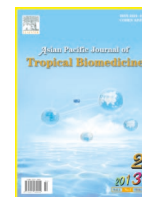




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The effects of co-infection with human parvovirus B19 and *Plasmodium falciparum* on type and degree of anaemia in Ghanaian children

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PEER REVIEW

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Comments

There are conflicting reports on whether co-infection with malaria and B19V can exacerbate anaemia. Some findings suggest that such co-infection is associated with anaemia, while others suggest not. This report finds that co-infection of B19V and malaria is associated with microcytic anaemia, which is novel and calls for further investigations to establish this effect.

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ABSTRACT

Objective: To determine the extent to which parvovirus B19 (B19V) and co-infection of B19V and malaria contribute to risk of anaemia in children. **Methods:** B19V DNA and malaria parasites were screened for 234 children at the PML Children's Hospital in Accra. The role of B19V and co-infection with B19V and malaria in anaemia was evaluated by analysing full blood cell counts, malaria and B19V DNA results from these children. **Results:** The prevalence of B19V, malaria and co-infection with B19V and malaria was 4.7%, 41.9% and 2.6%, respectively. Malaria posed a greater risk in the development of mild anaemia compared to severe anaemia ($OR=5.28$ vs 3.15) whereas B19V posed a higher risk in the development of severe anaemia compared to mild anaemia ($OR=4.07$ vs 1.00) from a non-anaemic child. Persons with co-infection with B19V and malaria had 2.23 times the risk (95% $CI=0.40-12.54$) of developing severe anaemia should they already have a mild anaemia. The degree of anaemia was about three times affected by co-infection (Pillai's trace=0.551, $P=0.001$) as was affected by malaria alone (Pillai's trace=0.185, $P=0.001$). B19V alone did not significantly affect the development of anaemia in a non-anaemic child. Microcytic anaemia was associated with B19V and co-infection with B19V and malaria more than normocytic normochromic anaemia. **Conclusions:** B19V was associated with malaria in cases of severe anaemia. The association posed a significant risk for exacerbation of anaemia in mild anaemic children. B19V and co-infection with B19V and malaria may be associated with microcytic anaemia rather than normocytic normochromic anaemia as seen in cases of B19V infection among persons with red cell abnormalities.

KEYWORDS

Malaria, Human parvovirus B19, Anaemia, Ghana, Children

1. Introduction

The aetiology and pathogenesis of anaemia is complex and cannot be accurately represented by one simple mechanism. It may be due to a single causative agent or factor or an interaction among several causative agents or factors. In most cases, it is due

to two or more causative agents rather than a single one^[1–3]. Malaria, bacteraemia, helminthiasis, human parvovirus B19 (B19V) infection and other infections such as tuberculosis and HIV/AIDS have been widely reported as important factors contributing to the high prevalence of anaemia in many populations^[3–16]. Even though some individual causes have been identified,

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the effect of their interaction to anaemia or progression to severe anaemia is rather complex and remain mostly unknown. Studies from Tanzania and Papua New Guinea have indicated that malaria and iron deficiency are the two major causative factors of anaemia^[17,18]. In a more recent study from Ifakara, Tanzania, however, a larger range of risk factors for hospitalisation for anaemia were listed. These included, malnutrition, *Plasmodium falciparum* (*P. falciparum*) infection, living >10 km from the hospital, a history of previous blood transfusion and increased recent health expenditure^[19].

The multi-factorial aetiology of anaemia in a patient may quickly result in severe anaemia and is likely to vary from region to region or among different age groups or populations within a region^[20–26]. This is because prevalence of the factors leading to anaemia are not the same for all regions or areas. Whilst hookworm may be an important cause of severe anaemia in rural areas associated with low living standards and poor hygienic conditions, it may not be a causative agent in urban areas. Likewise HIV infection may be important in adults in urban areas but not in rural areas^[1]. Furthermore, the aetiology and pathogenesis of anaemia will vary between pregnant and non-pregnant women^[23,25]. Due to the complex interaction and variability of factors that contribute to aetiology and pathogenesis of anaemia, it is difficult to adopt one common approach to deal with the anaemia situation^[1,3]. Whilst investigating all factors in one single study could be challenging in resource-limited settings, a model that adopts one or two major contributors to the aetiology of severe anaemia *e.g.*, malaria, iron deficiency or malnutrition, in a particular population and another parameter which is not well characterized as a major contributor *e.g.*, B19V to severe anaemia could help rule out or include potential contributors. A review of all such studies will elicit the multi-factorial aetiology of severe anaemia in a particular population.

