Original article

Interest of serum Copper/Zinc ratio in oxidative stress assessment on a population of senegalese sickle cell children


*Laboratoire de Biochimie Pharmaceutique-Faculté de Médecine de Pharmacie et d’Odontostomatologie, Université Cheikh Anta Diop, Dakar, Sénégal
**Centre Hospitalier National d’Enfants Albert-Royer, Dakar, Sénégal

ARTICLE INFO

Keywords:
Cu/Zn ratio
Inflammation
Oxidative stress
Sickle cell disease

ABSTRACT

Introduction: Sickle cell disease is a genetic disease characterized by lipid peroxidation. This lipid peroxidation is evaluated by the Cu/Zn ratio, a parameter positively correlated to several pro-oxidant markers (Cuivre, ...). This work aims to test the value of the Cu/Zn ratio as a marker of oxidative stress and inflammation. Materials and methods: This was a prospective case-control study conducted in 64 homozygotic sickle cell patients (31 in stationary phase; 33 in crisis) and 37 controls, aged 1 to 16 years. Total antioxidant status (TAS), cupremia, zincemia, CRP and Cu/Zn ratio were determined. The statistical analysis was carried out with the R version 3.4 software. Results: TAS and zinc averages were similar in patients and controls groups. Cupremia and Cu/Zn ratio were significantly increased in sickle cell patients compared to controls [(1,85±0,53 mg/l vs 1,42±0,48 mg/l; p=8.10-5) ; (3,22±1,92±2,25 ; 2,23±0,91 ; p=6.10-4)], but also in homozygous in crisis compared to controls [(2,01±0,56 mg/l vs 1,42±0,48 mg/l ; p=7,55.10-6) ; (3,9±2,25 ; 2,23±0,91 ; p=4,71.10-5)]. This ratio was not correlated with TAS (r = -0,03 ; p > 0,05), but, negatively correlated with zinc (r =-0.73; p =0.05), itself positively correlated to TAS (r = - 0,5; p = 0,05). The Cu/Zn ratio was positively correlated with copper (r = 0,59; p< 0,05) and CRP (r = 0,3; p< 0,05). Conclusion: The Cu / Zn ratio is not only a pro-oxidant marker, but also a witness of antioxidant control and inflammatory response.

1. Introduction

Sickle cell disease is an autosomal recessive pathology due to the substitution of a GAG codon by a GTG codon at the β globin gene. This mutation results in the replacement of glutamic acid in position 6 by valine, and in the formation of an abnormal and unstable hemoglobin: hemoglobin S. This hemoglobinopathy is the most widespread genetic disease in the world. [1]. Eighty percent of children suffering from sickle cell disease live in Africa, where the disease accounts for up to 2% of births (sub-Saharan Africa) [2]. In Senegal, the prevalence of sickle cell trait ranges between 8 and 10% [3].

Moreover, sickle cell disease is characterized by multiple physiopathological aspects, the first of which is oxidative stress, a condition resulting from an imbalance between antioxidant defense systems and the production of oxygen free radicals. Indeed, sickle cell disease is marked by haemolysis and vaso-occlusive events, associated with tissue oxygenation disorders that favor the exaggerated production of free radicals and lipid peroxidation [4-7]. This lipid peroxidation, at the origin of the structural and functional alterations of the erythrocyte membrane [8-10] is evaluated by numerous markers such as conjugated diene, aldehydes and more recently isoprostanes.

According to some authors, the Cu / Zn ratio is also a good marker for lipid peroxidation [11-13]. In fact, trace elements appear to be involved in the pathogenesis of many diseases [14-18] as an activator or inhibitor of enzymatic reactions [19], especially those involved in oxidative stress. Thus, zinc and copper participate in the destruction of free radicals via a system of reaction cascades [20]. In addition, these two metals are an integral part of about forty metalloenzymes, including superoxide dismutase, an enzyme known for its antioxidant and anti-
inflammatory activity [21,22]. Zinc exerts its antioxidant action by protecting thiol groups from proteins and inhibiting transition metal-induced EOA formation reactions such as iron and copper. Indeed, if at low concentration copper behaves like an antioxidant, at high dose, it becomes prooxidant and promotes the production of EOA likely to generate not only lipid peroxidation, but also, an oxidation of proteins, sugars and nucleic acids. There is therefore an antagonistic interaction between copper and zinc, the latter inhibiting the radical reactions induced by the cuprous ion. Therefore, the Cu / Zn ratio could be an indicator of the oxidative stress state of an individual in general [12,13].

