areas with high burden of the disease. We report this uncommon skeletal manifestation of sickle cell disease in a school age Nigerian boy.

II. CASE REPORT

A 6-year old boy with sickle cell anaemia presented at the pediatric haematology clinic of UNIMEDTH with two week history of pain and swelling over the lower back and pain involving both lower limbs. Swelling over the back had not significantly increased since it was noticed. Pain in the limbs was severe enough to cause impairment in walking and standing in an erect posture. There was no history of fever, cough, weight loss, night sweats or trauma to the lower back. He was diagnosed of sickle cell anaemia at four years and has had a fairly regular clinic attendance. He has had seven hospitalizations, mostly for vaso occlusion and haemolytic crisis. He has also had four previous blood transfusions. His last hospitalization was two months prior to the present complaint for which he was managed for vaso occlusion and haemolytic crisis with sepsis.

Physical examination revealed no fever (T-36.9◦C), there was obvious swelling with kyphosis over the lower back in the lumbar region (Fig 1) which was not tender, with no differential warmth. There was no tenderness along both lower limbs. There was normal muscle bulk.

Power and reflexes were normal in both lower limbs. He was unable to stand erect, had an anteflexion posture with an abnormal gait. He had hepatosplenomegaly. All other systemic examination findings were normal.

Blood investigation revealed a packed cell volume of 20%, raised total white blood count of 26.2 times 10^3mm and erythrocyte sedimentation rate (ESR) of 4mm/hr (1-10mm/hr). Mantoux test result was 1mm (0-4mm). A spine X-ray (Fig 2) revealed reversal of normal lumbar lordosis, osteolysis of L2-L4 with reduction in vertebral size. There was reduced intervertebral space with collapse at L2-L3. Magnetic resonance imaging and bone scans were not done in this patient, on account of financial constraints and limitation of facilities for bone scan. A diagnosis of Vertebral Osteonecrosis in childhood sickle cell anaemia was made based on clinical findings and the radiological investigations we were able to do in this patient

Conservative management included rest, analgesics, antibiotics and skeletal support with the use of lumbar braces. Patient subsequently got relieved of the pain in the lower limbs, with improved gait and was able to maintain an erect stance. Mild swelling still persists over the lower back, being site of angulation of the collapsed vertebrae. Patient is currently being followed up in the orthopaedic and haematology unit.

IV. DISCUSSION

Back pain is uncommon in the pediatric and adolescent age group unlike adults in whom it occurs commonly.[5] The presentation of low back pain however uncommon in children could suggest osteonecrosis in a child with sickle cell anaemia. The lumbar vertebrae have been reported as the commonest site of spine infarction among adults and children and this was demonstrated in this patient. Multiple spinal vertebrae involvement could occur in osteonecrosis. This patient had multiple spinal lumbar vertebrae involvement with associated vertebral collapse.

Bone infarction is a debilitating illness with significant impact in children with sickle cell anaemia. The repeated sickling of red blood cells results in irreversible and rigid red cells with resulting obstruction in microcirculation. This resultant effect of tissue ischaemia and hypoxia results in eventual cell death in the affected bone.
The presentation of bone infarction and infection could be difficult to distinguish apart from each other, due to similarity in clinical presentation. Spinal infections such as spondylitis and acute osteomyelitis occur commonly among people with sickle cell anaemia. Acute osteomyelitis is usually due to Staphylococcal aureus and Salmonella infection. In the case presented, fever was absent, and ESR was not elevated. However this patient had an elevated total white blood count. The patient had a mantoux test done to exclude tuberculosis, considering its high burden among the study population. There were no other features deferrable to tuberculosis in this child, and mantoux result was within normal limits, thus anti-tuberculous therapy was not considered in this patient.

Magnetic resonance imaging (MRI) is considered as the best imaging option when pathology involving the spine is being considered.[5] Marrow infiltration and H-shaped vertebrae as a result of central growth plate infarction has been reported as MRI findings in vertebral bone osteonecrosis.[6,7] Lumbar-sacral spine x-rays are useful in diagnosis where there are constraints to obtaining a MRI as it was in the reported case. The fish vertebrae sign and vanishing vertebrae have been demonstrated on X-ray.[8] The differences in the blood supply of the central part of the vertebrae and the peripheral parts are responsible for this findings. The longer arteries supplying the central vertebrae are more affected by infarction and the consequent bone destruction.[9] The findings of osteopenia, with reduced bone height, and vertebral collapse were highly suggestive in this case.

Vertebral osteonecrosis is amenable to conservative management including bed rest, analgesia and use of vertebral braces as it was in this case. A course of antibiotics was considered in this patient on account of elevated WBC. There is

a possible interplay of infection and infarction in sickle cell disease, also bone infarction and necrosis creates a nidus for bacterial growth and spread.[1]

Emodi anoye[10] had earlier reported two cases of nine year old Nigeria girls with sickle cell anaemia that had vertebral collapse, both were managed conservatively similar to this study. The patient in this study is however 6years and thus younger than the cases they reported. In a retrospective study of osteoarticular manifestation of sickle cell anaemia in Nigeria children, Chinawa et al reported vertebral collapse in one child in the 6-10years age group out of twenty –five children with musculoskeletal complications of sickle cell anaemia. However details of clinical presentation and management was not discussed in their study

V. CONCLUSION

Vertebral osteonecrosis is an uncommon presentation in children with vaso-occlusion. It should be considered in young children with sickle cell anemia, presenting with low back pain. Careful evaluation and exclusion of other common causes of low back pain should be done with imaging and other relevant studies. Early orthopaedic consultation and conservative management as seen in this case is associated with good patient outcome.

