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Co-infection of Parvovirus B19 and *Plasmodium falciparum* among Sickle Cell Disease Patients in Benin City, Nigeria

[1]Moses-Otutu, I. M., [2]Okojie, R. O., [1*]Akinbo, F. O., and [2]Eghafona, N. O.

[1]Department of Medical Laboratory Science, School of Basic Medical Sciences,
University of Benin, Benin City, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences,
University of Benin, Benin City, Nigeria.
*Correspondence to: fgbengang@yahoo.com

Abstract:

Background: Infections by parasites, bacteria, viruses such as human parvovirus B19 amongst others, have been widely reported as contributing to high prevalence of anaemia in many populations. This study was conducted to determine the co-infection of *Plasmodium falciparum* and human parvovirus B19 among sickle cell disease (SCD) patients in Benin City, Edo State, Nigeria. **Methodology:** A total of 400 participants consisting 300 SCD patients (134 males, 166 females) and 100 (38 males, 62 females) apparently healthy subjects with haemoglobin AA (which served as control) who were contacted in homes, schools and offices, were enrolled for the study. The age of the participants ranged from 1 to 54 years. Venous blood was collected for detection of *P. falciparum* using Giemsa stain while parvovirus B19 was detected with enzyme linked immunosorbent assay (ELISA). Full blood count was estimated using Sysmex KX-21N haematology auto-analyzer. **Results:** An overall prevalence of parvovirus B19 and *P. falciparum* co-infection observed among SCD patients in this study was 3.0% while single infection was 14.0% for *P. falciparum* and 26.7% for parvovirus B19. Religion was associated with 0 to 22 fold increased risk of acquiring co-infection of *P. falciparum* and parvovirus B19. Gender was significantly associated with *P. falciparum* infection (p=0.0291) while tribal extraction, platelet index and seasonal variation were significantly associated with single parvovirus B19 or co-infection of *P. falciparum* and parvovirus B19 (p<0.05). **Conclusion:** The provision of strict regulatory policy concerning the screening of whole blood or pooled plasma before the use of blood products and transfusion of SCD patients is advocated.

Keywords: parvovirus B19, Benin City, *P. falciparum*, sickle cell disease

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Co-infection par le parvovirus B19 et *Plasmodium falciparum* chez des patients atteints de drépanocytose à Benin City, au Nigéria

[1]Moses-Otutu, I. M., [2]Okojie, R. O., [1*]Akinbo, F. O., and [2]Eghafona, N. O.

[1]Département des sciences de laboratoire médical, École des sciences médicales de base,
Université du Bénin, Benin City, Nigéria

[2]Département de microbiologie, faculté des sciences de la vie,
Université du Bénin, Benin City, Nigéria
*Correspondance à: fgbengang@yahoo.com

Abstrait:

Contexte: Il a été largement rapporté que les infections par des parasites, des bactéries, des virus tels que le parvovirus humain B19, contribuent à la prévalence élevée de l'anémie dans de nombreuses populations. Cette étude visait à déterminer la co-infection de *Plasmodium falciparum* et du parvovirus humain B19 chez des patients atteints de drépanocytose à Benin City, dans l'État d'Edo, au Nigéria.
Méthodologie: Un total de 400 participants comprenant 300 patients atteints de MCA (134 hommes, 166 femmes) et 100 (38 hommes et 62 femmes) des sujets apparemment en bonne santé avec l'hémoglobine AA (qui servait de contrôle) qui ont été contactés à la maison, dans les écoles et au bureau inscrit à l'étude. L'âge des participants allait de 1 à 54 ans. Le sang veineux a été recueilli pour la détection de *P. falciparum* à l'aide de la coloration de Giemsa, tandis que le parvovirus B19 a été détecté par un test d'immunosorbant lié à une enzyme (ELISA). La numération globulaire totale a été estimée à l'aide de l'auto-analyseur d'hématologie Sysmex KX-21N.
Résultats: La prévalence globale de la co-infection au parvovirus B19 et à *P. falciparum* observée chez les patients atteints de MCs dans cette étude était de 3,0%, tandis que l'infection simple était de 14,0% pour *P. falciparum* et de 26,7% pour le parvovirus B19. La religion était associée à un risque accru de contracter la co-infection à *P. falciparum* et au parvovirus B19 de 0 à 22 fois plus élevé. Le sexe était significativement associé à l'infection à P. *falciparum* (p = 0,0291), tandis que l'extraction tribale, l'indice plaquettaire et la variation saisonnière étaient significativement associés à un parvovirus simple B19 ou à une co-infection à *P. falciparum* et au parvovirus B19 (p <0,05)
Conclusion: La mise en place d'une politique réglementaire stricte concernant le dépistage du sang total ou du plasma réuni avant l'utilisation du produit sanguin et la transfusion de patients atteints de MCS est recommandée.