In order to test this hypothesis, it seemed to us advisable to compare this ratio with another parameter of evaluation of the stress, more precisely the TAS (Total antioxidant status) which appreciates all the antioxidant capacities of the organism. This comparison is justified since previous studies [11,12] have already demonstrated a positive correlation between this report and several pro-oxidant markers [TBARs (substances reacting with thiobarbituric acid), MDA (malonaldehyde), etc.] In addition, given the involvement of copper and zinc in inflammatory phenomena, it is likely that their relationship is also associated.

In view of all these considerations, we have performed this work to test the relevance of using the Cu / Zn ratio as a marker of oxidative stress in general and a control of inflammation.

MATERIAL AND METHOD

This was a prospective longitudinal study conducted at the Albert Royer Children’s Hospital according to the Helsinki Declaration of 1975 as revised in 1996.

Subjects: This work involved 64 homozygous sickle cell patients, 31 of whom were in the stationary phase and 33 in crisis. Inclusion criteria were 1 to 16 years of age, a positive Emmel test, and an SS electrophoretic profile. Sickle cell disease with associated inflammatory pathology, supplemented with zinc or transfused within three months prior to recruitment were excluded. The state of stationary phase or crisis was determined mainly on the presence or not of clinical manifestations corroborated by the biological data in particular the concentration of CRP (the limit being fixed at 10 mg/l).

The control population consisted of 37 matched age and sex-matched sickle cell children and all controls were included on the basis of negative TE and CRP<6 mg/l.

The mean age was 8.94 ± 4.60 in the overall population; 9.48 ± 4.44 in sickle cell patients in the stationary phase; 8.75 ± 4.69 in sickle cell patients in crisis and 8.65 ± 4.73 in controls. The sex ratio M / F was 0.90 for the entire population. For each subject, the free and informed consent of the parents was obtained.

Samples:

They were taken at the level of the elbow fold and the blood collected in an EDTA tube for the completion of the hemogram and the Emmel test. A dry tube was also filled for the determination of lipid parameters and the assays for CRP, TAS, Cu and Zn. The dry tube was centrifuged and the serum aliquoted and stored at -20 °C because the assays were delayed.

Measurement:

The total antioxidant status was measured by an enzymatic method using 2,2’- azino-di- [3-ethylbenzothiazoline sulfonate] (ABTS) in the presence of a peroxidase; RANDOX kit NX2331, Paris (France) Serum concentrations of copper and zinc were also measured by colorimetry with the RANDOX CU2340 and ZN2607 respectively. All assays were performed on the Urit8021A PLC in Shanghai, China (Mainland), except for the measurement of copper levels that was performed on a UV-visible spectrophotometer: STATFAX 3300, India (New Delhi).

Statistical analysis:

The analysis of the different variables was carried out with the STUDENT t- test, the ANOVA test (followed by the TUKEY test), as well as the Pearson correlation coefficient.

All the statistical analyzes were carried out with the software R version 3.4, the threshold of significance being fixed for a value of p<0.05.

1. The comparison of the mean values of the studied parameters between homozygous and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Homozygous sickle cell patients (average±SD)</th>
<th>Controls (average±SD)</th>
<th>p-value (t-Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol/l)</td>
<td>0.88±0.32</td>
<td>1.00±0.3</td>
<td>7.2.10^-2</td>
</tr>
<tr>
<td>Zn (mg/l)</td>
<td>0.7±0.3</td>
<td>0.7±0.27</td>
<td>9.7.10^-1</td>
</tr>
<tr>
<td>Cu (mg/l)</td>
<td>1.85±0.53</td>
<td>1.42±0.48</td>
<td>8.10^-4</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>3.22±1.92</td>
<td>2.23±0.91</td>
<td>6.10^-4</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>36.46±43.34</td>
<td>3.00±2.98</td>
<td>5.64.10^-4</td>
</tr>
</tbody>
</table>
The comparison of the mean values of the studied parameters between homozygous and controls children with sickle cell disease (Table I) revealed significant differences (p<0.001) between the two groups, concerning CRP, cupremia and Cu/Zn ratio.