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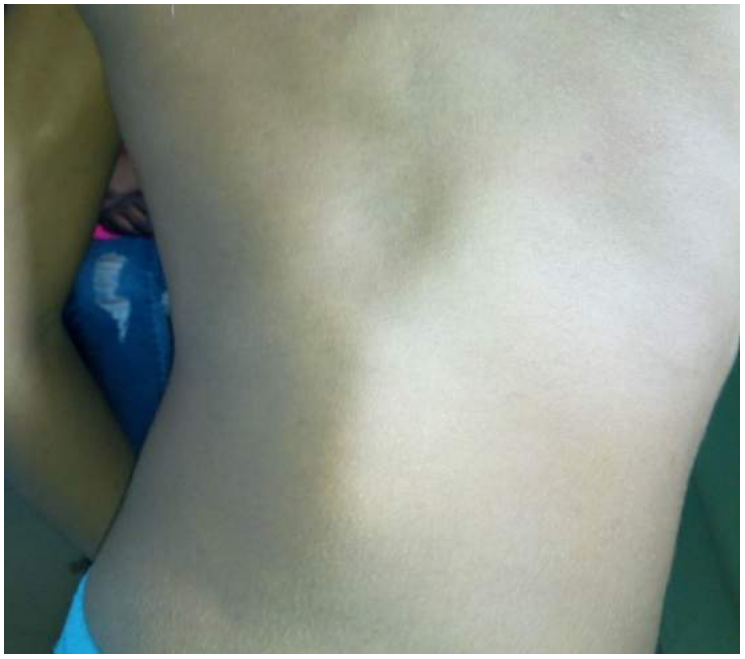

Figure 1: Swelling over the lower back and lateral curvature of the spine

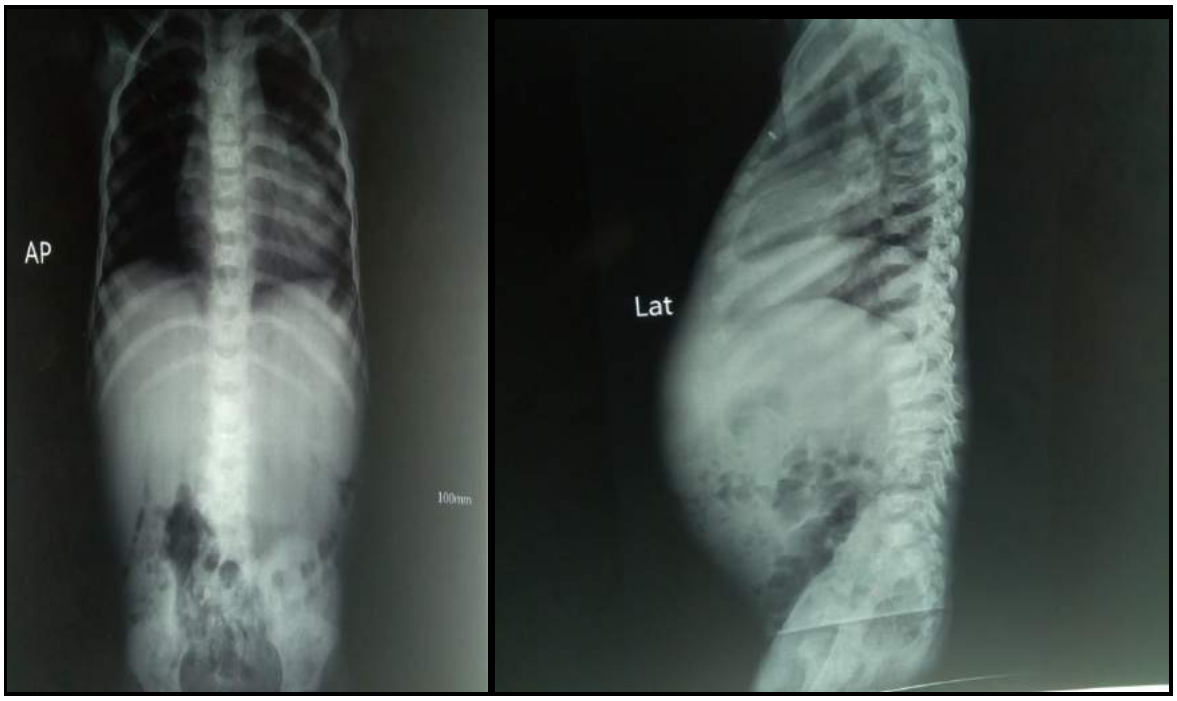

Figure 2: AP and lateral X-ray view of spine showing mild scoliosis, reduction in vertebral heights L2-L5 with irregular outlines and reduction in intervertebral disc space with collapse of L2-L3

Lived Experiences of Pregnancy among Women with Sickle Cell Disease Receiving Care at Mulago Hospital, Kampala, Uganda: A Qualitative Study

Tumwesige Kenneth, Namagembe Imelda, Kayiga Herbert, Munube Deogratias & Rujumba Joseph

Makerere University

ABSTRACT

Background: Women with sickle cell disease in Mulago National Referral Hospital face challenges when they become pregnant and they receive the same care as all other high risk pregnant women who come to the hospital. This study explored the lived experiences of pregnancy among women with sickle cell disease receiving care at Mulago National Referral Hospital.

