Zinc Deficiency is Associated with Elevation of High Sensitive C-reactive Protein in Hemodialysis Patients

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Background: Numerous studies claim that the oxidative stress is elevated in hemodialysis patients. The aim of this study is to investigate the serum level of zinc in hemodialysis patient in Isfahan, Iran.

Method: In this study 100 hemodialysis patients from hemodialysis section of Alzahra hospital and Isfahan Noor hospital among 2013-2014 years were recruited. Serum level of zinc, albumin and high sensitive C-reactive protein (hs-CRP) was measured by atomic absorption, colorimetric and agglutination method, respectively.

Result: Patients serum zinc level was 96.7± 20µg/dl and 12% of hemodialysis patients showed a zinc deficiency. Serum Albumin was significantly lower than normal (3.73±0.51) and hs-CRP was significantly higher (16.78±9.71). Statistical analysis showed a significant relation between serum zinc and hs-CRP.

Conclusion: this study demonstrates there is an inverse relation between serum level of zinc and hs-CRP. This relation was statistically significant. This study insists on the importance of nutritional supplementation of zinc in hemodialysis patients.

Introduction

End stage renal disease (ESRD) describes the clinical condition with a non-reversible reduction of renal function. In this stage patients can’t live without permanent dependence to dialysis or kidney transplantation. ESRD presents with water electrolyte disturbance, endocrine, gastrointestinal, hematological and immunological disorders. Some of these disorders improve by dialysis, however, others are continued or get worse and even some of these disorders initiated with dialysis (1). Hemodialysis (HD) is the main treatment for patients with renal failure that are waiting for kidney transplantation or don’t allow for kidney transplantation (2). HD treatment is associated with risk of disability and mortality in these patients (3). HD patients are at risk to essential trace element deficiency that can affect their health condition and quality of their life. Zinc is the one of essential trace elements that is required for numerous important biological activities in the body such as oxidative metabolism (4). Zinc also is one of the main participants of immunological activities (5). Growth and development of organisms somehow depend on this element. There is about 2-4 gr of zinc distributed in the human body and that’s concentration in plasma is about 12-16 µmol/l. Although its solubility in water is very low, but the mobility of this element is very high which indicates the immunological importance of zinc (6). In serum, zinc is mainly bounded to serum proteins like Albumin (7). It has recently been declared that free zinc intracellular concentration is about femtoliter that indicates the high ability of zinc to get bounded (8). Zinc deficiency in patients occurs in a mild form and causes decrease in immune system activity. So this shows the important role of zinc in the immune system (9). Also zinc deficiency can result in some non-specific signs such as: anorexia, decrease in appetite, bad taste feeling, sexual and cognition dysfunction (10). HD patients have changed the level of trace element, so they are coincident with trace element deficiency side effects. Zinc has an antioxidant function for example as a part of super oxide dismutase (SOD). SOD protects biological membranes from oxidative stress (4). Anti-inflammatory effects of zinc such as regulation of B and T lymphocyte, also reported. Zinc is one of the most important trace elements that is present on the catalytic site of more than 300 enzymes and plays structural and biological role in many proteins, peptides, hormones, cytokines, transcription and growth
factors. The participation of this element in nucleic acid and protein synthesis and its role in membrane structure is the cause to which zinc considered as defense element. According to previous studies trace element related disorders occurs in many uremic patients. Serum level of zinc in HD patients is lower than normal levels. Many studies show that zinc increases the effects of metallothionines, which absorb OH free radicals. So this effect also can be important in antioxidant role of zinc (11). One of the important functions of zinc is the competitive role of this element against the iron and copper free ions. Free ions of iron and copper cause to OH free radical production from H\textsubscript{2}O\textsubscript{2} (Weis-Heber reaction). So these ions increase in oxidative stress mediated by this reaction. Zinc ions as a competitive agent inhibits this reaction and decreases oxidant agents (12).