Taking into account the clinical status of the patients, we found that the mean values of the parameters obtained in sickle cell patients in the stationary phase and in the controls (p>0.05) were superimposable from one group to another.

Table II: Comparison of the averages of the studied parameters between homozygotes in crisis and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sickle cell patients in crisis (average±SD)</th>
<th>Controls (average±SD)</th>
<th>p-value (t-Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol/L)</td>
<td>0.86±0.32</td>
<td>1.00±0.3</td>
<td>1.5.10^-2</td>
</tr>
<tr>
<td>Zn (mg/L)</td>
<td>0.65±0.29</td>
<td>0.7±0.27</td>
<td>7.2.10^-1</td>
</tr>
<tr>
<td>Cu (mg/L)</td>
<td>2.01±0.56</td>
<td>1.42±0.48</td>
<td>7.5.10^-6</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>3.9±2.25</td>
<td>2.23±0.91</td>
<td>4.7.10^-4</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>65.37±43.69</td>
<td>3.00±2.98</td>
<td>3.08.10^10</td>
</tr>
</tbody>
</table>

Table III: Comparison of the averages of the studied parameters between homozygotes in crisis and homozygotes in steady state.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sickle cell patients in crisis (average±SD)</th>
<th>Sickle cell patients in steady state (average±SD)</th>
<th>p-value (Anova)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol/L)</td>
<td>0.86±0.32</td>
<td>0.91±0.33</td>
<td>7.1.10^-4</td>
</tr>
<tr>
<td>Zn (mg/L)</td>
<td>0.65±0.29</td>
<td>0.76±0.3</td>
<td>2.5.10^-4</td>
</tr>
<tr>
<td>Cu (mg/L)</td>
<td>2.01±0.56</td>
<td>1.67±0.44</td>
<td>1.7.10^-3</td>
</tr>
<tr>
<td>Cu/Zn</td>
<td>3.9±2.25</td>
<td>2.5±1.15</td>
<td>1.0^9</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>65.37±43.69</td>
<td>5.65±2.99</td>
<td>3.08.10^-10</td>
</tr>
</tbody>
</table>

The results of the correlation studies revealed a positive correlation between the Cu/Zn ratio and the copper ratio (r = 0.59, p = 0) in homozygous sickle cell patients and a negative correlation between this ratio and zincemia. A positive correlation between cupremia and plasma CRP was also identified (Table IV).

Table IV: Correlations between the different markers of oxidative stress among homozygous sickle cell patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TAS</th>
<th>Cu</th>
<th>Zn</th>
<th>Cu/Zn ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>-0.13</td>
<td>*0.32</td>
<td>-0.1</td>
<td>*0.31</td>
</tr>
<tr>
<td>TAS</td>
<td>—</td>
<td>0.05</td>
<td>0.23</td>
<td>-0.03</td>
</tr>
<tr>
<td>Cu</td>
<td>—</td>
<td>—</td>
<td>-0.15</td>
<td>**0.59</td>
</tr>
<tr>
<td>Zn</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>**-0.73</td>
</tr>
</tbody>
</table>

It also appears that, in the sickle cell group in the stationary phase (Table V) and in the critical period (Table VI), cupremia and the Cu/Zn ratio move in the same direction, whereas the fluctuations in zincemia go opposite to those of the Cu/Zn ratio.

