Association between haemoglobin variants S and C and Mycobacterium ulcerans disease (Buruli ulcer): a case-control study in Benin

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Summary
Risk factors for Buruli ulcer (BU) are poorly understood. We conducted a case-control study in southern Benin to investigate the association between haemoglobin variants S or C and BU, and particularly the association between haemoglobinopathies HbSS/SC and BU osteomyelitis. We compared the haemoglobin genotype of 179 patients with BU and 44 with BU osteomyelitis to that of 242 community controls. We found no evidence of an increased risk of BU according to the presence of haemoglobin variants S and/or C [odds ratio adjusted for sex, age, region of residence and ethnicity: 1.24 (95%CI: 0.80–1.93), \( P = 0.34 \)]. Haemoglobin variants S and C are unlikely to play a role in the BU burden. However, haemoglobinopathies HbSS/SC were more frequent among BU osteomyelitis patients than among controls (6.8% vs. 1.0%, Fisher’s exact \( P \)-value = 0.045), which may suggest that those disorders facilitate growth of Mycobacterium ulcerans in the bone matrix.

keywords Case-Control Studies, Mycobacterium ulcerans, Benin, Haemoglobin S, Haemoglobin C, Osteomyelitis

Introduction
Mycobacterium ulcerans disease or Buruli ulcer (BU) is an emerging skin disease of public health concern in West Africa (Marston et al. 1995; Amofah et al. 2002; Debacker et al. 2004a; Sizaire et al. 2006). The clinical lesion usually starts as a subcutaneous nodule, papule, plaque or oedema that secondarily ulcerates. Spontaneous healing can occur, but without appropriate early treatment, lesions often evolve towards extensive skin destruction. Bone involvement is increasingly reported (Portaels et al. 2003).

There is no specific vaccine against BU and control strategies are limited owing to lack of knowledge of potential risk factors. The mode of transmission of M. ulcerans to humans remains unclear (Duker et al. 2006). BU predominantly affects rural populations living near swampy environments (Barker 1973; Johnson et al. 2005b). The role of insects is increasingly suspected (Portaels et al. 1999; Marsollier et al. 2002; Kotlowski et al. 2004) but it is commonly postulated that M. ulcerans could enter the skin from contaminated environments through superficial injuries (Meyers et al. 1974; Debacker et al. 2002).

Host-related factors are also poorly understood. BU affects mainly children, but in Benin, higher detection rates were also found in adults aged 60–79 years than in younger adults, with a predominance in males (Debacker et al. 2004b). Severe forms of BU occur in HIV-positive patients (Johnson et al. 2002; Toll et al. 2005), but HIV has not been reported as a factor increasing the risk of developing BU. Schistosomiasis haematobium infection, which could shift the immune response towards increased
susceptibility to mycobacteria, does not appear to be associated with BU (Scott et al. 2004; Stienstra et al. 2004). However, concurrent S. haematobium infection could influence BU disease severity (Scott et al. 2004).

As M. ulcerans grows preferentially in microaerobic environments (Palomino et al. 1998), its proliferation in human tissues could be enhanced by reduced oxygen content and impaired microcirculation associated with haemoglobin variants S and C. Pszolla et al. (2003) suggested sickle cell trait as a factor promoting the systemic spread of M. ulcerans with multifocal cutaneous lesions and BU osteomyelitis in an Angolan patient. Moreover, haemoglobinopathies HbSS (sickle cell anaemia) and HbSC are responsible for osteonecrosis (Weatherall 1997; Nagel et al. 2003) and therefore could be associated with BU osteomyelitis. Although prevalence of haemoglobin variants S and C is high in West Africa (Embry et al. 1994), no epidemiological study has addressed this issue so far.

We therefore conducted a case-control study to investigate the association between the presence of haemoglobin variants S and/or C and BU, and particularly the association between haemoglobinopathies HbSS/SC and BU osteomyelitis. This study was approved by the Beninese Ministry of Public Health.