B19V infection leads to a suppression of the bone marrow^[27,28]. In patients with underlying haemolysis, acute B19V infection may lead to transient aplastic crisis, an abrupt and severe anaemia due to failure of red-cell production^[29,30]. In addition, to a cessation of the erythroid production, other blood cell lineages can be affected leading to clinical manifestations such as thrombocytopenia, neutropenia or pancytopenia^[31–37]. Anaemia may persist until the immune response clears the infection, then the red cell production resumes and eventually normalizes followed by lifelong immunity in most cases^[38,39]. It has been hypothesized that, since erythroid precursor cells represent a common target for both B19V and *P. falciparum*, a predisposing factor to viral invasion and infection could be a depression of

cellular immunity induced by *P. falciparum*^[40]. A high incidence of co-infection with malaria and B19V and an association with anaemia has been reported in malaria endemic areas^[41–45]. In addition, some case studies have also reported of B19V induced severe anaemia following malaria infection and successful treatment with antimalarials^[46–50]. Though it is primarily known to cause erythematous rash, foetal hydrops and other involvements in various haematological disorders, B19V is also involved in other conditions^[34,51–57]. B19V in many occasions have been reported as a bystander or contributor to many conditions such as hepatitis, myocarditis, arthritis among others^[58–67]. Its contribution to such conditions however is largely unknown.

In Ghana, a high seroprevalence of B19V has been reported among adult blood donors^[68,69]. In another study, the prevalence of B19V DNA among Ghanaian children hospitalized for severe anaemia was 11.5%^[45]. Similar studies from Papua New Guinea and Kenya have reported that B19V contributes to severe anaemia among children in malaria endemic areas^[42,43]. However, studies conducted among Malawian children report that B19V may not be a significant contributor to severe anaemia^[12,70]. We therefore set out to investigate the presence of B19V among Ghanaian children presenting with varying degrees of anaemia as well as a group with no anaemia and determine the effects of co-infection with B19V and malaria on the type and degree of anaemia.

2. Materials and methods

2.1. Study site and sample selection

The study sample was selected from children at Princess Marie Louis Children's Hospital in Accra, Ghana. A total of 234 subjects comprising 74 children with haemoglobin below 8.0 g/dL and two other similar groups of 80 each, similar for age and gender with haemoglobin 8.0–10.9 g/dL and 11.0 g/dL and above. Groupings were informed by the likelihood of transfusion as was the practice in the hospital. The groups were thus named as "Likely to be transfused" (LTBT), "Not likely to be transfused" (NLTBT) and Non-anaemic. The sample was collected between October and December 2010 and included all children presenting with severe anaemia within the period. B19V seasonality is not known in Ghana. However, the malaria peak season in Ghana is from April to July. Sampling was performed during the off-peak season of malaria to rule out potential effects of a high malaria burden.

Within the study period, all severe anaemia subjects

that went to the hospital laboratory were included in the study provided they did not satisfy any of the exclusion criteria. The exclusion criteria were: (a) children who were identified as malnourished or attended the hospital unit that saw malnourished children, (b) children who had HIV and sickle cell determined by light microscopy using the sodium metabisulphite method, (c) children who were older than twelve years old. The other two groups (NLTBT and non-anaemic) were selected by matching for age and gender. A *t*-test for means was performed to ensure that there was no statistical difference between the ages of the groups selected. A similar comparison was done for gender proportions. Retrospective samples were used in this study and data on the outcome of individual admissions could not be traced because the laboratory did not record folder numbers of patients.