Table V. Correlations between the different markers of oxidative stress among homozygous sickle cell patients in steady state

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TAS</th>
<th>Cu</th>
<th>Zn</th>
<th>Cu/Zn ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>-0.07</td>
<td>0.008</td>
<td>-0.23</td>
<td>0.22</td>
</tr>
<tr>
<td>TAS</td>
<td>—</td>
<td>0.05</td>
<td>**0.5</td>
<td>-0.16</td>
</tr>
<tr>
<td>Cu</td>
<td>—</td>
<td>—</td>
<td>-0.99</td>
<td>**0.71</td>
</tr>
<tr>
<td>Zn</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>**-0.67</td>
</tr>
</tbody>
</table>

Table VI. Correlations between the different markers of oxidative stress among homozygous sickle cell patients in crisis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TAS</th>
<th>Cu</th>
<th>Zn</th>
<th>Cu/Zn ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>-0.14</td>
<td>0.17</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>TAS</td>
<td>—</td>
<td>0.1</td>
<td>-0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Cu</td>
<td>—</td>
<td>—</td>
<td>-0.09</td>
<td>**0.48</td>
</tr>
<tr>
<td>Zn</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>**0.81</td>
</tr>
</tbody>
</table>

DISCUSSION

Sickle cell disease is marked by a quasi-permanent prooxidant environment [6, 23,24]. Many studies have reported the involvement of oxidative stress during this pathology. Our work revealed collapsed levels of TAS 0.88 ± 0.32 mmol / l) in homozygotes in general (usual values: 1.30 to 1.77 mmol / l). These results are consistent with those of several authors [23-25]. Indeed, in the face of a constant oxidative aggression, the antioxidant molecules would be regularly solicited and their rate would eventually drop. Therefore, the measurement of the total antioxidant status makes it possible to quantify the state of these defenses and to deduce the degree of severity of the radical attack. This determination can therefore be a first step followed, if the antioxidant status is lowered, the specific dosage of certain antioxidants.

In our series, TAS concentrations are lowered in sickle cell patients as well as in controls (Table I). Low TAS values have also been found in studies in Africa on homozygous sickle cell populations [24,25,26]. Thus, in Cameroon, Moor et al. [24] published mean TAS values of 0.76...
Senegalese sickle cell child.

shows indeed the effectiveness of the oxidative aggression in [32]. In fact, the hypercupremia found in our homozygous patients antioxidant function, becomes prooxidant in high concentrations. Dismutase. However, this trace element, despite its proven stress, particularly because of its role as cofactor of superoxide generate a lipidoperoxidation of the erythrocyte membrane with externalization of phosphatidylserine on the surface of red blood cells. What follows is a phagocytosis of red blood cells with intravascular hemolysis and hyperbilirubinemia.

Finally, there was a significant difference between stationary and sick patients in crisis with regard to average copper concentrations. It follows from this observation that vaso-occlusive episodes favor an increase in copper levels. Indeed, hemolytic phenomena, accentuated during CVOs, would be accompanied by a release of trace elements such as copper or iron. However, these metals, known for their pro-oxidative properties, are likely via the Fenton reaction [34] to increase oxidative stress and lipid peroxidation, already latent in sickle cell disease. In addition, copper concentrations are modulated by ceruloplasmin levels that would increase in response to the inflammatory process associated with vaso-occlusive seizures.

It is now clearly recognized that plasma fluctuations of copper and zinc occupy an important place in human pathology [35-37]. Indeed, like most transition metals (iron, chromium, cobalt, etc.), copper is able to produce, within biological systems, free radicals such as superoxide anion or hydroxyl radical. Zinc, on the other hand, although it is a transition metal, occupies a special place insofar as it participates in the antioxidant fight. In fact, an imbalance between the concentrations of these two trace elements seems to condition the level of oxidative stress of an individual [14].

The disturbances of the Cu/Zn ratio have thus been associated with numerous pathological states including solid cancers and other neoplasias, aging, cardiovascular diseases, dermatoses, neurodegenerative diseases, etc. In the context of hemoglobinopathies plasma levels of copper (Cu) and zinc (Zn) play a significant role. Indeed, the impairment of immune function and the growth retardation noted during these conditions suggest a relationship between hemoglobinopathies and the concentrations of these two trace elements.