Materials and methods

Setting

This study was conducted in southern Benin. Until 1998, the ‘Centre Sanitaire et Nutritionnel Gbemoten’ (CSNG) at Zagnanado, in the Zou region, was the only reference centre for BU treatment in this area. In 1998, the ‘Centre de Dépistage et deTraitement de l’Ulcére de Buruli’ (CDT/UB) at Lalo, in the Couffo region, was progressively involved in BU treatment and active case finding. More than 5700 BU cases have been reported in southern Benin since 1989 (WHO 2004; Debacker et al. 2004a) and in 1999, the BU detection rate in the Zou region was estimated at 21.5 per 100 000 inhabitants (Debacker et al. 2004a). In 1992, Chippaux et al. (1992) estimated the prevalence of haemoglobin variants (S and/or C) at 28% in Cotonou and 35% in the Zou region.

Cases

Two case series were included in our study. First, we planned to recruit all consecutive patients clinically diagnosed with BU (all clinical forms) by medical officers specialized in BU treatment at the CSNG and the CDT/UB from March to August 2003. Patients living outside Benin were excluded as were those living in the cities of Cotonou and Porto-Novo, as it is unlikely that they contracted BU in those urban areas. Among the 166 BU patients hospitalized during the study period at the CSNG, one died and 125 (75.3%) provided a blood sample for haemoglobin genotyping. Fifty-four of the 55 patients hospitalized during the study period at the CDT/UB provided a blood sample for haemoglobin genotyping. Overall, therefore, we included in the present study 179 patients with all forms of BU (125 from CSNG and 54 from CDT/UB). Lesions were classified according to the clinical form: nodule, plaque, oedema, ulcer, mixed and other lesions. Specimens of tissue and of exudates from some patients were analysed by the following laboratory tests to confirm the clinical diagnosis: Ziehl-Neelsen examination, culture and IS2404 PCR and histopathologic examination (WHO 2001).

Second, we aimed to recruit retrospectively a series of 79 patients who were treated at the CSNG between 1996 and August 2003 for a M. ulcerans osteomyelitis confirmed by at least two laboratory tests performed on bone tissue (WHO 2001; Portaels et al. 2003). We could find and include 47 (59.5%) of those 79 patients.

Controls

Controls were recruited as part of a larger case-control study conducted in the same area to estimate BCG vaccine effectiveness against BU (Nackers et al. 2006) and to investigate other potential BU risk factors. The larger study recruited four individually age-, sex- and neighbourhood-matched controls for each patient diagnosed with BU from August 2002 to May 2003 at the CSNG and from August 2002 to August 2003 at the CDT/UB. Participating controls were examined to rule out active or healed BU and were invited to provide a blood sample that was stored on filter paper. Individual refusal rate was estimated at 25.7%. We included in the present study the first control who accepted to provide a blood sample in each matched set of the study investigating BCG and other BU risk factors, i.e. 242 controls. The controls are therefore a random selection of the population from which the cases came, but with an age, sex and area distribution matched to BU patients, and restricted to those who agreed to give a blood sample.

The recruitment period of the BCG and BU risk factors study partly overlapped with the recruitment period of patients with all forms of BU in the present study. As a consequence, a subgroup of the 242 controls are individually matched to a subgroup of the 179 cases with all forms of BU included in this study, giving 82 matched pairs.

F. Nackers et al.   Haemoglobin variants S and C and Buruli ulcer
Data collection

Age, sex, region of residence and ethnicity were recorded for each participant. Clinical information for the cases was abstracted from medical records. After consent of the participant (or guardian for children <15-year-old), a blood sample was collected on filter paper to detect S (β⁶Glu → Val) and C (β⁶Glu → Lys) mutations. Allelic discrimination by real-time PCR (TaqMan®) using minor groove binder (MGB) probes was used on extracted DNA. This novel genotyping method has been validated by comparison with conventional polymerase chain reaction–restriction fragment length polymorphism using the Bsu36I restriction enzyme. (This validation and technical details, including optimization for amplification of DNA extracted from filter paper, will be reported elsewhere.) Each identified SC, SS and CC genotype was confirmed by sequence analysis.