Methods: This was a qualitative phenomenological study conducted with 15 participants who were women with sickle cell disease with the experience of pregnancy. In-depth audio recorded interviews were conducted to collect data from women who were pregnant at time of study or had ever been pregnant aged 16 to 38 years of age with sickle cell disease. Recorded data was transcribed and analyzed using content thematic approach.

Keywords: lived experiences, sickle cell disease, pregnancy.

Classification: NLMC CODE: WH 170

Language: English

London Journal of Medical and Health Research

Volume 21 | Issue 2 | Compilation 1.0

Lived Experiences of Pregnancy among Women with Sickle Cell Disease Receiving Care at Mulago Hospital, Kampala, Uganda: A Qualitative Study

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ABSTRACT

Background: Women with sickle cell disease in Mulago National Referral Hospital face challenges when they become pregnant and they receive the same care as all other high risk pregnant women who come to the hospital. This study explored the lived experiences of pregnancy among women with sickle cell disease receiving care at Mulago National Referral Hospital.

Methods: This was a qualitative phenomenological study conducted with 15 participants who were women with sickle cell disease with the experience of pregnancy. In-depth audio recorded interviews were conducted to collect data from women who were pregnant at time of study or had ever been pregnant aged 16 to 38 years of age with sickle cell disease. Recorded data was transcribed and analyzed using content thematic approach.

Results: This study revealed that pregnant women with sickle cell disease faced both negative and positive health care experiences and individual lived experiences of pregnancy. The few positive individual lived experiences were joy of motherhood and giving birth to child free of sickle cell disease whereas the negative individual lived experiences reported were recurrent painful crises, pregnancy loss, premature delivery, stigma and discouragement, relationship discord and desertion by spouse. There were few positive healthcare experiences reported in this study which included: dedicated care and support from health workers, referral to specialist services, support from their male partners and the negative healthcare experiences reported were delay to get medical assistance, informal hospital charges, unsatisfactory care, and advocacy for caesarian section Vs normal delivery.

Conclusion: Pregnant women with sickle cell disease lived with great expectation and in fear of being further weakened by the disease. Their experiences were in general negative at both individual, social and health system levels.

Keywords: lived experiences, sickle cell disease, pregnancy.

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I. BACKGROUND

Sickle cell disease (SCD) is defined as an autosomal recessive hemoglobinopathy that includes SCD (HbSS) and various compound heterozygous genotypes (e.g. sickle cell carrier (HbSC) or sickle cell b-thalassemia disease (HbSb-thal) characterized by chronic hemolytic anemia and vaso-occlusive complications [1]. Sickle cell disease is the commonest hereditary disorder and affects 30 million people worldwide [2] and about 80% of SCD cases are believed to be in Sub-saharan Africa [3] with 300,000 children born with the condition each year [4] and in Uganda alone 20,000 babies per year are born with SCD [5]. Therefore, it's against this background that we explored the lived experiences of pregnancy among patients with SCD in MNRH.

Pregnancy is a potentially serious condition among women living with SCD and can leave them even more fragile and insecure [6]. The fear of death and the death of their child is justified by the history of women who experience these complications. The women become aware of this fear from the reports of other women affected by the disease or from those who have had similar experiences in the family [7, 8]. Pregnancy is a potentially serious condition among women living with SCD and can leave them even more fragile and insecure[6]. During pregnancy, 50% to 70% of them require at least one hospitalization and 30-40% requires transfusion. Pulmonary hypertension which affects 6%-11% of SCD patients is especially morbid in pregnancy due to cardiopulmonary demands of gestation and has previously led to 30%-50% maternal mortality[9]. Fear and anxiety marked the pregnancy of women suffering from SCD while dilemmas permeated the decision to abort[10], and the desire to be a mother was frustrated anticipated complications of frequent hospitalizations, urinary tract infections, painful crises, blood transfusions, orthopedic complications and miscarriage but also due to the lack of equipment and lack of social support for exercising motherhood[11].

Health care services and health care professionals are poorly prepared to care for these women, especially during pregnancy, which can contribute to increasing the insecurity and fear they experience in this life stage. Access to quality prenatal care is often hampered for women suffering from SCD, either by physical and economic limitations to access services, or by the discrimination suffered because of their race, gender and low social class, in which these women find themselves. The difficulty of access to hospitals is one of the main factors responsible for maternal deaths [6].

However, while many previous studies have explored how SCD affects pregnancy outcomes [12], few studies have explored what women with SCD go through during pregnancy. This presents a missed opportunity for understanding the experiences that these women go through during pregnancy and impedes our ability to assist them to enjoy the same health care services that other women without SCD enjoy. With this study we bridged this gap by exploring the lived experiences of pregnancy among women with SCD receiving care from MNRH.