Thus our study showed a highly significant (p = 6.10-4) increase in the Cu/Zn ratio in the sub-group of homozygous sickle cells as a

\[ \pm 0.07 \text{ mmol} /l \]; El-Ghamrawy’s team [25] found in Egyptian children an average TAS of 1.03 ±

0.10 mmol / l; another Egyptian team [26] found mean concentrations of TAS of 1.00 ± 0.10 mmol /lin boys and 1.02 ± 0.08 mmol /lin girls.

All this leads us to raise the issue of the necessity to define African usual values, even Senegalese ones. Indeed, the usual international values are generally determined from Caucasian populations that have genetic and socio-economic environments different from those of our black populations. It should be noted that in the context of our developing countries, the regular consumption of fruits and vegetables is the preserve of a few privileged. In addition, children often live under diktats forbidding them the consumption of this or that other food which constitutes a bed favorable to the installation of any kind of deficiency including a deficit in antioxidants [27,28]. Regarding the low levels of TAS found in homozygous sickle cell patients in general, they would reflect the oxidative aggression and lipid peroxidation described during this pathology [4, 29, 30].

Nevertheless, the interpretation of the TAS must be done with circumspection to the extent that a significant difference was noted according to the state of the patients (in crisis or not). This insignificant difference could be explained on the one hand by the relatively small size of our study population, on the other hand by the fact that certain non-enzymatic antioxidants would be increased in a crisis situation, thus reducing the decline in TAS. Indeed, the TAS includes certain non-enzymatic antioxidants such as albumin, bilirubin, uric acid, carotene, tocopherols, ascorbic acid and certain trace elements such as zinc [26, 31], so that any disruption of any of these parameters would have an impact on the TAS. In other words, the concentration of TAS in sickle cell patients in crisis could be increased. By way of example, the haemolysis observed during CVOs causes the degradation of the heme and is often accompanied by hyperbilirubinemia. The phenomena of ischemia-reperfusion lead to xanthine oxidase oxidation of xanthine or hypoxanthine, which leads to uric acid accumulation.

Plasma copper is another parameter for evaluating oxidative stress, particularly because of its role as cofactor of superoxide dismutase. However, this trace element, despite its proven antioxidant function, becomes prooxidant in high concentrations. [32]. In fact, the hypercupremia found in our homozygous patients shows indeed the effectiveness of the oxidative aggression in Senegalese sickle cell child.

In addition, copper mean values in stationary phases sickle cells, as well as in crisis patients, are increased relative to controls (Tables II and III). Our results are corroborated by many other studies [33] which, it must be said, have not taken into account the clinical state of sickle cell disease. These elevations of cupremia, which have been observed interictally as well as during critical episodes, suggest that the presence of hemoglobin S alone would promote the onset of oxidative stress. It is indeed known that Hb S self-oxidizes 1.7 times faster than Hb A and causes the formation of superoxides and hydrogen peroxide. In general, these reactive oxygen species generate a lipidoperoxidation of the erythrocyte membrane with externalization of phosphatidylserine on the surface of red blood cells. What follows is a phagocytosis of red blood cells with intravascular hemolysis and hyperbilirubinemia.

Finally, there was a significant difference between stationary and sick patients in crisis with regard to average copper concentrations. It follows from this observation that vaso-occlusive episodes favor an increase in copper levels. Indeed, hemolytic phenomena, accentuated during CVOs, would be accompanied by a release of trace elements such as copper or iron. However, these metals, known for their pro-oxidative properties, are likely via the Fenton reaction [34] to increase oxidative stress and lipid peroxidation, already latent in sickle cell disease. In addition, copper concentrations are modulated by ceruloplasmin levels that would increase in response to the inflammatory process associated with vaso-occlusive seizures.

It is now clearly recognized that plasma fluctuations of copper and zinc occupy an important place in human pathology [35-37]. Indeed, like most transition metals (iron, chromium, cobalt, etc.), copper is able to produce, within biological systems, free radicals such as superoxide anion or hydroxyl radical. Zinc, on the other hand, although it is a transition metal, occupies a special place insofar as it participates in the antioxidant fight. In fact, an imbalance between the concentrations of these two trace elements seems to condition the level of oxidative stress of an individual [14].

