Evaluation of Creatine Kinase Activity and Inorganic Phosphate Concentration in Adult Nigerian Homozygous and Heterozygous Hemoglobin Phenotypes

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Abstract

Background: Biochemical parameters vary in subjects with different hemoglobin phenotypes, compared with normal controls. Aim: The aim was to evaluate serum creatine kinase (CK) activity and inorganic phosphate concentrations in Nigerian adults with homozygous and heterozygous hemoglobin phenotypes. Subjects and Methods: A prospective study, carried out at the hematology out-patient clinic of our hospital, a community health center and a private hospital, all in Anambra state. Subjects included hemoglobin phenotypes AA, AS, and SS, in steady state and vaso‑occlusive crisis (VOC). Samples were collected for serum CK activity and inorganic phosphate concentrations. Data obtained were analyzed using SPSS 16.0 (SPSS Inc., Chicago IL, USA). Means were compared using the Student’s t-test and statistical significance was set at \( P < 0.05 \). Results: A total of 100 subjects participated in the study. There was a statistically significant difference in the means of serum CK activity in hemoglobin SS (HbSS) in VOC versus hemoglobin AA (HbAA) \( (P = 0.01) \) and HbSS in steady state versus HbAA \( (P = 0.02) \) but not in hemoglobin AS (HbAS) versus HbAA \( (P = 0.79) \) and HbSS in VOC versus HbSS in steady state \( (P = 0.06) \). A statistically significant difference was noted in the means of serum inorganic phosphate concentration in HbSS in VOC versus HbAA \( (P = 0.01) \), but not in HbSS in steady state versus HbAA \( (P = 0.43) \), HbSS in VOC versus HbSS in steady state \( (P = 0.09) \) and HbAS versus HbAA \( (P = 0.20) \). Conclusion: Sickle cell disease is a predictor for high serum CK activity and low serum concentration of inorganic phosphate, particularly in VOC. There may be a need to monitor serum CK activity in HbSS subjects presenting with major VOC.

Keywords: Serum creatine kinase activity, Serum inorganic phosphate concentration, Sickle cell disease, Steady state, Vaso‑occlusive crisis

Introduction

Sickle cell disease (SCD) is an umbrella terminology which includes sickle cell anemia, and other groups of conditions characterized by the inheritance of the sickle(S) gene together with another abnormal hemoglobin gene such as C, E, D and the thalassemia genes.\(^1\) It has a wide geographical distribution, involving parts of the Mediterranean, Middle East, and Africa.\(^2\)

The asymptomatic carrier state, hemoglobin AS (HbAS), has reported prevalence of 25% in Nigeria, confers some immunity to severe falciparum malaria infection and is responsible for the continued presence of the sickle gene in parts of Africa.\(^3\)\(^4\)

The course of SCD includes periods of “crises” interspersed by periods of apparent normality known as steady states. More so, various complications, including end organ damage, have also been reported.\(^5\) Vaso‑occlusive crisis (VOC) is one of the...
established crises of SCD, the hallmark of which is vascular occlusion and tissue (including muscle) ischemia.[6]

Creatine kinase (CK) is a ubiquitous enzyme with various isoforms. Serum levels of the muscle (MM) isoform have been found to be increased in conditions associated with tissue hypoxia and muscle necrosis.[7]

Inorganic phosphate is found free in plasma, is actively re-absorbed by the renal tubules and as such serum concentrations are dependent on renal function.[8]

The aim of this study was to evaluate serum CK activity and serum inorganic phosphate levels in adult homozygous and heterozygous hemoglobin phenotypes in Nigeria.

Subjects and Methods

This study was conducted at three different locations, namely; the hematology out-patient clinic of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, a community health center and a private hospital, all in Anambra state. A total of 100 subjects were studied, including 30 known hemoglobin SS (HbSS) subjects in steady state (steady state was defined as the absence of any form of crises and steady clinical conditions at least 2 weeks prior to recruitment). Ten HbSS subjects in VOC, 30 confirmed HbAS and 30 hemoglobin AA (HbAA), subjects were also included.

Each participant had 5 ml of venous blood collected following standard methodology. Of this, 2 ml of venous blood was transferred into ethylene diamine tetra acetic acid bottle for hemoglobin electrophoresis; 3 ml was captured in plain bottle allowed to clot and the serum extracted by centrifugation at 5000 rpm for 5 min for estimation of serum CK activity and inorganic phosphate concentration.