Statistical analysis

Proportions were compared using Pearson’s chi-squared and Fisher’s exact tests. Mann-Whitney test was used for the comparison of age. Confidence intervals (CI) of crude odds ratios (OR) were calculated using exact methods. OR were adjusted for age, sex, ethnicity and region of residence using logistic regression. Data for the subsample of individually matched cases and controls were analysed using conditional logistic regression. Associations and interactions in all logistic models were tested using the likelihood ratio test (LRT). Given the shorter life expectancy of subjects suffering from sickling disorders in Africa, analyses to investigate the association between haemoglobinopathies HbSS/SC and BU osteomyelitis were restricted to subjects aged less than 40 years. Risk difference per cent was calculated as (OR-1)/OR and population attributable fraction as [Prevalence of exposure among cases × (OR-1))/OR under a rare disease assumption. Data were entered in Epi-info (version 6.04) and analysed with STATA (version 9).

Results

All forms of Buruli ulcer

Patients recruited for haemoglobin genotyping at the CSNG had a similar age distribution to those not recruited but they were more likely to be females and had a different distribution of regions of residence (Table 1). Among the 179 patients recruited from both centres (CSNG and CDT/UB), ulcer was the most frequent clinical form of BU (48.0%) followed by plaque (26.8%) and the mixed forms (19.6%) (Table 2). Fifty-one percent of the BU cases were confirmed by at least one of the four laboratory tests. Eight patients with mixed forms had bone involvement.

Buruli ulcer osteomyelitis

Three of the 47 patients with BU osteomyelitis recruited were over 40 years old at diagnosis and were excluded from further analysis. Patients recruited were similar to those not recruited in terms of age and region of residence but not sex (Table 1). Reasons for non-recruitment were: 1 refusal, 2 deaths, 4 unreachable villages, 11 wrong addresses and 14 unknown. Of the 44 patients with BU osteomyelitis, the type of bone lesion was unknown for one patient. Among the others, 11 (26%) presented a bone lesion contiguous to a BU skin lesion and 32 (74%) presented metastatic bone lesions (i.e. bone lesions distant from a skin lesion and involving at least two bones).

Table 1 Comparison of cases included and not included in the study (Centre Sanitaire et Nutritionnel Gbemoten)

<table>
<thead>
<tr>
<th></th>
<th>BU (all forms)</th>
<th>BU osteomyelitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not included</td>
<td>Included</td>
</tr>
<tr>
<td></td>
<td>(n = 41)</td>
<td>(n = 125)</td>
</tr>
<tr>
<td>Female - n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>11.0 (0.6–70)</td>
<td>14.0 (1–80)</td>
</tr>
<tr>
<td>Region of residence – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouedé –Plateau</td>
<td>13 (31.7)</td>
<td>87 (69.6)</td>
</tr>
<tr>
<td>Zou</td>
<td>18 (43.9)</td>
<td>30 (24.0)</td>
</tr>
<tr>
<td>Mono-Couffo, Atlantique</td>
<td>10 (24.4)</td>
<td>8 (6.4)</td>
</tr>
</tbody>
</table>

*Pearson’s chi-squared.
†Mann-Whitney.
‡Fisher’s exact test.
BU, Buruli ulcer.
Haemoglobin variants S and C and Buruli ulcer

Socio-demographic characteristics

Age ranged from 1.5 to 83 years among controls, from 1 to 80 years among patients with all forms of BU and from 4 to 30 years among patients with BU osteomyelitis. Characteristics of the participants are shown in Table 3. Patients with all forms of BU were compared with the 242 controls while the 44 BU osteomyelitis patients were compared with the subgroup of controls aged less than 40 years, i.e. 195 controls. Age and sex distributions were similar among patients with all forms of BU, BU osteomyelitis and controls. Compared with controls, patients with all forms of BU were less likely to belong to the Fon ethnic group while BU osteomyelitis patients were more often from the Zou and Atlantique regions.

Haemoglobin genotype

Allele frequencies for the beta globin gene were similar in controls, patients with all forms of BU and BU osteomyelitis (Table 4). Among controls, the haemoglobin genotypes appeared overall in Hardy-Weinberg equilibrium. There was no evidence that the distributions of haemoglobin variants among controls was 27.3% of the controls had abnormal haemoglobin (S or C or both) (Table 5) and there was no evidence of an increased risk of BU with the presence of haemoglobin variants S and/or C (OR 1.15, 95% CI: 0.73–1.80). The OR estimation changed very little after adjustment for age, sex, ethnicity and region of residence (adjusted OR 1.24, 95% CI: 0.80–1.93, Table 5), when analysis was restricted to the majority Fon ethnicity (adjusted OR 1.10, 95% CI: 0.67–1.82), laboratory-confirmed cases (adjusted OR 1.20, 95% CI: 0.69–2.10) or when matched analysis was performed on the 82 sets matched on sex, age and neighbourhood (OR adjusted on ethnicity: 1.20, 95% CI: 0.59–2.45). Associations were similar in males and females (P-value for interaction = 0.98) and in different age groups (P-value for interaction = 0.50).