The disturbances of the Cu/Zn ratio have thus been associated with numerous pathological states including solid cancers and other neoplasias, aging, cardiovascular diseases, dermatoses, neurodegenerative diseases, etc. In the context of hemoglobinopathies plasma levels of copper (Cu) and zinc (Zn) play a significant role. Indeed, the impairment of immune function and the growth retardation noted during these conditions suggest a relationship between hemoglobinopathies and the concentrations of these two trace elements.

Thus our study showed a highly significant (p = 6.10-4) increase in the Cu/Zn ratio in the sub-group of homozygous sickle cells as a
Cu / Zn ratio in the group of patients (Table II). Indeed, it is generally accepted that the increase in the Cu level and the decrease in the Zn level lead to an increase in the Cu / Zn ratio [39]. However, copper is a prooxidant and zinc has the distinction of being both an antioxidant and an anti-inflammatory [40]. The increase in their ratio would therefore be in favor of oxidative stress and increased inflammatory phenomena.

The comparison of the results of the homozygous group in the stationary phase and those of the controls did not reveal a significant difference in the Cu / Zn ratio. In fact, the patients in the stationary phase had the same inflammatory profile as the controls, as attested by the CRP means (p = 0.9). It emerges from this observation that, if it is present, the oxidative stress borne by the people with sickle cell disease during the intercritical period would be close to that to which the control population is subjected. In other words, in sickle cell disease, the balance between oxidizing species and antioxidant defense systems would be balanced in the intercritical period.

Regarding the comparison between homozygous sickle cell crisis patients and controls, we observed a significant increase in the Cu / Zn ratio in the group of patients (Table II). Similar results were also found by comparing sickle cell crisis and stationary phase sickle (Table III).

This elevation of the Cu / Zn ratio results from increased cupremia and decreased zinc levels.

The increase of the Cu / Zn ratio in the homozygous crisis population of our series confirms the inflammatory process associated with the sickle cell crisis, already underlined by the CRP results which were significantly higher in sickle cell patients in crisis compared to patients in phase steady state (Table III) and controls (Table II). Indeed, hypozincemia and increased plasma copper concentrations have been reported in chronic and acute inflammatory conditions [41,42].

Moreover, knowing the involvement of inflammatory phenomena in atherogenesis in sickle cell patients, the increase of the copper / zinc ratio in our homozygotes in crisis leads us to consider the use of this parameter as a marker of atherosclerotic phenomena. In fact, cardiovascular complications such as cerebral vasculopathy are more frequent in homozygotes, particularly after several years of repeated vaso-occlusive episodes. These vaso-occlusive bone or organ attacks are accompanied by occlusion of the micro-vessels. These micro-vascular lesions are mostly glomerulopathies, retinopathies, cerebral vasculopathies, etc. They can begin in childhood and are therefore regularly detected in homozygous children with sickle cell disease [43]. Therefore, the Cu / Zn ratio could be a marker of cardiovascular risk in homozygous children with sickle cell crisis. This cardiovascular risk is also found in our patients in stationary phase as in our controls if we consider the CRP in its low values (ultra-sensitive CRP). In fact, the American Heart Association / American College of Cardiology has proposed for the CRP a threshold <3 mg/l [44].

The correlation analysis showed no association between TAS and the other parameters of oxidative stress (copper, Cu / Zn ratio) or inflammation (CRP) (Tables V, VI, VII).

Nevertheless, we found in sickle cell patients a positive association between TAS and zinc (Table V). This association highlights the link between these two parameters, even if their proven antioxidant role, could not be demonstrated in this study, thus confirming their limits as antioxidant markers in sickle cell disease.

The Cu / Zn ratio is considered as a control of lipid peroxidation, in other words as a marker of oxidative aggression. As for the TAS, it determines the state of the body's antioxidant defense. The lack of correlation between these two parameters suggests that the Cu / Zn ratio does not reflect the state of the antioxidant stores. However, this hypothesis needs to be qualified because the TAS is subject to variations in the biochemical parameters that compose it as described above.