2.2. Full blood count and *P. falciparum* testing

Full blood count was performed using the Sysmex KX-21N haematology analyser (Sysmex Corporation, Japan) following manufacturer's instructions. It is an 18 parameter analyser in whole blood mode operation and 8 parameters in predilute mode with a throughput rate of 60 samples per hour. The whole blood mode was used to analyse all samples. The parameters in whole blood mode included the full blood count [white blood count, red blood count, hemoglobin (HGB), hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets] and a 3-part differential leukocyte count (Lymph %,#; Neut %,#; Mixed %,#). Further parameters derived from the cell volume histograms included RDW-SD, RDW-CV, platelet distribution width, MPV, P-LCR. Results were displayed on an LCD screen and printed on a thermal printer. Determination of HGB concentration is based on the non-cyanide method whereas the cumulative pulse height detection method is used for the haematocrit determinations. The direct channel detection principle, Sysmex's patented fluorescent flow cytometry technology is used for the white blood count, red blood count and platelets determination whereas the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values were computed using the red blood count/haematocrit, red blood count/HGB and HGB/haematocrit respectively.

All samples were placed on an electronic rotor prior to analysis. The sample was well mixed by gentle inversion prior to the test. The automated analysis was done following the manufacturer's operational guidelines. All samples were analysed within 30 minutes of collection.

P. falciparum infection was determined by

conventional light microscopy on Giemsa stained thick and thin blood films as well as qualitative detection *P. falciparum* Histidine-rich Protein 2 (HRP2) in whole blood specimens using the First Response® Malaria (HRP2) antigen detection card Test (Premier Medical Corporation, India).

2.3. DNA extraction and B19V semi-nested PCR

The high Pure Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany) was used for the isolation of viral DNA from plasma samples. Elution of viral DNA was done with 50 µL of elution buffer according to the manufacturer's instructions. 19 DNA was detected using a modified form of an in-house semi-nested PCR described earlier[71]. The semi-nested PCR targeted the C-terminal part of the VP1u region with a product size of 358 bp using outer primers GAPS 5'-ATG GAC AGT TAT CTG ACC ACC CCC-3' (fwd) and USTO 5'-GCT GGG GTA TTT TTC CGA GGC GT-3' (rev) and inner primer GAPSi 5'-GTA TTA TCT AGT GAA GAC TTA CAC AAG CCT GGG-3' (fwd) located within the VP1u region. The first round was performed in 50 µL of reaction with 10 µL of the extracted DNA and 5×reaction buffer with 7.5 mmol/L MgCl₂, 200 µmol/L dNTP, 2.5 IU of *Taq* DNA polymerase, 0.4 µmol/L forward, GAPS and reverse USTO primers. The second round was performed with the same concentrations of reagents but with 5 µL of the first round reaction and the other forward primer, GAPSi. The cycling conditions for both rounds were denaturation for 5 min at 95 °C, followed by 35 cycles at 95 °C for 30 seconds, 57 °C for 40 seconds, and 72 °C for 50 seconds, followed by final extension at 72 °C for 7 min. All reagents were from Roche Diagnostics, Mannheim, Germany.

2.4. B19V controls and standards

The performance of the B19V assay was first evaluated with DNA extracted from the First International Standard for B19V DNA plasma (code 99/800) from the National Institute for Biological Standards and Control (NIBSC) containing 10⁵ IU/mL B19V DNA. The PCR product of 358 bp was visualized on 1.5% agarose gel. The lower level of sensitivity was determined with a serial dilution of the B19V DNA control plasma as well as serial dilutions of B19V DNA containing 10⁵ and 10⁴ IU/mL. Ten fold dilutions were performed with each of the standards down to 10 IU/mL. The lower level of sensitivity was found to be 10³ IU/mL. An aliquot of diluted (10⁴ IU/mL) of the NIBSC 99/800 DNA plasma was included in each batch of DNA extraction performed as a positive control and also included in the subsequent PCR reaction.

Plasma dilution was performed using normal human plasma (Roche Diagnostics, Mannheim, Germany).

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS Version 19 software (IBM Corporation, Somers, New York, USA) and Epi Info™ version 3.5.3 (Centres for Disease Control and Prevention, Georgia, USA). The independent *t*-test and Mann Whitney *U* tests for testing two variables as well as the Kruskal–Wallis test for testing multiple variables were used in comparing the age and full blood count parameters, malaria, B19V DNA and the degree of anaemia. One-way ANOVA was used to determine mean variations between the full blood count parameters of the various groups of patients by the degree of anaemia. Odds ratio and general linear models were used to determine the association between anaemia, B19V and *P. falciparum* infection. Gender distribution and anaemia types were described summarized and described by bar charts whereas statistical difference between them was conducted using *t*-test for independent variables. All tests for significance were two sided, and *P* values of less than 0.05 were considered to indicate statistical significance.