Mots-clés: parvovirus B19, Benin City, *Plasmodium falciparum*, drépanocytose

Introduction:

Sickle cell disease (SCD) is known to consist of several disorders characterized by the presence of sickle haemoglobin (1). An estimated 300,000 children are born annually with SCD worldwide. This constitutes about 1% of the global population of SCD with over 75% in sub-Saharan Africa (2, 3). The high birth rate of SCD has highlighted the burden of SCD as a public health priority (3). However, there is a dearth of information on the burden of SCD to healthcare system and the significance on individual health (4).

Infection with Parvovirus B19 is common and can lead to a variety of clinical manifestations based on the immunological and haematological status of patients (5). Parvovirus B19 belongs to the family *Parvoviridae* which is sub-divided into *Parvovirinae* and *Densovirinae* depending on the type of the infected host (6). Parvovirus B19 has specific tropism for erythroid progenitor cells and is capable of causing temporary infection of the bone marrow resulting in transient arrest of erythropoiesis (7). In patients with underlying haemolysis or haemato-logical disorders such as sickle cell disease, acute B19 infection may cause transient aplastic anaemia, erythema infectiosum, hydrops fetalis, abrupt and severe anaemia due to failure of red blood cell production (8, 9, 10). This virus is transmitted mainly via respiratory droplets but can be spread by contaminated blood, organ transplantation and transmission from mother to foetus (11).

Malaria is one of the major causes of morbidity and mortality in tropical and sub-tropical countries and is caused by the protozoan parasites of the genus *Plasmodium* with *P. falciparum* being the most virulent species (12). Malaria causes over 200 million cases of febrile illness out of which over a million children living in sub-Saharan Africa die annually (13, 14).

It is widely seen as a major health challenge in Africans with SCD (15).

Parvovirus B19 infection can cause significant drop in haemoglobin concentration and reticulocyte count, conditions that could have serious consequences in patients particularly children with underlying malaria or those in malaria endemic regions (16). There are a number of studies that have emphasized the importance of co-infection with Parvovirus B19 in the etiology and pathogenesis of malaria in adults and children in non-sickle cell disease subjects (17-22). It is recognized that interactions between SCD and other infectious agents influence the health status of SCD patients. Parasites, bacteria, human parvovirus B19, and other infectious agents have been widely reported as important factors contributing to the high prevalence of anaemia in many populations (23, 24, 25, 26).

There is however a dearth of information on the co-infection of *P. falciparum* and Parvovirus B19 among SCD patients in our environment. Against this background, this study was conducted to determine the co-infection of these pathogens among SCD patients in Benin City, Edo State, Nigeria.

Materials and methods:

Study population

The study was conducted between September 2017 and July 2018 at the Sickle Cell Center, Benin City, Edo State. The Sickle Cell Center has a referral status for the management of SCD patients Edo, Delta, and other neighbouring states. A total of 400 participants consisting of 300 SCD patients (134 males and 166 females) and 100 (38 males and 62 females) apparently healthy subjects with haemoglobin AA that were contacted in homes, schools and offices (served as control), were enrolled for the study. The age of the participants ranged from 1 and 54 years.

A well-structured questionnaire was administered to collect bio-data and other demographic information from the participants. Informed consent was obtained from all subjects or the parents or guardians in the case of children prior to specimen collection. The protocol for this study was approved by the Ethics and Research Committee of the Ministry of Health, Edo State, Nigeria.

Specimen collection and processing

Venous blood sample of about 8 ml was collected from each participant, out of which 4.5 ml was dispensed into ethylene diamine tetraacetic acid (EDTA) bottle and thoroughly mixed. The remaining 3.5 ml sample was dispensed into plain container, allowed to clot, and serum separated for Parvovirus B19 analysis. *Plasmodium falciparum* was detected using a previously described method (27). Briefly, both thick and thin blood films were made from each blood specimen and allowed to air-dry. The blood films were stained in 3% Giemsa stain for 30 min, rinsed in tap water and allowed to air dry. The thick film was examined microscopically for presence of malaria parasite while the thin film was used to detect the species of *Plasmodium* using the oil immersion lens. A total of 200 fields per film were examined.