At least one allelic variant (S, C or both) was present in 29.5% (13/44) of the patients with BU osteomyelitis and 27.2% (53/195) of the controls (P = 0.75) (Table 4). There was weak evidence of an increased risk of BU osteomyelitis with haemoglobinopathies HbSS or HbSC (Table 5). Using the point estimates and assuming causality, the proportion of BU osteomyelitis attributable to the haemoglobinopathy in those with HbSS/SC (the risk difference per cent) was 83%. In the general population, the proportion of BU osteomyelitis attributable to HbSS/SC (the population attributable fraction) was estimated as 6%. When analysis was restricted to the 32 with metastatic osteomyelitis, the crude OR was 6.43 (exact 95% CI: 0.44–60.62; Fisher’s exact P = 0.096).

Discussion

We found similar frequencies of haemoglobin variants S and/or C in patients with all forms of BU and controls and we can exclude a strong association between the presence of single variants and the risk of developing BU.

This study was not fully individually matched, as blood sampling of the cases started later than originally planned. But we have no reason to believe that this biases the results as the controls were representative of the population providing BU cases in southern Benin. Prevalence of haemoglobin variants among controls was 27.3%. This is slightly lower but still consistent with previously published data of 35% in the Zou region and 28% in Cotonou (Chippaux et al. 1992).

Not all BU cases in the region were included in the study: ascertainment of cases in the region is unlikely to have been complete, and of the eligible hospital patients, not all were recruited. Among the controls, only 74% agreed to provide a blood sample. However, as refusal,
health-seeking behaviour and the recruitment process in hospitals are unlikely to differ with respect to asymptomatic or mild conditions such as haemoglobin genotype AS, AC or CC, we can exclude substantial selection bias. Also, patients recruited at the CSNG were more likely to be females than those not recruited. As sex was not found as a modifying factor for the association between BU and haemoglobin variants ($P$-value for interaction $= 0.98$), this should not have biased our results.

Only half of the patients with all forms of BU were laboratory-confirmed but restricting the analysis to the confirmed cases did not change the estimates. Cases

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Socio-demographic characteristics of the participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>Controls (all) (n = 242)</td>
</tr>
<tr>
<td>Female</td>
<td>110 (43.5)</td>
</tr>
<tr>
<td>&lt;6</td>
<td>19 (7.9)</td>
</tr>
<tr>
<td>6&lt;13</td>
<td>92 (38.0)</td>
</tr>
<tr>
<td>13&lt;20</td>
<td>33 (13.6)</td>
</tr>
<tr>
<td>20&lt;30</td>
<td>33 (13.6)</td>
</tr>
<tr>
<td>30&lt;39</td>
<td>18 (7.4)</td>
</tr>
<tr>
<td>40&lt;59</td>
<td>31 (12.8)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>16 (6.6)</td>
</tr>
<tr>
<td>Region of residence</td>
<td></td>
</tr>
<tr>
<td>Oue´me-Plateau</td>
<td>136 (56.2)</td>
</tr>
<tr>
<td>Zou</td>
<td>43 (17.8)</td>
</tr>
<tr>
<td>Mono-Couffo</td>
<td>49 (20.2)</td>
</tr>
<tr>
<td>Atlantique</td>
<td>14 (5.8)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Fon</td>
<td>208 (86.3)</td>
</tr>
<tr>
<td>Others</td>
<td>33 (13.7)</td>
</tr>
</tbody>
</table>