2.6. Ethical considerations

The study was approved by the Ethical and Protocol Review Committee (EPRC) of the University of Ghana Medical School (Protocol Identification No.: MS–Et/M.9–P.3.4/2009–10). Ethical Clearance was submitted to the management of the Princess Marie Louis Children’s Hospital after which approval was given to collect the archived samples used in this study.

3. Results

3.1. Patient characteristics, anaemia and malaria

A total of 234 subjects were analysed in the study. The mean age of all samples used was 34.37 months (range: 0.10–144.00 months). The group specific age characteristics are shown in Figure 1.

Of the 234 study subjects, 154 (65.81%) were anaemic. Normocytic normochromic anaemic was observed in 80 (51.9%) of the anaemic cases, the majority (63.7%) of whom had mild anaemia. Microcytic anaemia constituted 61 (39.6%) whilst macrocytic anaemia was observed in the remaining 13 (8.4%) of the anaemic cases. In the NLTBT group, normocytic normochromic anaemia was

predominant whereas in the LTBT group, microcytic anaemia was predominant. Severe anaemia (HGB<5 g/dL) was observed in 5.2% of the anaemia cases. Because the laboratory did not record folder numbers of patients, we couldn’t trace folders of the patients to access the individual outcomes of admissions. Data from the laboratory’s blood bank obtained by matching name, age and gender of selected samples with that in the blood bank records however indicated that cross-matching for transfusion was performed within 48 h for all the 74 severe anaemic samples. We are however unable to tell whether all were successfully transfused or otherwise.

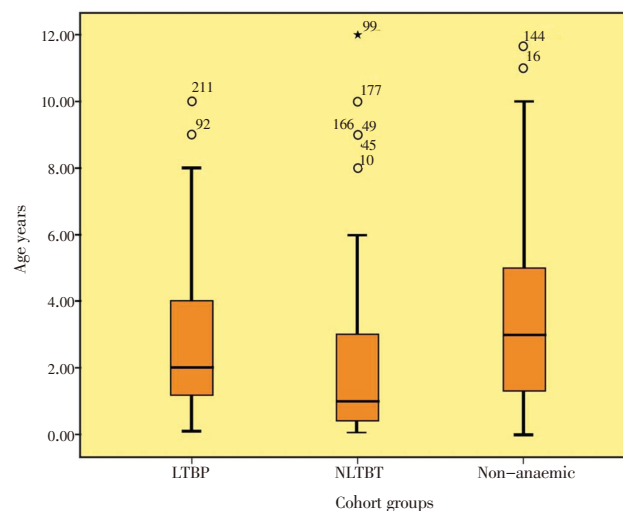


Figure 1. Age distribution of cohort groups used in the study. Age data was not available for four study subjects. Mean ages were 2.65 (SE=0.26, SD=2.21), 2.25 (SE=0.31, SD=2.72) and 3.65 (SE=0.32, SD=2.84) for 71, 79 and 80 LTBT, NLTBT and Non-anaemic subjects respectively. Outliers 92 and 211 were 9 years and 10 years respectively for the LTBT group whereas 10, 45, 49, 99, 166 and 177 as well as 16 and 114 were 8, 9, 9, 12, 9 and 10 years as well as 11 and 11.67 years for the NLTBT and Non-anaemic groups respectively.

Red cell morphology was predominantly microcytic in B19V as well as B19V and malaria co-infected individuals. Microcytic anaemia was the predominant type of anaemia for the malaria parasitized (34.7%), B19V infected (54.5%) and B19V and malaria co-infected (83.3%) subjects. Macrocytic anaemia was absent in B19V infected individuals. The prevalence of normocytic normochromic anaemia was higher in malaria parasitized individuals (40.8%) than non-parasitized individuals (29.4%) whereas in B19V infected individuals, the prevalence was rather slightly lower (27.3%) than the non-infected individuals (34.5%). A breakdown of the different anaemia types by infection status has been presented in Figure 2. The prevalence of thrombocytopenia, lymphocytopenia and leukopenia were significantly higher in malaria parasitized subjects compared to non-parasitized subjects (62.2%, 31.6%, and 18.4% versus 15.4%, 25.7%, and 15.4% respectively). Reference values for thrombocytopenia,

lymphocytopenia and leukopenia were obtained from literature[72].