Full blood count was analyzed using a Sysmex KX-21N haematology auto-analyzer (Sysmex Corporation, Japan). Whole blood specimen dispensed into EDTA container was used. Anaemia was defined using the WHO criteria as haemoglobin concentration <13 g/dl for males and <12 g/dl for females (28). Parvovirus B19 was detected using enzyme-linked immunosorbent assay (ELISA) technique (Serion classic Parvovirus B19 IgG/IgM Wuzburg, Germany). Briefly, each sample was assayed according to the manufacturer's instruction using peroxidase-labeled rabbit anti-human IgM as the secondary antibody, tetramethyl benzidine as a substrate, and 1M H_2SO_4 as a stop solution. The absorbance was read at 450 nm using a spectrophotometer. Index value between 10 and 15 was taken as reference value, with samples below the index range taken as negative while value

above this range was taken as positive for IgM.

Statistical analysis

The data generated were analyzed using Chi square (X^2) test for frequency data whereas the odd ratio was calculated for each potential risk factor. The statistical software used was INSTAT (GraphPad Software Inc, La Jolla, CA. USA).

Results:

The prevalence of 26.7% for parvovirus B19, 14.0% for *P. falciparum* and 3.0% for co-infection of both pathogens were reported among the SCD patients, while prevalence of 4.7% for B19 infection only was observed in the control subjects. Gender was not significantly associated with prevalence of B19 infection or co-infection of B19 and *P. falciparum* (p>0.05) (Table 1). However, gender was significantly associated with the prevalence of *P. falciparum* infection among the SCD patients (OR=0.445; 95% CI=0.2186, 0.9095; p=0.0291). The age of SCD patients was not associated with single infection as well as co-infection of B19 and *P. falciparum* in the study (p>0.05). Educational status and religion were also not significantly associated with single and co-infection of B19 and *P. falciparum* (p>0.05).

Tribal extraction was significantly associated with single and co-infection of B19 and *P. falciparum* with the Etsako subjects (Edo State) being the most infected (53.9%) by B19 (p=0.0065), Yoruba tribe had the highest prevalence of *P. falciparum* infection (45.5%) (p=0.0137) while the Hausa tribe had the highest prevalence (20.0%) of coinfect-ion of B19 and *P. falciparum* among the SCD patients (p=0.0012). Seasonal variation was not significantly associated with prevalence of co-infection of B19 and *P. falciparum* (p>0.05). However, rainy season significantly influenced the prevalence of B19 infection among SCD patients (OR=2.077; 95% CI=1.171, 3.684; p=0.0144)

Table 1: Factors associated with infection of parvovirus B19 and *Plasmodium falciparum* among sickle cell disease patients in Benin-City, Nigeria

Factors	No tested	No infected (%)	OR	95%CI	*p* value
Gender					
Parvovirus B19					
Male	134	36 (26.9)	1.019	0.6088,1.704	1.000
Female	166	44 (67.7)			
P. falciparum					
Male	134	12 (9.0)	0.4459	0.2186,0.9095	0.0291
Female	166	30 (18.1)			
Co-infection					
Male	134	5 (3.7)	1.570	0.4130, 0.5967	0.5194
Female	136	4 (2.4)			
Age group (years)					
Parvovirus B19					
1-10	89	14 (15.7)			0.0746
11-20	122	37 (30.3)			
21-30	53	16 (30.2)			
31-40	29	11 (37.9)			
41 & above	7	2 (28.6)			
P. falciparum					
1-10	89	14 (15.7)			0.0654
11-20	122	20 (16.3)			
21-30	53	4 (7.5)			
31-40	29	2 (6.9)			
41 & above	7	2 (28.6)			
Co-infection					
1-10	89	2 (2.2)			0.2357
11-20	122	3 (2.5)			
21-30	53	3 (5.7)			