Hemoglobin electrophoresis was done using the alkaline cellulose acetate method.[9]

Serum CK activity was assessed using the ultraviolet spectrophotometric method,[10] while serum inorganic phosphate concentration was assayed using the spectrophotometric method described by Henry et al.[11] Ethical approval for this study was obtained from our Institution Review Board, and all participants gave informed consent.

Data analysis was performed using SPSS version 16 computer software (SPSS Inc., Chicago IL, USA). Descriptive variables were expressed as means and standard deviations (SD).

The means of serum CK activity and inorganic phosphate were compared among hemoglobin phenotypes using the Student’s \( t \)-test and the difference between means was considered significant if \( P < 0.05 \).

Results

Creatine kinase activity

The means and SD of serum CK activity in HbSS subjects in steady state, in VOC, HbAS, and HbAA subjects were 585.3 (184.2) IU/L, 719.3 (200.9) IU/L, 457.3 (102.0) IU/L, and 467.2 (182.8) IU/L, respectively [Table 1].

There was a statistically significant difference in the means of serum CK activity in HbSS in VOC versus HbAA \( (P = 0.01) \) and HbSS in steady state versus HbAA \( (P = 0.02) \) [Table 1]. There were no statistically significant difference in the means of CK in HbAS versus HbAA \( (P = 0.79) \) and HbSS in VOC versus HbSS in steady state \( (P = 0.06) \) [Table 1].

Serum inorganic phosphate concentration

The means of serum inorganic phosphate concentrations in HbSS in steady state, in VOC, HbAS and HbAA subjects were 2.6 (1.2) IU/L, 2.0 (0.3) IU/L, 3.3 (2.0) IU/L, and 2.8 (0.7) IU/L, respectively [Table 2]. There was a statistically significant difference in the means of inorganic phosphate concentration in HbSS in VOC versus HbAA \( (P = 0.01) \) [Table 2]. No statistically significant difference was noted in the means of inorganic phosphate in HbSS in steady state versus HbAA \( (P = 0.04) \), HbSS in VOC versus HbSS in steady state \( (P = 0.09) \), and HbAS versus HbAA \( (P = 0.20) \) [Table 2].

<p>| Table 1: The means and SD of serum CK activity in the different hemoglobin phenotypes |
|---------------------------------|-----------------|---|</p>
<table>
<thead>
<tr>
<th>Hemoglobin phenotype</th>
<th>Means and SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAS ( \text{n} = 30 )</td>
<td>457.2 (102)</td>
<td>0.79</td>
</tr>
<tr>
<td>HbAA ( \text{n} = 30 )</td>
<td>467.2 (182.6)</td>
<td>0.02*</td>
</tr>
<tr>
<td>HbSS (steady state, ( \text{n} = 30 ))</td>
<td>585.3 (184.2)</td>
<td>0.06</td>
</tr>
<tr>
<td>HbAA ( \text{n} = 30 )</td>
<td>467.2 (182.6)</td>
<td>0.02*</td>
</tr>
<tr>
<td>HbSS (VOC, ( \text{n} = 10 ))</td>
<td>719.3 (200.9)</td>
<td>0.06</td>
</tr>
<tr>
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<td>0.06</td>
</tr>
</tbody>
</table>


<p>| Table 2: The means and SD of serum inorganic phosphate concentrations in the different hemoglobin phenotypes |
|---------------------------------|-----------------|---|</p>
<table>
<thead>
<tr>
<th>Hemoglobin phenotype</th>
<th>Means and SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbAS ( \text{n} = 30 )</td>
<td>3.3 (2)</td>
<td>0.20</td>
</tr>
<tr>
<td>HbAA ( \text{n} = 30 )</td>
<td>2.8 (0.7)</td>
<td>0.43</td>
</tr>
<tr>
<td>HbSS (steady state, ( \text{n} = 30 ))</td>
<td>2.6 (1.2)</td>
<td>0.06</td>
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<td>0.06</td>
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<td>HbSS (steady state, ( \text{n} = 30 ))</td>
<td>2.6 (1.2)</td>
<td>0.06</td>
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Discussion

Serum CK activity has been documented in previous studies to be dependent on race, level of physical exertion, muscle trauma, inflammation, and organ damage.