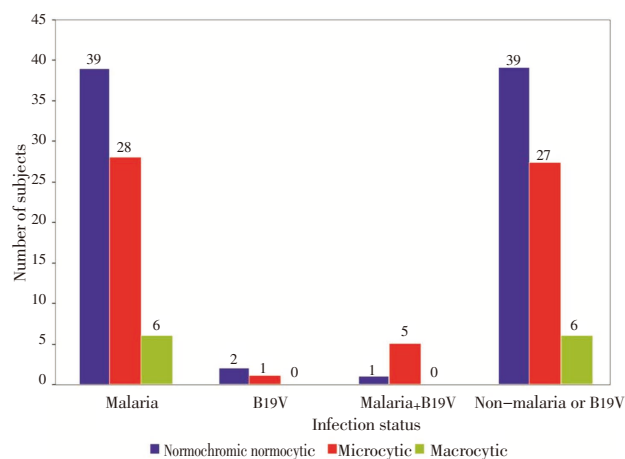


Figure 2. Breakdown of the different anaemia types by infection status.

Normocytic normochromic was predominant except among B19V/malaria co-infected subjects where microcytic anaemia was predominant.

Of the 234 study participants, 98 (41.9%, 95% CI=35.7–48.2) had malaria parasites in their peripheral blood. A statistically significant difference was observed between the haemoglobin ($P=0.001$) and platelet count ($P<0.001$) of parasitized and non-parasitized individuals. Red cell morphology showed a normocytic and normochromic picture in 40.8% and 29.4% of parasitized and non-parasitized individuals respectively. There was a significant positive correlation between age and malaria [$P<0.001$, 99% confidence level (CL)] whereas no significant correlation existed between gender and malaria. There were significant correlations between malaria and erythrocyte counts ($P=0.023$, 95% CL), haemoglobin ($P=0.001$, 99% CL), haematocrit ($P=0.015$, 99% CL) and red cell distribution with ($P=0.002$, 99% CL).

3.2. Co-infection with B19V and malaria and its association with anaemia

The semi-nested B19V PCR detected 11 (4.7%, 95% CI=2.6%–8.3%) of the 234 study subjects had B19V viraemia. Of these, 7 (63.6%) were moderately to severely anaemic with 57.1% of them presenting with malaria as well. Two (18.2%) of the B19V viraemic subjects had mild anaemia, both of whom had malaria as well whereas

the remaining 2 (18.2%) of the B19V infected individuals were non-anaemic and did not have malaria either. The prevalence of B19V among moderate to severely anaemic children was 9.5% (95% CI=4.4% to 18.5%) and was higher than the 2.5% (95% CI=0.2%–9.2%) observed in mild anaemic and non-anaemic children (Table 1). In all the prevalence for B19V and malaria co-infection was 2.6% (95% CI=1.1%–5.6%) of which all were persons with anaemia. None of the B19V/malaria co-infected subjects was non-anaemic.

In the absence of B19 IgG and IgM data in this study and in view of the current consensus that the duration of B19V viraemia is much longer than expected[73–77]. A box plot was generated to determine the distribution of B19V infection (Figure 3).

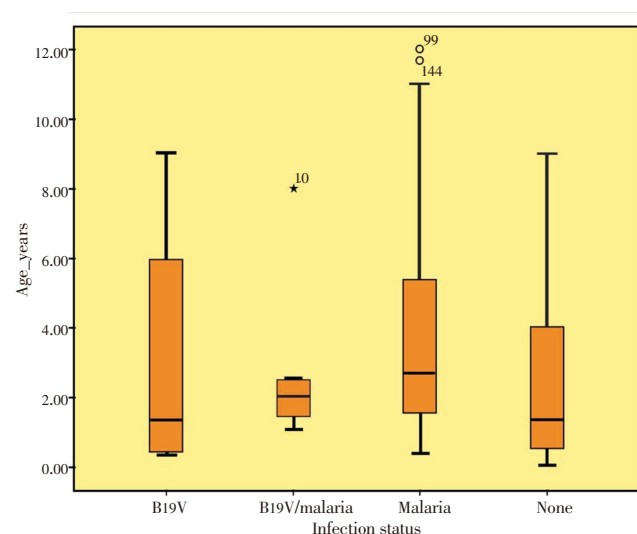


Figure 3. Age distribution of study subjects with malaria, B19V or co-infection with malaria and B19V.