II. METHODS

2.1 Study design

This was a qualitative phenomenological study that explored the lived experiences of pregnant women with SCD receiving care in MNRH in Kampala, Uganda. Data collection took place between October to December 2018. The choice of the qualitative approach was based on the fact that this approach provides the opportunity to document lived experiences of pregnancy among women with sickle cell disease through more in-depth and detailed narratives.

2.2 Study setting

The study was conducted in MNRH in Kampala, Uganda's Capital City. The selection of the study site was based on the fact that this is the national referral hospital for the country and handles all the high risk pregnant mothers and therefore would be able to get a big number of women with sickle cell disease that were pregnant at the time of study or had ever been pregnant. At the time of study, MNRH was under restructuring and the department of obstetrics and gynecology was in Kawempe Hospital and the hematology clinic was in Kiruddu Hospital.

III. RECRUITMENT OF PARTICIPANTS

Mulago National Referral Hospital records offices were searched to get patient files from outpatients unit, ward records books and antenatal books so as to identify women with SCD managed before and those who were receiving care from the hospital at that time. From the findings a total of 113 women with SCD in the reproductive age had received care from both Kawempe and Kiruddu Hospitals. 113 phone contacts were extracted from the hospital records and phone calls made to the participants inviting them to the clinics.

From Kawempe Hospital, Department of Obstetrics and Gynecology 46 possible participants'

phone numbers were extracted and only 7 of these participants were women with history of pregnancy accepted to be part of the study. Four women had interviews in the hospital and 3 at home. Antenatal and postnatal women with SCD admitted on the ward or attending antenatal care during the period of the study were recruited after they had stabilized or given appointments to return after discharge.

From Kiruddu Hospital, the hematology clinic record files were searched to look for women with SCD who were in the reproductive age and a total of 67 phone contacts of women with SCD were extracted and 8 women met the criteria to participate and so appointments were fixed by the PI with options of interviews done from hospital (5 women), home (1 woman) or at their place of work (2 women),

For interviews that were conducted in the hospital setting, we secured a consultation room in both Kawempe and Kiruddu Hospitals and requested the in-charges to give us the room for the interview without any other activity going on in that room.

For interviews conducted at home, we agreed with the mothers to fix a date when she had enough help at home from someone who took away the baby for a few minutes and we sat in the house to avoid interference.

For interviews at the workplace we scheduled interviews at hours that were not busy, one was a hotel manager while the second participant had a boutique shop in town and both recommended interviews are done early morning hours.

IV. DATA COLLECTION

A qualitative approach of semi-structured in depth interviews was used to explore the lived experiences of pregnancy among women with SCD. The interview guide was in English and translated to Luganda to make it easy for participants to understand. Thus an appropriate language was used for each participant. Eight participants were called and accepted to come to the hospital and we requested those who couldn't come to the clinic to give us appointments and four participants preferred interviews done at their homes, two participants gave us appointments at their workplaces while only one participant was admitted in the hospital after delivery and she was interviewed on the day of discharge. This approach emphasis was to focus on capturing the participants' perspective, enlisting the participant as the expert knower, describing the participants lived experience from their own perspective and searching for the meanings individuals give to their particular lived experiences. The responses of each participant were audio recorded using a recorder so that no information was lost.

V. DATA ANALYSIS

This study used a descriptive phenomenology approach which favors thick description and close analysis of the lived experiences to understand how meaning is created through embodied perception[11]. Data analysis followed the six steps of data analysis advanced by Colaizzi [13].

Each interview was transcribed verbatim by a research assistant with experience in qualitative research to maintain contextual information. The individual identifiers were masked and transcripts were coded as P1-P15 to maintain anonymity. Each transcript was reviewed by the principal investigator alongside the audio-file for accuracy. The principal investigator then read through each transcript in order to become familiar with the collected data.

A code book was generated to give description of codes, significant and research relevant phrases were extracted from the transcripts by coding. This involved generating an initial list of concepts and themes by reading the data and creating as many relevant codes as possible and grouping the data accordingly.

The codes/themes were sorted according to each participant using a coding framework and matrix to explore whether they have an indication of similar or disparate experiences. Each of the above steps was repeated for each transcript and organized to illustrate an aggregated meaning. The main themes that emerged from the above steps of analysis of the interviews were described and used to give an exhaustive description of the

investigated phenomenon to understand the lived experience of women with SCD regarding pregnancy.

VI. ETHICAL CONSIDERATION

Permission to do the research was sought from School of Medicine Research Ethics Committee (SOMREC), Uganda National Council for Science and Technology, Department of Obstetrics and Gynecology and Hematology department of Mulago National Referral Hospital.

The study was individually explained to each participant and they were also encouraged to ask questions and seek clarification on anything related to the study. Informed consent was obtained from all study participants. Each study participant gave an informed consent and this obtained from the study participants before data was collection. Study participants under 18years were considered as emancipated minors gave their own consent for participation in the study.