31-40	29	0			
41 & above	7	1 (14.3)			
Educational status					
Parvovirus B 19					
Primary	120	21 (17.5)			
Secondary	98	32 (32.7)			
Tertiary	82	27 (32.9)			
P. falciparum					
Primary	120	16 (13.3)			
Secondary	98	19 (19.4)			
Tertiary	82	7 (8.5)			
Co-infection					
Primary	120	2 (1.7)			
Secondary	98	4 (4.1)			
Tertiary	82	3 (3.7)			
Religion					
Parvovirus B19					
Christian	282	74 (26.2)	0.7115	0.2577, 1.964	0.5828
Muslim	18	6 (33.3)			
P. falciparum					
Christian	282	39 (13.8)	1.246	0.3447, 4.505	0.7257
Muslim	18	3 (16.7)			
Co-infection					
Christian	282	9 (3.2)	1.285	0.7191, 22.969	1.000
Muslim	18	6 (33.3)			
Tribe					
Parvovirus B 19					
Igbo	48	14 (29.2)			0.0065
Yoruba	11	4 (36.4)			
Ibibio	21	8 (38.1)			
Bini	164	31 (18.9)			
Esan	20	7 (35.0)			
Etsako	26	14 (53.9)			
Hausa	10	2 (20.0)			
P. falciparum	48	4 (8.3)			0.0137
Igbo	11	5 (45.5)			
Yoruba	21	2 (9.5)			
Ibibio	164	19 (11.6)			
Bini	20	4 (20.0)			
Esan	26	6 (23.1)			
Etsako	10	0			
Hausa					
Co-infection					
Igbo	48	2 (4.2)			0.0012
Yoruba	11	2 (18.2)			
Ibibio	21	0			
Bini	164	2 (1.2)			
Esan	20	0			
Etsako	26	1 (3.85)			
Hausa	10	2 (20.0)			
Season					
Parvovirus B19					
Rainy	190	60 (31.6)	2.077	1.171, 3.684	0.0144
Dry	110	20 (18.2)			
P. falciparum					
Rainy	190	31 (16.3)	1.755	0.8436, 3.650	0.1669
Dry	110	11 (10.0)			
Co-infection					
Rainy	190	7 (3.7)	2.066	0.4213, 10.127	0.4939
Dry	110	2 (1.8)			

Table 2: Effect of haematological factors on co-infection of Parvovirus B19 and *Plasmodium falciparum* among Sickle Cell Disease patients in Benin-City, Nigeria

Factors/Patients	No tested	No infected	OR	95% CI	*p* value
Transfusion					
Parvovirus B19					
Yes	176	46 (26.1)	0.9367	0.5577, 1.573	0.8946
No	124	34 (27.4)			
P. falciparum					
Yes	176	26 (14.8)	1.170	0.5985, 2.287	0.7363
No	124	16 (12.9)			
Co-infection					
Yes	176	6 (3.4)	1.424	0.3490, 5.806	0.7406
No	124	3 (2.4)			
Anaemia					
Parvovirus B19					
Anaemia	292	76 (26.0)	0.3519	0.08584, 1.442	0.2160
No anaemia	8	4 (50.0)			
P. falciparum					
Anaemia	292	40 (13.7)	0.4762	0.09282, 2.443	0.3110
No anaemia	8	2 (25.0)			
Co-infection					
Anaemia	292	8 (2.7)	0.1972	0.02162, 1.798	0.2186
No anaemia	8	1 (12.5)			
Platelet count (cells/μL)					
Parvovirus B19					
< 150,000	24	6 (25.0)	0.9099	0.3478, 2.381	1.000
≥ 150,000	276	74 (26.8)			
P. falciparum					
< 150,000	24	4 (16.7)	1.253	0.4058, 3.866	0.7576
≥ 150,000	276	38 (13.8)			
Co-infection					
< 150,000	24	4 (16.7)	10.840	2.696, 43.580	0.0031
≥ 150,000	276	5 (1.8)			

History of blood transfusion was not significantly associated with the prevalence of single or co-infection of B19 and *P. falciparum* among the SCD patients (p>0.05) (Table 2). Anaemia was also not significantly associated with single or co-infection of B19 and *P. falciparum* (p>0.05). However, platelets count was significantly associated with co-infection of B19 and *P. falciparum* among the SCD patients especially with platelet count of<150 cells/μL (OR 0.840, 95%CI 2.696, 43.580, p=0.0031).

Discussion:

Sickle cell disease runs a variable clinical course ranging from mild disease to severe life threatening complications (29). Individuals with SCD are known to be susceptible to infectious agents (30, 31). This study examined parvovirus B19 and *P. falciparum* infections in SCD patients in our locality. To our knowledge, this is the first study on this in Edo State. It has been hypothesized that depression of cell-mediated immunity in *P. falciparum* infection might favour co-infection with opportunistic pathogens including Parvovirus B19 (32). An overall prevalence of parvovirus B19 and *P. falciparum* co-infection observed in this study was 3.0% whereas the single infection was 14.0% for *P. falciparum* and 26.7% for B19. The prevalence of co-infection of B19 and *P. falciparum* observed in our study is lower than the 14.21% observed in non-SCD patients in Gabon (33). This difference in prevalence rates may be related to population studied, geographical location and seasonal variation.