Age range for children with B19V DNA and malaria was 0.33 to 9.00 years and 0.33 to 12 years respectively whereas that of children with co-infection with B19V and Malaria was 1.04 to 8.00 years. Six children had co-infection and the specific ages are 2.00, 2.00, 1.42, 2.50, 1.04 and 8.00 years.

It was realized that though B19V DNA was present in children of all age groups, co-infection with malaria only occurred in children who were 2.00, 2.00, 1.42, 2.50, 1.04 and 8.00 years. It is unlikely that the children of two and a half years old and below have lived through a previous B19V epidemic since there is a general consensus that B19V infection occurs usually within ages up to 2 years[78]. The presence of B19V DNA therefore could be an infection rather than persistence of B19V DNA. Excluding the single older child (8 years)

Table 1

Prevalence and pairwise comparison of B19V and *P. falciparum* infection among study cohorts.

Conditions	Entire population		LTBT		NLTBT		Non-anaemic		LTBT/NLTBT			LTBT/Non-anaemia			NLTBT/Non-anaemic		
	Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI	Prevalence	95% CI	P-value	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value	Odds ratio	95% CI
B19V	11/234 (4.7%)	2.6%–8.3%	7/74 (9.5%)	4.4%–18.5%	2/80 (2.5%)	0.2%–9.2%	2/80 (2.5%)	0.2%–9.2%	0.065	4.07	0.82–20.282	0.065	4.07	0.82–20.28	1.000	1.00	0.14–7.28
<i>P. falciparum</i>	98/234 (41.9%)	35.7%–48.2%	34/74 (45.9%)	35.1%–57.2%	47/80 (58.8%)	47.8%–68.9%	17/80 (21.3%)	13.6%–31.5%	0.106	0.60	0.32–1.13	0.001	3.15	1.56–6.37	0.000	5.28	2.63–10.59
B19V/ <i>P. falciparum</i>	6/234 (2.6%)	1.1%–5.6%	4/74 (5.4%)	1.7%–13.5%	2/80 (2.5%)	0.2%–9.2%	0 (0%)	0.0%–5.5%	0.352	2.23	0.40–12.54	–	–	–	–	–	–

LTBT: Likely to be transfused (moderate and severe anaemic children); NLTBT: Not-likely to be transfused (mild anaemic children); –: Cannot be calculated because outcome for B19V/malaria in non-anaemic group equalled zero (0).

with co-infection with malaria did not significantly affect the outcome of analysis.

There was a significant difference between the proportion of malaria infection between each of the anaemic groups and the control (non-anaemic) group (Table 1). Of the 6 B19V/malaria co-infected subjects, five had predominantly microcytic hypochromic cells. Microcytic hypochromic cells were common in all B19V infected subjects.

From univariate analysis, it was observed that B19V did not significantly affect haemoglobin, leukocytes, platelets, lymphocytes and neutrophils. However, malaria and co-infection with B19V and malaria significantly affected those parameters ($P < 0.001$ in all cases). In the multivariate analysis however, it was observed that the degree of anaemia was about three times affected by co-infection (Pillai's trace=0.551, $P=0.000$) as was affected by malaria alone (Pillai's trace=0.185, $P=0.000$). The effect of B19V on the degree of anaemia however was weak and insignificant (Pillai's trace=0.004, $P=0.963$).

The risk of developing a more severe anaemia in the event of B19V infection was not different whether the child had a mild anaemia or was non-anaemic ($OR=4.07$, 95% $CI=0.82-20.28$). The risk of an exacerbated anaemia in the event of B19V infection was further lower ($OR=1$, 95% $CI=0.14-7.28$) for a child that was not anaemic. The risk of developing mild anaemia in a non-anaemic person was higher ($OR=5.28$, 95% $CI=2.63-10.59$) than the risk of developing severe anaemia by a non-anaemic person. The risk of developing severe anaemia was further lower ($OR=0.60$, 95% $CI=0.32-1.13$) for a non-anaemic person.