The means of serum CK activity in HbSS patients in our study was significantly higher than in HbAS and HbAA subjects. There was a statistically significant difference in the means of serum CK activity in HbSS subjects both in VOC and steady state and HbAA controls. Hunt et al. have reported an increase in CK activity in HbSS patients presenting in VOC; however, their cohort of patients in steady state were reported to have normal CK activity. This is in contrast with the findings of this report in which HbSS subjects in steady state had elevated serum CK levels. It may thus appear that our HbSS study population in steady state has other factors that lead to a high CK level. The level of physical exertion is documented to increase serum level of CK activity even though, we did not assess the level of physical exertion of our patients in this study, it may be likely that our group of SCD patients exert themselves more, which may account for the high CK activity noted in them, even in steady state conditions. Though our study noted a higher serum CK activity in HbSS subjects presenting in VOC than those in steady state (719.3 [200.9] IU/L vs. 585.3 [184.2] IU/L), this was not statistically significant (P = 0.06). The lack of statistical significance in serum CK activity in the two study populations may stem from the fact that our steady state HbSS subjects already had elevated serum enzyme activity, as earlier alluded. This is at variance with the report of Agarwal et al., who reported a significant increase in serum CK activity in SCD patients, presenting in VOC. Muscle ischemia and membrane perturbation, that may follow VOC, with subsequent release of enzymes into the serum are thought to be directly responsible for this increase. Extensive muscle perturbation following strenuous exercise in individuals with sickle cell trait has been variously reported to cause rhabdomyolysis, disseminated intravascular coagulopathy, renal failure and even death. Such extensive muscle perturbation resulting from VOC (especially major forms of it) may thus predispose these subjects to this potentially fatal complication. Interestingly, serum CK levels has been shown to be a better predictor of acute renal failure due to rhabdomyolysis than serum creatinine. It is therefore important that such subjects should have their serum CK activity and urine output monitored so as to enable early identification of those likely to develop this complication. When detected early, appropriate therapeutic interventions are known to prevent progression to acute renal failure.

Hemoglobin SS patients in our study population, including those presenting in VOC and those in steady state had lower serum concentrations of inorganic phosphate compared to HbAS and HbAA subjects. Particularly in HbSS patients in VOC, a statistically significant difference was noted between the means of their inorganic phosphate and that of HbAA controls (P = 0.01, Table 2). There were however no statistically significant difference in serum concentration of inorganic phosphate in HbSS in steady state versus HbAA, HbSS in steady state versus HbSS in VOC or HbAS versus HbAA (P > 0.05).

Sickle cell disease patients are known to have increased tubular re-absorption of phosphate and this may account for a higher serum levels than controls. Our finding of lower concentration of serum inorganic phosphate concentration in HbSS subjects compared to normal controls is at variance with findings of De Jong et al. and Oladipo et al. both reported high levels in adults and children, respectively with SCD. On the contrary, Al-Harbi et al. have reported a low serum concentration of inorganic phosphate in a population of Saudi children with SCD. This was attributed to high levels of parathyroid hormone; a hormone known to lower renal re-absorption of phosphate. While the reason for the low serum levels in our population of SCD patients, in VOC, who were adult cohorts, was not apparent from this study, it is tempting to hypothesize that in VOC, acute reduction in blood to the kidney may cause a reduction of absorption of inorganic phosphate from the nephron. It is however needful to further study the renal functional status as well as serum levels of parathyroid hormone in our patients in VOC, as both factor is known to reduce serum phosphate levels.

Conclusion

Sickle cell disease, whether in VOC or in steady state, appears to be a significant predictor of higher CK activity compared with normal controls, while VOC appears to significantly predict for lower serum concentration of inorganic phosphate in our data set. Monitoring of serum CK activity in HbSS subjects with major VOC is advocated to enable early detection of rhabdomyolysis, a complication which may lead to renal damage. The low serum inorganic phosphate concentration reported in our HbSS subjects in VOC may be as a result of elevated parathyroid hormone and/or acute reduction in blood flow, but will need to be further confirmed in subsequent studies, with a larger cohort of patients.

Limitations of the study

The study is limited by the small number of SCD patients in VOC that were studied.

References


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