Gender was not significantly associated with co-infection of *P. falciparum* and B19 although it was significantly associated with *P. falciparum* infection among the SCD patients. Similarly, age was not significantly associated with single infection or co-

infection of B19 and *P. falciparum* among the SCD patients. These observations in our study may indicate adherence of SCD patients or their parents or guardians or relatives to health information that can aid quality of life of SCD patients, usually provided by their clinicians.

Patients or individuals living in malaria endemic regions are known to be at increased risk of serious complications with co-infection of B19 and *P. falciparum* (34). In individuals with SCD who have tolerated chronic anaemia, there could be rapid worsening of the anaemia, which can present as an emergency (4). Under these circumstances, anaemia becomes life threatening and requires prompt treatment with blood transfusion to reduce the deleterious effects of haemoglobin S and improve outcome (4). Parvovirus B19 and *P. falciparum* are easily transmitted by blood transfusion and transfusion with plasma derived products (35). SCD patients are known to be at high risk of transfusion-transmissible infections since they receive frequent, often unplanned, emergency blood transfusion (36, 37). Surprisingly, history of blood transfusion was not significantly associated with single and co-infection of B19 and *P. falciparum* among our SCD patients. The reason for this finding is unclear.

In this study, religion and educational status of our SCD patients were not significantly associated with single or co-infection of B19 and *P. falciparum*. However, tribal extraction was significantly associated with single or co-infection of B19 and *P. falciparum* among the SCD patients, with the SCD patients of Etsako tribe in Edo State having the highest prevalence (53.9%) of B19 infection, the Yoruba tribe had the highest prevalence (45.5%) of *P. falciparum* while the Hausa tribe had the highest prevalence of co-infection (20.0%) of B19 and *P. falciparum*. The reasons for these tribal differences remain to be elucidated. Seasonal variation in prevalence of malaria is well established with highest prevalence during the rainy season (38, 39). Surprisingly, seasonal variation was not significantly associated with prevalence of *P. falciparum* malaria and co-infection of B19 and *P. falciparum* in our SCD patients, but was significantly associated with the prevalence of B19 infection, with highest prevalence (31.6%) in the raining season compared to the dry season (18.2%) (p=0.0144).

Both immunological and non-immunological destructions of platelets have been implicated to cause thrombocytopenia, resulting from consumptive coagulopathy, platelet sequestration in spleen, antibody mediated platelet destruction and oxidative stress. Platelet may also act as cofactor to trigger severe malaria, and abnormalities in platelet structure and function have been described as a consequence of malaria and in rare instances, platelets can be invaded by malaria parasites (40, 41, 42). Previous studies have indicated the involvement of white cells and platelets in single infection and co-infection of B19 and *P. falciparum* (43, 44, 45, 46). In our study, platelet index was not significantly associated with prevalence of single infection of B19 or *P. falciparum*. However, platelet count of <150 cells/μL was a risk factor as it was associated with a 2 to 43 fold increased risk of acquiring co-infection of B19 and *P. falciparum* among our SCD patients. In addition, platelet index was significantly associated with the prevalence of co-infection of B19 and *P. falciparum* among the SCD patients. Our findings are in agreement with the previous report of Girei *et al*. among SCD patients with B19 infection in Jos (46).

Parvovirus B19 and *P. falciparum* co-infections have been reported to cause severe anaemia, which can be fatal particularly among SCD patients (11, 47, 48). Parvovirus B19 causes anaemia because it selectively inhibits and lyse actively replicating erythroid progenitor cells (9, 49) which are targets of *P. falciparum*, co-infection of the two pathogens therefore result in severe anaemia (19, 50, 51). Surprisingly, anaemia was not significantly associated with single infection or co-infection of

parvovirus B19 and *P. falciparum* among our SCD patients. Our finding is consistent with the previous study of Toan *et al*. (33) who also did not observe significant difference in haemoglobin concentration among non-SCD patients with co-infections of B19 and *P. falciparum*. The reason for this finding remains unclear.

Conclusion:

An overall prevalence of parvovirus B19 and *P. falciparum* co-infection of 3.0% was observed in our SCD patients in this study, and single infection of 14.0% for *P. falciparum* and 26.7% for B19 were similarly reported. While gender was significantly associated with *P. falciparum* infection among our SCD patients, tribal extraction, platelet index and seasonal variation were significantly associated with single parvovirus B19 infection or co-infection of B19 and *P. falciparum*. The provision of strict regulatory policy concerning the screening of whole blood or pooled plasma before transfusion of blood or blood products in SCD patients is advocated.

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