4. Discussion

In this study co-infection with B19V and malaria was identified to have a potential of exacerbating anaemia though the presence of B19V DNA alone did have such potential. The approach used in this study was designed to systematically determine within different cross-sections of children with varying degrees of anaemia the potential of B19V and/or co-infection with B19V and malaria to cause a rather more severe form of anaemia. This study is in contrast to other B19V studies in which investigations were structured to prove that co-infection with B19V and malaria had a similar potential by investigating only cross-sections of children with severe anaemia and those without severe anaemia[41–44]. This study is the first to evaluate B19V's contribution to anaemia in a malaria endemic area in this manner. The results show a pattern of changes that

occur in the haemoglobin levels as well as the number of other leukocytes in non-anaemic, mild anaemic and moderate/severe anaemic children.

On the basis of the finding that the presence of B19V DNA in the plasma of children used in this study was associated with microcytic anaemia other than normocytic normochromic anaemia, we thus report that B19V infection in persons without aplastic crisis, sickle cell disease or other red cell malignancies may result in microcytic hypochromic erythrocytes. Though real time PCR was not used to determine B19V viraemia in this study, the low sensitivity of the semi-nested B19V assay (103 IU/mL) indicates that infections detected are all acute infections rather than persistent infections (B19V DNA <103 IU/mL).

Severe anaemia for many years has remained a major cause of morbidity and mortality in Ghanaian children[79,80]. In this study, malaria was found to be closely associated with anaemia, making it a major causative factor consistent with previous studies[79,81,82]. There has been a recent scale up of malaria control interventions such as insecticide-treated bed nets and artemisinin-based combination therapy to curb the ever increasing burden of malaria in Ghana and several other endemic areas in Africa[81,83–89]. This has resulted in a decrease in the prevalence of malaria in most areas[90–93]. This study showed a decrease in the burden of malaria as compared to previous studies in Ghana[94], and other African countries[95]. This should however be viewed in the context of an off-peak malaria season when it was conducted. This study was conducted in a hospital that is known to receive a lot of malnourished children, hence the contribution of malnutrition to anaemia cannot be overlooked though the contribution of malnutrition and other parasitic infections to anaemia in malaria endemic regions has been found to be minimal compared to malaria[21,96].

In a Ghanaian blood donor population the seroprevalence of B19V was found to be 83% ($n=1\ 000$) with a relatively low viraemic prevalence of 1.3%[69]. In another study, B19V genotype three seroprevalence was similarly high in Ghanaian pregnant mothers[97]. In this study, B19V DNA prevalence in the total study population made up of three cohorts of moderate to severely anaemic children, mild anaemic children and non-anaemic children was slightly higher than that observed in the adult blood donor population by other investigators[45,69,98]. These observations are consistent with the transmission patterns of B19V as reviewed to be higher in children than in adults[78].

It is also evident from this study that B19V prevalence in moderate to severely anaemic children is relatively high as reported earlier[45]. This finding also confirms

the 11.8% DNA prevalence of B19V among severely anaemic children (HGB<5.0 g/dL) from Papua New Guinea^[43]. The results are however different from that observed in Malawi where B19V DNA was not demonstrated in 159 severely anaemic children with malaria^[70]. The differences may be accounted for by the type of samples used in these studies. While the study in Malawi used dried blood spots, the other studies used either stored whole blood or plasma for the investigation. The type of children may not be a factor since in both the Malawi and Papua New Guinea studies the children were basically persons with malaria. These studies however had different results on the prevalence and association of B19V with anaemia and malaria. Even though no study have compared the suitability of different samples for use in such studies, it appears the Malawi study may have been affected by the use of dried blood spots.

The association of B19V with malaria is significant because erythroid precursor cells are a common target for both *P. falciparum* and B19V^[40]. The association of B19V with aplastic crisis is extensively reported but its role in malaria remains a controversy. This study confirms studies conducted in Nigeria^[41], Papua New Guinea^[43], and Kenya^[42], where B19V co-infection with malaria was found to contribute significantly to anaemia. This observation could be explained by the changes in the climatic conditions as well as absence of underlying conditions to exacerbate severe anaemia. Underlying conditions may include a background mild anaemia seen in a large population of children in developing tropical countries. The risks of having anaemia exacerbated in some form have been clearly demonstrated in this study.

