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

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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

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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 subdivided 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 haematological 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 H2SO4 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 co-infection 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)         |          |                  |           |
Co-infection of parvovirus B19 and Plasmodium falciparum

|               | 31-40 | 41 & above | 0 | 1 (14.3) |
|---------------|-------|------------|---|----------|

**Educational status**

|                | Parvovirus B19 |          |          |          |
|----------------|----------------|----------|----------|----------|
|                | Primary        | Secondary | Tertiary |          |
| Parvovirus B19 | 120            | 98       | 82       |          |
|                | 21 (17.5)      | 32 (32.7)| 27 (32.9)|          |
| P. falciparum  | 120            | 98       | 82       |          |
|                | 16 (13.3)      | 19 (19.4)| 7 (8.5)  |          |
| Co-infection   | 120            | 98       | 82       |          |
|                | 2 (1.7)        | 4 (4.1)  | 3 (3.7)  |          |

**Religion**

|                | Parvovirus B19 |          |          |          |
|----------------|----------------|----------|----------|----------|
|                | Christian      | Muslim   |          |          |
| Parvovirus B19 | 282            | 18       | 6 (33.3)|          |
|                | 74 (26.2)      | 39 (13.8)| 39 (13.8)|          |
|                |                |          |          |          |
| P. falciparum  | 282            | 18       | 6 (33.3)|          |
|                | 9 (3.2)        | 3 (1.7)  | 3 (1.7)  |          |
| Co-infection   |                |          |          |          |
|                |                |          |          |          |
| Tribe          |                |          |          |          |
| Parvovirus B19 |                |          |          |          |
|                | Igbo           | Yoruba   | Ibibio   | Bini     |
|                | 48             | 11       | 21       | 164      |
|                | 14 (29.2)      | 4 (36.4) | 8 (38.1) | 31 (18.9)|
|                |                |          |          |          |
|                |                |          |          |          |
| P. falciparum  | 48             | 11       | 21       | 164      |
|                | 4 (8.3)        | 5 (45.5) | 2 (9.5)  | 19 (11.6)|
| Co-infection   |                |          |          |          |
|                |                |          |          |          |
|                |                |          |          |          |
| Season         |                |          |          |          |
| Parvovirus B19 |                |          |          |          |
|                | Rainy          | Dry      |          |          |
|                | 190            | 110      | 20 (18.2)|          |
|                | 60 (31.6)      | 20 (18.2)| 2.077    | 1.171, 3.684| 0.0144|
| P. falciparum  | 190            | 110      | 20 (18.2)|          |
|                | 31 (16.3)      | 11 (10.0)| 1.755    | 0.8436, 3.650| 0.1669|
| Co-infection   | 190            | 110      | 20 (18.2)|          |
|                | 7 (3.7)        | 2 (1.8)  | 2.066    | 0.4213, 10.127| 0.4939|
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)    |        |              |         |
| **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-
effect 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
parovirus 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 parovirus 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 parovirus 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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**References:**

1. Onwubalili, J. K. Sickle cell anaemia and reincarnation beliefs in Nigeria. Lancet. 1983; 2 (8364): 1423.
2. Diallo, D., and Tchernia, G. Sickle cell disease in Africa. Curr Opin Hematol. 2002; 9 (2):111–116
3. World Health Organization. *Management of Birth Defects and Haemoglobin Disorders: Report of a Joint WHO-March of Dimes Meeting*. Geneva, Switzerland: World Health Organization; 2006.
4. Makani, J., Ofori-Acquah, S. F., Nnodu, O., Wonkam, A., and Ohene-Frempong, K. Sickle Cell Disease: New Opportunities and Challenges in Africa. Scientific World J. 2013; 1-16. [http://dx.doi.org/10.1155/2013/193252](http://dx.doi.org/10.1155/2013/193252)
5. Plummer, F. A., Hammond, G. W., Forward, K., Sekla, L., Thompson, L. M., Jones, S. E., Kidd, I. M., and Anderson, M. J. An erythema infectiosum-like illness caused by human parovirus infection. N Engl J Med. 1985; 313: 74–79
6. International Committee on Taxonomy of Viruses (ICTV). Virus taxonomy: 2007 release. Virology division, IUMS. 2007. Available at [http://www.ictvonline.org](http://www.ictvonline.org)
7. Ozawa, K., Kurtzman, G., and Young, N. Replication of the B19 parovirus in human bone marrow cell culture. Science. 1986; 233: 883-886.
8. Serjeant, G. R., Serjeant, B. E., Thomas, P. W., Anderson, M. J., Patou, G., and Pattison, J. R. Human parovirus infection in homozygous sickle cell disease. Lancet. 1993; 341 (8855): 1237–1240
9. Young, N. S., and Brown, K. E. Parovirus B19. N Engl J Med. 2004, 350 (6): 586–597.
10. Carzavec, D., Gacina, P., Vasilij, A., Katovic, S. K. Aplastic crisis induced by human parovirus B19 as an initial presentation of hereditary spherocytosis. Coll Antropol. 2010; 34: 619-621.
11. Heegaard, E. D., and Brown, K. E: Human parovirus B19. Clin Microbiol Rev. 2002; 15 (3):485–505.
12. Koukovikila-Koussounda, F., Ntoumi, F., Ndounga, M., Tong, H.V., Abena, A. A., and Velavan, T. P. Genetic evidence of regulatory gene variants of the STAT6, IL10R and FOXP3 locus as a susceptibility factor in uncomplicated malaria and parasitaemia in Congolese children. Malar J. 2013; 12: 9
13. Murray, C. J., and Lopez, A. D. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. Lancet. 1997; 349:1436–1442.
14. Snow, R. W., Craig, M. H., Newton, C. R. J. C., and Steketee, R. W. The public health burden of *P. falciparum* malaria in Africa: deriving the numbers. Bethesda, MD: Fogerty International Center, National Institutes of Health, 2003.
15. Aidoo, M., Terlouw, D. J., Kolczak, M., McElroy, P. D., ter Kuile, F. O., Kariuki, S., Nahlen, B. L., La, A. A., and Udhayakumar, V. Protective effects of the sickle cell gene against malaria morbidity and mortality. Lancet. 2002; 359 (9314):1311–1312.
16. Pasvol, G. Parovirus infection, malaria and anaemia in the tropics – a new hidden enemy. J Infect Dis. 2006; 194: 141–142
17. Jones, P. H., Pickett, L. C., Anderson, M. J., and Pasvol, G. Human parovirus infection in children and severe anaemia seen in an area endemic for malaria. J Trop Med Hyg. 1990; 93 (1): 67–70.
18. Lortholary, O., Eliaszewicz, M., Dupont, B., and Courouce, A. M. Parovirus B19 infection during acute *Plasmodium falciparum* malaria. Eur J Haematol. 1992, 49 (4): 219.
19. Wildig, J., Mueller, I., Kiniboro, B., Maraga,
Co-infection of parvovirus B19 and Plasmodium falciparum