Finally, the lack of correlation between the Cu / Zn ratio and the TAS does not allow us to consider this parameter as a marker of oxidative stress in its entirety.

Speaking of copper, correlation studies in homozygous sickle cell patients, regardless of their clinical situation, showed no relationship between this trace element and the other parameters, with the exception of CRP and the Cu / Zn ratio. As an antioxidant,
copper is believed to trap free radicals to prevent damage from them [45]. In view of the absence of a positive correlation between the cuprous ion and the antioxidant markers (TAS, Zinc), it appears that in sickle cell disease, it is the pro-oxidant component of copper which is expressed the most to the detriment of the antioxidant one. Indeed, although it is generally bound to proteins, this trace element is likely to be released and to participate in the formation of highly reactive species such as hydroxyl radical. The data obtained in vitro as well as cell cultures have, moreover, largely demonstrated the ability of copper to initiate oxidative damage [32].

The positive correlation recorded between copper and plasma CRP (Table V) attests to the involvement of the cuprous ion in inflammatory phenomena. Indeed, the relationship between oxidative stress and inflammation is well established. As a result, during sickle cell disease, copper, despite its proven antioxidant capacity, would intervene more by its pro-oxidative and pro-inflammatory properties.

Positive correlations noted between copper and Cu / Zn ratio were observed in homozygous sickle cell patients as a whole (Table IV), regardless of their clinical status (stationary phase or seizure) (Tables VI and VII).

These were strong correlations in the sickle cell group in general \((r = 0.59, p = 0.00)\) and in the sickle cell phase \((r = 0.71, p = 0.00)\); and a mean correlation in sickle cell crisis patients: \(r = 0.48; p = 0.005\).

Knowing the pro-oxidative effects of copper, this report would contribute to evaluate the oxidative stress and more particularly to highlight the radical attack. In addition, copper, which is an integral part of this report, has been widely described as an agent whose pro-oxidative effects favor the atheromatous process [46, 47]. The Cu / Zn ratio thus constitutes in the sickle cell child a marker of lipid peroxidation or even atherosclerosis.

On the other hand, concerning the ratio Cu / Zn and Zinc, we observed significant negative correlations between these two parameters in the three subgroups concerned. These correlations, which were strong, were found as much in the homozygotes in the critical phase and in the stationary period as in the homozygotes in general (Tables VII, VI and V), thus circumventing the limits that zinc presents, in isolation, as an antioxidant marker. As a result, this ratio could supplement zinc, or even constitute an antioxidant marker, given the limits of this trace element taken alone, in the assessment of the antioxidant response (Tables I, II, II, and IV). This assertion seems to be confirmed by some authors who describe the Cu / Zn ratio as being a much more sensitive index than the exclusive determination of plasma zinc [48, 49]. In the same vein, it would be relevant to study the behavior of this ratio against other antioxidant markers such as glutathione, uric acid, etc.

Thus, the ratio Cu / Zn would be of double interest in the context of the evaluation of oxidative stress in sickle cell disease. Indeed, being composed of two biochemical entities that oppose (Cu, Zn), it would inform not only the radical attack, but also the reaction of the body to this aggression. Nevertheless, in our study, the correlations between the Cu / Zn ratio and zinc were higher, in absolute value, than those observed between this ratio and plasma copper (Tables V and VII). It would therefore be more relevant to use this marker as an antioxidant parameter than an indicator of oxidative aggression. Moreover, the Cu / Zn ratio was positively correlated not only with copper, but also with plasma CRP (Table IV). Therefore, this parameter plays a key role in highlighting the inflammatory process. Thus, the ratio Cu / Zn would also be a control of inflammation as that was described in other pathologies.

REFERENCES:

2. OMS. Epidémiologie mondiale des troubles de l'hémoglobine et indicateurs des services dérivés. [Internet]. WH.O.http://www.who.int/bulletin/volumes/86/6/06-036673-ab/fr/