Confidentiality was maintained for all participants by ensuring that all the members of the research team understood what confidentiality meant and ensured that all the participants' information was only accessed by the study staff and the consent forms and transcripts were kept under lock and key. To further maintain confidentiality, the study participants' names were not used but rather study numbers were assigned to keep their identity anonymous.

VII. RESULTS

7.1 Participant Characteristics

A total of 15 women with sickle cell disease participated in the study, of whom 12 had a history of pregnancy and 3 were pregnant at the time of the study. Seven of the study participants were from Kawempe hospital and 8 were from Kiruddu hospital. The age range of participants was 16-38, most of the women with sickle cell disease in this study were single and majority had attained secondary education. Majority of the par- ticipants had prior pregnancy experience and only a few were currently pregnant at the time of the study (see table 1).

Table 1: Characteristic of women with sickle cell disease

Categories	Number	Percentage (%)
Age		
15-25	4	26.6%
26-35	7	46.6%
36-45	4	26.6%
Marital status		
Single	8	53.3%
Married	7	46.6%
Education		
Primary	1	6.6%
Secondary	12	80.0%
Tertiary	2	13.3%
Pregnancy status		
Prior pregnancy	12	80.0%
Currently pregnant	3	20.0%
Place of interview		
Hospital	9	60.0%
Home	4	26.6%
Work place	2	13.3%

VIII. LIVED EXPERIENCES OF PREGNANCY AMONG WOMEN WITH SCD RECEIVING CARE FROM MNRH

The study explored lived experiences of pregnancy among women with SCD receiving care from MNRH and the findings are summarized under two major themes 1) Individual lived experiences and 2) health care service experiences as shown in table 2.

Table 2: Lived Experiences of Pregnancy Among Women With Scd Receiving Care From Mnrh

Sub themes	Theme	Phenomenon
POSITIVE EXPERIENCE • Joy of motherhood • Giving birth to a baby without SCD	Individual lived experiences	Lived experiences of pregnancy among women with SCD
NEGATIVE EXPERIENCES • Recurrent painful crises • Pregnancy loss • Premature delivery • Stigma and discouragement • Relationship discord and desertion by spouse		
POSITIVE EXPERIENCES • Dedicated care and support from health workers • Referral to specialist services • Support from their male partners	Health care service experiences	
NEGATIVE EXPERIENCES • Delay to get medical assistance • Informal hospital charges • Unsatisfactory care • Advocacy for caesarian section Vs normal delivery		

IX. INDIVIDUAL LIVED EXPERIENCES

Women with SCD in this study expressed both positive and negative individual lived experiences of pregnancy. A few of the study participants had positive lived experiences like Joy of motherhood and giving birth to a baby without sickle cell disease like the mother. Most of the women had negative lived experiences such as recurrent painful crises, pregnancy loss, premature delivery, stigma and discouragement, relationship discord and desertion by spouse.

9.1 Joy of motherhood

Only a few of the participants reported great desire to have a baby regardless of the advice to abort and were willing to risk their lives to become a mother as this would make them happy as in the excerpt below;

> *"Okay there is a certain doctor who told me that a woman with sickle cell disease should not get pregnant because if you get pregnant, you can easily lose your life or the baby will die. So it is best we remove the baby now. Then I just told the doctor, ahh it's just a gift from God. So, if it goes well that's what God wants but if I die, that's what God wants but for me, I will have my baby." (pg 2 line 51-58) P1*

9.2 Giving birth to a baby without sickle cell disease like the mother

One Mother who had given birth to a child without sickle cell disease expressed happiness knowing that her child will not be affected by SCD as narrated below;

> *"I remember I received like 3 units of blood, yeah but the good chance I got was the baby*

was not affected with the sickness." (pg 2 line 38-41) P6

Most of the mothers in our study narrated the dark side of their individual experiences of pregnancy as described in the following subsections.

9.3 Recurrent painful crises

Most of the mothers narrated how they had experienced painful crises during the pregnancy, having severe body pains and struggling with their health status often resulting into frequent hospitalization and blood transfusions conditions uncommon to their non-sickle cell disease counterparts as the mothers explained;

"I went through a lot of painful experiences; I could be feeling dizzy, joint pains...I even got an attack and could get severe headaches every now and then. It is during this time that I went to hospital and was admitted." (Page 1 Line 12-14) P10

For most mothers keeping a stable health status was an uphill task, especially the mother who had been pregnant more than once noted that the complications in SCD such as pain, fever, blood transfusions increased during pregnancy as explained below by one of them.

"Again while pregnant I got a fever, and when I went to the clinic the doctor reported that I was anemic. I was transfused with 5 bottles of blood and 4 of drip water. I had fear for my life because I had heard from people that blood from [mentions name of hospital] is infected; with this wrong information I ended up running out of hospital." (Pg1 Line 11-14) P8

9.4 Pregnancy loss

Three of the participants had experienced a miscarriage or intrauterine fetal death a process they described as difficult and painful as one was quoted in the excerpt below;

"Okay my condition was not good so they gave me some injections and suddenly they told me that my baby had died in the stomach and yet it was okay from before because I went for a scan on Monday then again on Friday. They told me to go back again for scanning. Then when I went there, they told me that the baby died and yet the baby had been okay. It was painful" (pg 1 line 12-18) P1.