S., Siba, P., and Cossart, Y. Seroprevalence of antibodies to parvovirus B19 among children in Papua New Guinea. Am J Trop Med Hyg. 2007; 77 (2): 354–357.

20. Bensch, C., Kempf, C., Mueller, I., Manning, L., Laman, M., Davis, T. M., and Ros, C. Chloroquine and its derivatives exacerbate B19-associated anemia by promoting viral replication. PLoS Negl Trop Dis. 2010; 4 (4): e669.

21. Inggrassia, F., Gadalaeta, A., Maggi, P., and Pastore, G. Plasmakum falciparum malaria and Parvovirus B19; a case of acute co-infection. BMC Infect Dis. 2010; 10: 87.

22. Dudu, K. O., Sagoe, K. W., Ayeh-Kumi, P. F., Affrim, R. B., Adiku, T., and Huat, L. B. The effects of co-infection with human parvovirus B19 and Plasmodium falciparum on type and degree of anaemia in Ghanaian children. Asian Pac J Trop Biomed. 2013; 3 (2): 129–139.

23. Dreyfuss, M. L., Stoltzfus, R. J., Shrestha, J. B., Pradhan, E. K., LeClerq, S. C., Khtry, S. K., Shrestha, S. R., Katz, J., Albonico, M., and West, K. P. Jr. Hookworms, malaria and vitamin A deficiency contribute to anaemia and iron deficiency among pregnant women in the plains of Nepal J Nutr. 2000; 130: 2527-2536.

24. Brooker, S., Akhwale, W., Pullan, R., Estambale, B., Clarke, S. E., Snow, R. W., and Hotez, P. J. Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anaemia, and prospects for combining control. Am J Trop Med Hyg. 2007; 77: 88-98.

25. van Hensbroek, B. M., Calis, J. C., Phiri, K. S., Vet, R., Munthali, F., Kraaijenhagen, R., van den Berg, H., Faragher, B., Bates, I., and Molyneux, M. E. Pathophysiological mechanisms of severe anaemia in Malawian children. PLoS One. 2010; 5: e12589.

26. Carraturo, A., Catalani, V., Ottaviani, D., Menichelli, P., Rossini, M., Terella, D., and Biondi, B. Parvovirus B19 infection and severe anaemia in renal transplant recipients. Sci. World J. 2012; 2012: 102829.

27. Cheesbrough, M. District Laboratory Practice in Tropical Countries. 2nd Edition, Cambridge University Press, Cambridge, 1999.

28. Beutler, E., Waalen, J. The definition of anaemia: what is the lower limit of normal of the blood haemoglobin concentration? Blood. 2006; 107: 1747–1750.

29. Weatherall, D. J. Genetic disorders of Haemoglobin. In: Hoffbrand, A. V., Lewis, S. M., and Tuddenham, E. G. D. (eds). Postgraduate Haematology 4th edition. Great Britain. Butterworth-Heinemann, 1999; 91-119.