The ability of B19V to exacerbate anaemia in persons with haemoglobinopathies may not be different in both temperate and tropical regions since has a worldwide distribution and it does not have a geographic endemicity^[78]. However, the effect will be higher in tropical regions where there are underlying conditions or factors that will favour severe anaemia. The contribution of B19V to severe anaemia is also supported by the understanding that it causes a 2.0 g/dL decrease in haemoglobin level, even in adult volunteers with haemolytic disease who were previously healthy^[27]. With this finding, the hypothesis that B19V may exacerbate severe anaemia in persons with underlying mild anaemia cannot be overemphasised. In Ghana therefore where the prevalence of anaemia is far above 60% in children as observed in this study and the demographic health survey^[80], it is not surprising to find an association of B19V with malaria making a significant contribution to severe anaemia.

Association of B19V with malaria and their contribution to other haematological disorders has been under reported. The finding that B19V and its co-infection with malaria was associated with microcytic anaemia other than normocytic normochromic anaemia as may occur in patients with aplastic crisis is important. The presence of normocytic normochromic anaemia in non-B19V infected mild anaemia individuals and microcytic anaemia in B19V infected as well as B19V/malaria co-infected individuals suggests a possible involvement of B19V with microcytic anaemia. Microcytic anaemia results from deficient or defective heme or globin synthesis and may include iron deficiency anemias, iron-transport deficiency anemias, iron-utilization anemias and thalassemyas^[72,99]. An important limitation of this study is the absence of serum iron or a direct estimate of iron deficiency in the children. This could clarify whether the microcytic anaemia is due to a defect in haem synthesis due to B19V replication or the reduced half-life of erythrocytes are contributing to B19V replication or otherwise. Further studies could help clarify this.

The association of co-infection with B19V and malaria with severe anaemia observed in this study suggests that the prevention of B19V infections is likely to result in a significant reduction in the burden of severe anaemia in young children in Ghana and other malaria endemic areas. This will greatly respond to the call to reduce or prevent severe anaemia^[100]. The need therefore to strengthen efforts to develop safe and effective vaccines, some of which are underway for use in areas where there is a high burden of background mild anaemia alongside other prevention strategies to reduce malaria, helminth infections and iron deficiency anaemia cannot be overemphasised^[101].

In conclusion, B19V plays a very important role in the aetiology of severe anaemia in persons with mild anaemia. Additionally, it supports the hypothesis that B19V may be associated with malaria in cases of severe anaemia in Ghana. These findings highlight B19V as important in the effort to prevent severe anaemia in malarial anaemia cases.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Anaemia is a public health problem among children in Ghana. Besides *P. falciparum* as the causative pathogen, Ghanaian children are also co-infected with B19V. This original paper analysed the extent to of B19V and its co-infection with malaria on anaemia of 234 children warded at PML Children Hospital in Accra, Ghana.

Research frontiers

This is probably the first study in Ghana which investigated the association between malaria and B19V co-infection on the type and degree of anaemia among Ghanaian children.

Related reports

Parsyan *et al.* (2006) reported that ~11% of hospitalised Ghanaian children infected with B19V had severe anaemia. However, Wildig *et al.* (2006; 2010) revealed that B19V may not be a contributing factor to anaemia among Malawian children.

Innovations and breakthroughs

This study utilized a combination of molecular and routine techniques to study the association between malaria–B19V co-infection and anaemia among hospitalized Ghanaian children.

Applications

This study highlighted the significant effects of *P. falciparum* and B19V co-infection on anaemia among Ghanaian children. Awareness among clinicians on the effects of the co-infection will enable them to improve the management of anaemia among children.

Peer review

There are conflicting reports on whether co-infection with malaria and B19V can exacerbate anaemia. Some

findings suggest that such co-infection is associated with anaemia, while others suggest not. This report finds that co-infection of B19V and malaria is associated with microcytic anaemia, which is novel and calls for further investigations to establish this effect.

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