9.5 Premature delivery

One of the mothers narrated that carrying the child to full term was a challenge as she ended up having to give birth earlier than expected and cesarean delivery was done to save the baby as well as her life and the fear of death was evident for mothers in the study.

"They said this child is about to get delivered before it's mature but another doctor said let us first give her this medicine in the meantime so that the lungs can grow. So they injected me with medicine. That medicine was very painful but I didn't care about its pain. I just prayed to God for one thing that he may deliver me and my child. And I stayed in Mulago for a week because she was premature." pg 2 line 50-53) P3.

9.6 Stigma and discouragement

Apart from the community beliefs and expectation that women with SCD can hardly conceive and bear children, pregnant mothers also shared experiences of being stigmatized; receiving discouraging and negative comments from those around them. This created feelings of being alone and not cared for as one woman mention;

"In most cases, the medical people blame you. You are a woman with sickle cell disease, why did you go for it? You knew, you think you can carry this pregnancy. You can't. You knew you were a woman with sickle cell disease, why did you engage in sex to get pregnant?" (pg 9 line 285-287) P4

Another woman with sickle cell disease carrying her first pregnancy narrated how she was being criticized and discouraged by health workers for being pregnant she reported as quoted below;

"When I went to the hospital for the first time, the doctor that attended to me told me not to

get pregnant again and she went on to tell me I shouldn't have gotten pregnant because women with sickle cell disease don't give birth and die at the time of delivery." (Page 4 Line 75-77) P12

The stigma, discouragement and criticism received by pregnant mothers often led to anxiety and depression as one of the mothers carrying her second pregnancy expressed in the excerpt below;

"When my first born was 4years I conceived again because I could get different advice from different people not to at least stop at one child; while pregnant with this child, I became ill because I was depressed… There reached a time when I could be depressed that I will not make it. And on one occasion while I was pregnant I remember I lost my consciousness and after gaining it after 3days I realized they had operated on me and removed the baby after getting an attack that almost took my life." (Pg 1 Line 9-10) P8

9.7 Relationship discord and desertion by spouses

Most mothers with SCD were single mothers as they reported being deserted by their male partners. It's should be noted that a positive relationship between pregnant women and their partners is vital as being deserted by their partners put a strain on their well being as one of the participants explained;

"Now me, the one who said he would take responsibility when he knew that I was pregnant, he took off. So when he took off, family members were suggesting that I imprison him. But I refused. The man ran away completely….. When I tried to contact the family, for them they were saying ah ah we can't manage you. So I was left like that. So I carried the pregnancy up to, it was like even up to term." (pg 2 line 18-35) P4

Some male partners after realizing the health condition of their partners fail to take their roles as fathers and this resulted in separation as one of the mothers narrated;

"The biggest problem is with men, after impregnating you they deny you after knowing you are having sickle cell disease. There is a friend of mine previously who consulted him [participant's partner] asking why he left and he was telling him that I am sick of HIV because of the sickle cell crises I was getting so throughout the pregnancy, the 9 months, he refused to give care. He was just quarrelling so I decided to give up with him because even the baby had died." (pg 2 line 40-42) P9.

A few of the mothers noted that they had experienced desertion even from family members who at such times would have been a great source of support especially for those who had been abandoned by their partners as one of them is quoted below;

"For even my family members disappeared. The bad thing is my mother and dad; they died a long time ago and my sisters also gave up on me… " (pg 2 line 43-46) P4

Abandonment left these women lonely and without anyone to care for them especially where admission was involved as one of the women explained;

"I was very okay. But this one came due to stress. So I was admitted. I was there alone. I was left alone…. After he left I felt lonely and all alone. It was not a nice experience. That is the first thing I told you. I had no one to take care of me." (pg 4 line 151-154) P4.

X. HEALTH CARE EXPERIENCES

While participants recognized the importance of access to health care, there were several concerns that related to their experience with the facilities and the healthcare providers. A few were positive experiences divided into sub themes which included: dedicated care and support from health workers, referral to specialist services and support from their male partners while the majority had negative experiences with the health care they received summarized into sub-themes including: delay to get medical assistance, unsatisfactory care and inappropriate health care systems.

XI. POSITIVE EXPERIENCES

11.1 Dedicated care and support from health workers

Empathy, humanity, understanding and respect were qualities of health care providers, including doctors and nurses that a few of the mothers appreciated and credited as in the narrative below;

> *"Those people, they always attended to us in time. The results would come back on time, the feedback on how to solve the problem. They made sure that the baby inside is very fine, it was their routine to do that whenever we visited. And at least, they would look out for us." (pg 5-6 line 163-165) P7*

11.2 Referral to specialist services

Most of the mothers acknowledged that they were a special population from other women and required special care and attention. Thus attending a health facility where they were considered priority, having personnel offering a service and doing everything in their power to see that the mother is well created a favorable experience. However few of the mothers reported that they got linkage to specialist care which they received at MNRH as seen in the narratives below;

> *"So when I went to the hospital, there is also our clinic, a sickle cell clinic. There is a doctor. He connected me to a specialist that I went to see. When I went, he told me some things and I did them".... (pg 3 line 78-81) P2.*