30. West, T. B., West, D. W, and Ohene-Frempong, K. The presentation, frequency, and outcome of bacteraemia among children with sickle cell disease and fever. Pediatr Emerg Care. 1994; 10 (3): 141–143.

31. Wierenga, K. J. J., Hambleton, I. R., Wilson, R. M., Alexander, H., Serjeant, B. E., and Serjeant, G. R. Significance of fever in Jamaican patients with homozygous sickle cell disease. Arch Dis Childhood. 2001; 84 (2): 156–159.

32. Ho, M., Webster, H. K., Looareesuwan, S., Supanaranond, W., Phillips, R. E, Chanthavanich, P., and Warrell, D. A. Antigen-specific immunosuppression in human malaria due to Plasmodium falciparum. J Infect Dis. 1986; 153: 763-771.

33. Toan, N. L., Sy, B. T, Song, L. H., Luong, H. V., Binh, N. T., Binh, V. Q., Kandolf, R., Velavan, T. P., Peter, G., Kremsner, P. G., and Bock, C-T. Co-infection of human parvovirus B19 with Plasmodium falciparum contributes to malaria disease severity in Gabonese patients. BMC Infect Dis. 2013; 13: 375-384.

34. Slavov, S. N., Kashima, S., Silva-Pinto, A. C., and Covas, D. T. Genotyping of human parvovirus B19 among Brazilian patients with hemoglobinopathies. Can J Microbiol. 2012; 58: 200-205.

35. Modrow, S., Wenzel, J., Schimanski, S., Schwarzbeck, J., Rothe, U., Oldenburg, J., Jilg, W., and Eis-Hubinger, A. Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. Vox Sang. 2010; 100: 351–358.

36. Hassan, H., Hasan S., Giday, S., Alamgir, L., Banks, A., and Frederick, W. Hepatitis C virus in sickle cell disease. Journal of the National Medical Association. 2003; 95 (10): 939–942.

37. Thilolo, L. M., Mukendi, R. K., and Wembanduya, S. O. Blood transfusion rate in Congolese patients with sickle cell disease Indian J Pediatr. 2007; 74:735–738.

38. Riley, E. M., Wagner, G. E., Akamori, B. D., and Koram, K. A. Review Do maternally acquired antibodies protect infants from malaria infection? Parasite Immunol. 2001; 23 (2): 51-59.

39. Achidi, E. A., Apinjoh, T. O., Mbuwne, E., Besingi, R., Yafi, C., Wanjighe, A. N., Ajuwa, A., and Anchang, J. K. Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in school children resident at different altitudes, in south-western Cameroon. Ann Trop Med Parasitol. 2008; 102 (2): 103-118.

40. Jadhav, U. M., Patkar, V. S., and Kadam, N. N. Thrombocytopenia in malaria-correlation with type and severity of malaria. J Assoc Physicians India. 2004; 52: 615-618.

41. Rasheed, A., Saeed, S., and Khan, S. A. Platelet counts in malaria. Pak J Phytopathol. 2008; 19: 86–88 42. Faseela, T. S., Roche, Anita, K. B., Malli, C. S., and Rai, Y. Diagnostic value of platelet
43. Doran, H. M., and Teall, A. J. Neutropenia accompanying erythroid aplasia in human parvovirus infection. Br J Haematol. 1988; 69: 287-288

44. Inoue, S., Kinra, N. K., Mukkamala, S. R., and Gordon, R. Parvovirus B19 infection: aplastic crisis, erythema infectiosum and idiopathic thrombocytopenic purpura. Pediatr Infect Dis J. 1991; 10: 251-253

45. Olumese, P. E., Adeyemo, A. A., Ademowo, O. G., Gbadegesin, R. A., Sodeinde, O., and Walker, O. The clinical manifestations of cerebral malaria among Nigerian children with the sickle cell trait. Ann Trop Paediatr. 1997; 17: 141-145

46. Girei, A. I., Alao, O. O., Joseph, D. E., Damulak, D. O., Banwat, E. B., Nwadioha, S. I., and Jombo, G. T. A. Human parvovirus B19 infection among children with sickle cell anaemia in Jos, North Central Nigeria. Journal of Hainan Medical University. 2010; 10.

47. Broilden, K., Tolfvenstam, T., and Norbeck, O. Clinical aspects of parvovirus B19 infection. J Intern Med. 2006; 260: 285-304

48. Booth, C., Inusa, B., and Obaro, S. K. Infection in sickle cell disease. A review. Intern J Infect Dis. 2010; 14: 2-12.

49. Brown, K. Anaemia, parvovirus, and malaria. Lancet. 2006; 368 (9537): 714–716.

50. Scarlata, F., Gianelli, E., Miceli, S., Galimberti, L., and Antinori, S. Acute parvovirus B19 infection and anemia during Plasmodium falciparum malaria. Clin Infect Dis. 2002; 35 (11): 1449–1451.

51. Gupta, R., and Singh, T. Parvovirus B19 coinfection with falciparum malaria: a cause of severe anemia. Haematologica. 2005; 90 (12): ECR41