*Pearson’s chi-squared.
†Missing value for one control older than 40 years.
‡Fisher’s exact test.
BU, Buruli ulcer.
*aAge at BU diagnosis for BU osteomyelitis.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Allele frequencies and crude associations between Buruli ulcer (BU) and haemoglobin genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td>Controls (all) (n = 242)</td>
</tr>
<tr>
<td>n (%)</td>
<td>OR [95%CI]†</td>
</tr>
<tr>
<td>βA</td>
<td>413 (85.3)</td>
</tr>
<tr>
<td>βS</td>
<td>44 (9.1)</td>
</tr>
<tr>
<td>βC</td>
<td>27 (5.6)</td>
</tr>
<tr>
<td>Haemoglobin genotype</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>176 (72.7)</td>
</tr>
<tr>
<td>AS</td>
<td>41 (16.9)</td>
</tr>
<tr>
<td>AC</td>
<td>20 (8.3)</td>
</tr>
<tr>
<td>SS</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>SC</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>CC</td>
<td>3 (1.2)</td>
</tr>
</tbody>
</table>

*Pearson’s chi-squared.
†Fisher’s exact test – 95% CI exact limits.
OR, odds ratio; CI, confidence interval.
differed from controls in ethnicity which could be an important confounder when studying genetic variants (Rothman & Greenland 1998). Controlling for ethnicity or restricting the analysis to the Fon ethnic group changed the estimates very little. Estimates were also controlled for region of residence. This uses wide administrative boundaries while BU incidence and environmental exposure to M. ulcerans are known to vary across smaller areas (Johnson et al. 2005a; Stoffel et al. 2005). Estimation remained the same when we analysed the 82 sets matched on neighbourhood.

In conclusion, we found no evidence of an increased risk of BU with the presence of haemoglobin variants S and/or C and this is unlikely to be explained by confounding or bias. Our data do not support the hypothesis that microcirculation abnormalities associated with the presence of haemoglobin variants S and/or C could enhance survival of M. ulcerans in human subcutaneous tissue. Haemoglobin variants S and C are thus unlikely to play a role in the burden of BU in Benin.

Haemoglobinopathies SS and SC tended to be more frequent in BU osteomyelitis (6.8%) cases than in controls (1.0%). Haemolytic anaemia, immune-deficit and impaired microcirculation associated with those disorders may facilitate haematogenous spread and growth of M. ulcerans in the bone matrix.

Metastatic BU osteomyelitis suggests a haematogenous spread of M. ulcerans infection. This is consistent with the pathway of bone infection that can be expected in sickling disorders. Although contiguous bone lesions are less likely to fit with this pathway, long-term follow-up of patients with contiguous osteomyelitis shows that most of them will develop metastatic bone lesions later (F. Portaels, personal communication). Our main analysis therefore included all types of bone lesions. Among the patients with BU osteomyelitis and a sickling disorder, two presented metastatic bone lesions on lower limbs. The third patient presented two bone lesions (on the humerus and the collar bone although only the humerus sample tested positive for M. ulcerans). Unfortunately, the location of BU skin lesion(s) of this patient was unknown preventing us from classifying the osteomyelitis as contiguous or metastatic. Excluding this patient and the patients with contiguous osteomyelitis from the analysis only slightly reduced the OR.

HbSC disorder was more common in our sample than HbSS which is consistent with a better prognosis of HbSC compared with HbSS disease (Embury et al. 1994; Nagel et al. 2003) while osteonecrosis is as frequent in both diseases (Nagel et al. 2003). All osteomyelitis cases were confirmed by at least two tests positive for M. ulcerans, excluding any misclassification of disease status. This is a strong feature of this study. Indeed, including unconfirmed cases would have led to bias as non-BU osteomyelitis is known to be associated with HbSS/SC. Consequently, the sample size for BU osteomyelitis was small and sickling syndrome HbSS/SC was rare in the survey sample. As a result, we only have weak evidence of an association between haemoglobinopathies SS and SC and BU osteomyelitis. However, even relying on small numbers, our data suggest a possible strong association between sickling disorders and BU osteomyelitis. Although, owing to their low prevalence, HbSS/SC disorders could only be responsible for a small part of all BU osteomyelitis occurring in the population of southern Benin, they could be responsible for up to 83% of the BU osteomyelitis occurring in the affected people.