> *"And the doctor told me you should go to Mulago and get this person but I didn't know that [mentions specialist's name] was there and I received specialized care. So, it was just God. It was just God." (pg 7 line 209-211) P2.*

11.3 Support from their male partners

The other important positive health care experience that was revealed was having a supportive partner. This was important because the male partners would provide care and love which is key in the wellbeing of the pregnant mother in the hospital as one mothers narrated in the excerpt below;

> *"They tell us that we can't but me I have seen that if you have good support, and when you are getting support, you are cared for, then you can carry the pregnancy up to term, that one, it is only care and love. When I was weak, he was there for me." (pg 3 line 63-71) P4*

XII. NEGATIVE EXPERIENCES

12.1 Delay to get medical assistance

Most mothers had great delays in receiving medical assistance when they arrived at the health facility however these delays weren't unique for the mothers with SCD. For some delays in care resulted in worsening of the pain crises as expressed in the excerpts below

> *"In the morning, the doctor told me we have to have an operation and get the baby out. There was a doctor who came to check on me and told the nurse, take this one to the theatre so she can be operated upon at 2 pm.So I was there, waiting, knowing that at 2pm, I am going to theatre but I spent two solid days and was not taken yet the doctor had even already signed the forms. Until at night, I got an attack. I am pregnant; I started getting pain in the stomach. Remember, the time had elapsed. I was in so much pain...." (pg 2 line 56-70)P5*

Delayed medical assistance was not only for in-patient mothers but similar experiences were witnessed by out-patient mothers who often became exhausted and frustrated as one participant expressed below.

> *"You spend a long time waiting at the hospital... You come and sit for long, waiting. Sometimes you even go back without seeing someone...Sometimes you find the doctors tired. You find someone and he's not in the moods, he's not in the moods at all. And he is like "Ah you people, we are tired. Come back next time."(pg 7 line 214-225) P2*

12.2 Informal hospital charges

Some of the delays in receiving health care related to inability to pay informal charges. One mother also noted that they were discriminated against on the basis of inability to pay the informal charges as she narrates below;

> *They didn't treat me well, even worse than the last time. We gave them the money. The one you give the money does not work at night. There was a lot of money spent. We had to plead with them. Then when you give him 10,000shs and the other person has given him 20,000shs so the one who gave him 10,000shs is left and he goes with the one that gave him a lot more.(pg 4 line 30-38) P11*

The notion 'No money, No care' seemed to resonate with some of the mothers who could not afford to offer some money and 'oil the health worker's hands ' [bribe] to the health worker in order to receive care and or receive care urgently even in circumstances of life and death.

> *"There was a friend who was also brought. She has SCD and twins. As for her, she got pressure and even her children died but they didn't care. She was a poor woman.... the doctors would bypass her just like that until the girl got pressure. But if they had rushed her, they [the twins] would not have died. That is when I started getting worried. The things we go through are terrible. If you don't have money, you cannot give birth."(pg 3 line 84-89) P13*

12.3 Unsatisfactory care

Despite waiting for long to see a health worker and being faithful to return to the clinic on their scheduled hospital visit days, the mothers relented that some of their visits were less informative and were not handled well by the health workers as most of the mothers narrated below; at the end of the day did not value their coming to the health facility or seeking medical assistance.

> *"But for the pregnancy, I used to come here, on the day they told you to come back. You come, they check on you, but I don't know whether they used to estimate. They used to test the pressure, and they are telling me it is normal, it is normal but you are not well, till when I saw that it is too much and I said that let me go and try out at sickle cell clinic. Maybe I will get help." (pg 7 line 203-207) P2*

Two of the mothers narrated that many times during their hospital visits, they were attended to by a doctor who did not know much about SCD or did not know how to handle a pregnant woman with SCD. The mothers expressed the concern of limited knowledge by the healthcare workers more so handling pregnancy situations among women with SCD as reported in excerpt below;

> *"It is just that when I get an attack; the problem is the other people don't really know much about SCD, and worse still when you are pregnant because I have been in so many health facilities, I have similar experience. You can reach the facility and you are the one to give them details that, 'when am like this, they do this and that and treat me in this way' laughs." (Pg 3 Line 78-78) P13*

As a result of being less experienced in handling SCD patients, most mothers shared that many health care workers gave less care and attention to them despite their vulnerability. The mothers narrated that in some cases, the health workers were afraid or scared to handle/ care for a mother with such condition.

> *"...even recently, they took me when I was heavy and in crisis, but reaching the emergency section at [mentions name of regional referral hospital], the nurses and doctors were just looking at me. No one even came near my bed.*
> *Of course, the medical people we meet many times don't understand sickle cell and yet they are the medical people who should know the condition but they don't understand." (pg 7 line 239-240) P4*

Certainly, as a result of receiving suboptimal care and attention, seeking antenatal care and support became unpopular to most mothers who never

perceived the need for ANC if they had no complications.