Table 5

<table>
<thead>
<tr>
<th>Haemoglobin genotype</th>
<th>Controls (all)</th>
<th>BU (all forms)</th>
<th>Crude OR [95%CI] †</th>
<th>P</th>
<th>Adjusted OR [95%CI]</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>176 (72.7)</td>
<td>125 (69.8)</td>
<td>1</td>
<td>0.52*</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Others</td>
<td>66 (27.3)</td>
<td>54 (30.2)</td>
<td>1.15 [0.73–1.80]</td>
<td>1.24 [0.80–1.93]*</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>193 (99.0)</td>
<td>41 (93.2)</td>
<td>7.06 [0.77–85.98]</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>SS, SC</td>
<td>2 (1.0)</td>
<td>3 (6.8)</td>
<td>5.99 [0.72–50.02]*</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*K Pearson’s chi-squared.
† Fisher’s exact test – 95% CI exact limits.
‡LRT, likelihood ratio test.
a Adjusted by logistic regression on age, sex, region of residence and ethnicity.
b Ethnicity is missing for one control HbAC.
OR, odds ratio; CI, confidence interval.

Table 5 Associations between Buruli ulcer (BU) and haemoglobin variants S and/or C
Acknowledgements

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References


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**Association entre les variantes S et C de l’hémoglobine et l’infection à Mycobacterium ulcerans (ulcère de Buruli): une étude cas-témoins au Bénin**

Les facteurs de risque de l’ulcère de Buruli sont peu connus. Nous avons mené une étude cas-témoins dans le sud du Bénin pour investiguer l’association entre les variantes S et C de l’hémoglobine et l’ulcère de Buruli et, en particulier, l’association entre les hémoglobinopathies HbSS/SC et les ostéomyélites à *M.ulcerans*. Nous avons comparé le génotype de l’hémoglobine de 179 patients atteints d’ulcère de Buruli et de 44 patients avec une ostéomyélite à *M.ulcerans* à celui de 242 témoins de la communauté. Nous n’avons trouvé aucune évidence d’un plus grand risque d’ulcère de Buruli en présence des variantes S et/ou C de l’hémoglobine (OR ajusté pour le sexe, l’âge, la région de résidence et l’appartenance ethnique : 1,24 [IC95% : 0,80–1,93], P = 0,34). Il est peu probable que les variantes S et C de l’hémoglobine jouent un rôle dans le fardeau de l’ulcère de Buruli. Cependant, les hémoglobinopathies HbSS/SC étaient plus fréquentes chez les patients avec une ostéomyélite à *M.ulcerans* que chez les témoins (6,8% contre 1,0%, P-valeur du test exact de Fisher = 0,045), ce qui permet de suggérer que ces troubles de l’hémoglobine pourraient faciliter la croissance de *M. ulcerans* dans le tissu osseux.

**mots clés** Études cas-témoins, Mycobacterium ulcerans, Bénin, hémoglobine S, hémoglobine C, ostéomyélite

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**Asociación entre las variantes de hemoglobina S y C y la úlcera de Buruli: estudio caso-control en Benin**

Los factores de riesgo para la úlcera de Buruli no son bien conocidos. Hemos conducido un estudio de casos y controles en el sur de Benin para investigar la asociación entre las variantes de hemoglobina S o C y la úlcera de Buruli, en particular la asociación entre hemoglobinopatías HBSS/SC y la osteomielitis por úlcera de Buruli. Hemos comparado el genotipo de hemoglobina de 179 pacientes con úlcera de Buruli y 44 con osteomielitis por úlcera de Buruli, frente a 242 controles de la comunidad. No encontramos evidencia de un riesgo aumentado de úlcera de Buruli según las variantes de hemoglobina S y/o (OR ajustado para sexo, edad, lugar de residencia y etnia 1.24 [95%CI: 0.80–1.93], P = 0.34). Las variantes de hemoglobina S y C no parecen jugar papel alguno en la carga por úlcera de Buruli. Sin embargo, las hemoglobinopatias HBSS/SC eran más frecuentes entre pacientes con osteomielitis por úlcera de Buruli que entre los controles (6.8% versus 1.0%, valor P exacto de Fisher = 0.045), lo cual podría sugerir que estos desórdenes facilitan el crecimiento de M. Ulcerans en la matriz ósea.

**palabras clave** Estudios caso-control, Mycobacterium ulcerans; Benin, Hemoglobina S, Hemoglobina C, Osteomielitis