> *"People don't seem to care when you go. In the early months I had no complications whatsoever. My very first antenatal visit was at 7months. I was forced to go because I felt heaviness and I had started feeling unwell. If it wasn't for the pain, I would not have gone." (Page 1 Line 10-11) P10*

12.4 Advocacy for caesarian section Vs normal delivery

To some mothers, the feeling that the health workers were less experienced to handle mothers with SCD is the reason they exhibited less care and attention. They believed that the health workers wanted to simplify their lives when handling pregnant women with SCD in labor by resorting/ advocating for C-section against normal delivery.

> *"I went back for admission. My doctor promised to take me for a caesarean section with no explanation. So I was on the ward and I told the doctor that now it is full term and I have not heard any movement. But I had moved up to term so I could push." (pg 2 line 35-37) P4*

XIII. DISCUSSION

This phenomenological study explored the lived experiences of pregnancy among women with sickle cell disease. Most women had negative experiences at individual and health system levels. The dominance of the negative experiences among pregnant women with SCD in this study can be explained by inadequate support at family, community and health system levels. The negative experiences were worsened by the widespread belief that women with sickle cell disease shouldn't become pregnant because of the feared maternal and fetal complications. As a result women in this study lived in constant fear and had moments of depression. Our findings are similar to those of a study done in Brazil where pregnant women with SCD thus experienced sadness, depression and thought of interrupting the pregnancy [6].

The few women who described positive experiences drew on the joy of motherhood, giving birth to babies free of sickle cell disease and special treatments they occasionally received from some health workers. Many of the women with SCD report a desire to realize the dream of their companions – paternity but also the desire for children so as to constitute a biological family, which emphasizes the strength of the marriage bond and gender differences [14].

This study has revealed that women with SCD have their dreams of being a mother frustrated by their inability to effectively utilize health care services due to several barriers at individual, community and health system levels. Navigating the delicate balance between caution from community and health workers with the reality of pregnancy was a major barrier to health care service utilization among women with SCD. Women in this study feared to be blamed by the community members and health workers for getting pregnant which threatened their lives. In a study done by Ross et.al among the African-American group of women with sickle cell disease, participants reported that their family members and health care providers believed that they were too sick to bear children and they would become more sick or even die when pregnant[15].

In this context by becoming pregnant, women with SCD were viewed as those who didn't take heed of advice from their family, friends and health care providers and thus were stigmatized at home, in communities and at health care facilities. As a result of stigma most women with SCD were reluctant to seek health care services or did so with a lot of difficulty. Consistent with findings of this study, Xavier in Brazil reported that people suffering from SCD are stigmatized [6, 16, 17]. The implication here is that efforts to improve the quality of care for these women should address stigma as a key barrier to health care utilization.

This study also revealed that lack of partner support, inaccessible health facility, informal hospital charges, lack of drugs and other supplies were other key barriers faced by pregnant women with SCD in their effort to utilize health care services. These barriers reflect the general

inadequacies within the health care system where women with SCD seek to utilize reproductive health care services. These barriers to health care utilization were also documented in Jamaica by Asani et.al where it was found that most delays in care were due to patient factors or quality of care once the patient had been seen, with limitations in availability of blood, drugs and technical equipment[12]. While these constraints are faced by other pregnant women, those with SCD are more affected because they frequently utilize health care services for the complications they faced with.

XIV. CONCLUSIONS

Pregnant women with SCD receiving care from MNRH had both individual lived experiences and health care experiences which were mostly negative experiences with a few positive experiences during pregnancy. Stigma and discouragement with relationship discord were viewed as the most negative experiences whereas the joy of motherhood and giving birth to a child who was free from sickle cell disease were the main positive experiences for the mothers.

Abbreviations

SCD: Sickle cell disease; MNRH: Mulago national referral hospital, MUK: Makerere University; MUST: Mbarara University of Science and Technology.

Declarations

Ethics approval

Permission to do the research was sought from the School of Medicine Research Ethics Committee of Makerere University. The committee reviewed the proposal and granted approval on 24/04/2018 with reference number: #REC REF 2018-065.

Consent to participate

The study was individually explained to each participant and they were also encouraged to ask questions and seek clarification on anything related to the study. Informed consent was obtained from all study participants. It was emphasized to the participants that participation and withdrawal from the study was voluntary. Each study participant gave an informed consent and this obtained from the study participants before data was collection. Study participants under 18years were considered as emancipated minors and have their own consent for participation in the study.

Consent for publication

Not applicable because no images, videos or audio voices included.

Availability of data and materials

The data sets used for this study are available from the corresponding author upon a justified request.

Competing interests

The authors declare that they have no competing interests.

Funding

Self sponsored

Author's contributions

KT; Conceived the study, designed, collected data, participated in analysis and interpretation of data, wrote the first draft of the paper and was responsible for the final submission of the paper.
IN; Made substantial contributions to conception, design, data collection, analysis and interpretation of data.
HK; Made substantial contributions to conception, design, data collection, analysis and interpretation of data.
DM; Made substantial contributions to conception, design, data collection, analysis and interpretation of data.
JR; Made substantial contributions to conception, design, data collection, analysis and interpretation of data.
All authors have read and approved the manuscript.

ACKNOWLEDGEMENT

The authors would like to appreciate Mulago National Referral Hospital for allowing us to access the hospital records. We also appreciate women with sickle cell disease who receive care from MNRH for participating in the study.

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