Based on information from previous study the level of zinc decreases in HD patients and these patients are at risk of zinc deficiency. Decrease in zinc takes place with different mechanism which the most important of them is due to sever inflammatory responses and redistribution of this ion from the blood to other tissues. Circulatory dilution of zinc ions also decreases their intake (13).

High sensitivity C-reactive protein (hs-CRP) is a well-known marker of inflammation. Chronic inflammation is associated with cardiovascular disease and mortality in HD patients. Otherwise, it is reported that an elevated level of hs-CRP is a good index for prediction of cardiovascular mortality in HD patients. There are conflict evidences about the relation between serum levels of zinc with hs-CRP as so based on some reports there is a negative correlation between serum level of zinc and hs-CRP (14). But other studies didn’t show any relation between them. So the aim of this study was to investigate the possible relation between serum levels of zinc an hs-CRP (15).

Material and Methods

This study is a descriptive-analytic study that was done in 2013-2014 years in Isfahan, Iran. Study population (100) HD patient was selected from HD patients who were referred to Alzahra and Noor hospital. The method of this study was approved by the ethical committee of Isfahan University of medical science and all subjects gave their informed consent for entering to study. All HD patients were about 18 years old and had no background of the former inflammatory disease in last 3 months. They hadn’t admitted in hospital for the last three months and didn’t take any zinc supplementation during the above mentioned time.

Demographics including age, sex, HD vintage, cause of renal failure were obtained. Disposable plastic syringes and stainless steel needles were used to obtain 5ml venous blood, which was transferred to glass tubes, these had been made free of metal ion by immersion in dilute (1+19) nitric acid for 2h and then rinsed six times with deionized water and dried completely. Serum was separated by centrifugation in 1500g for 10 min at zinc free polycarbonate tubes at -20\textdegree c until analysis.

Previously described methods were used to determine the serum albumin, zinc and hs-CRP. Zinc determined by atomic absorption method and with perkin elmer 2380 set. Hs-CRP concentration was determined by assay based on a latex-enhanced turbidometric immunoassay method. When an antigen antibody reaction occurs between hs-CRP with sample and anti-CRP antibody, which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change (570 nm) with the magnitude of the change being proportional to the quantity of hs-CRP in the sample. The actual concentration is then determined by interpolation from calibration curve prepared from Calibrators of known concentration.

Albumin was determined by assay based on the selective interaction between Bromocresol green and albumin forming a chromophore that can be detected at 620 nm. The signal is directly proportional to the amount of albumin present in serum.

Statistical Analysis

Sets of data were analyzed by means of statistical tests include X\textsuperscript{2}, one way analysis of variance, independent t test and Pearson correlation test.

Result

In this study HD patients were recruited and investigated. Patients were aged 23-82 years (57.7± 12.3). Also 12 patients were under 40 years old, 14 patients 40-49 years old, 26 patients 50-59 years 28 patients 60-69 and 20 patients were 70 years old. 57 men (57%) and 43 women (43%). Mean HD vintage of 47.4± 43.4 months (range 1-156).

The primary causes of ESRD were diabetes (40%), hypertension (23%), idiopathic (11%) and 26% other causes such as polycystic kidney, Amiloidosis, lopus and…….

The mean serum level of zinc in patient was 96.7±20 µg/dl (range 59-136). Based on our finding 12% of them were lower than normal value and others (88%) were normal. Table 1 shows the mean and standard deviation of patient serum zinc levels based on their demographics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level of marker</th>
<th>HS-CRP</th>
<th>Serum level of albumin</th>
<th>Serum level of zinc</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>Mean</td>
<td>P</td>
<td>Mean</td>
</tr>
<tr>
<td>&gt;40</td>
<td>0.88</td>
<td>15/08±9/62</td>
<td>0.55</td>
<td>3/89±0/51</td>
<td>0.96</td>
</tr>
<tr>
<td>40-49</td>
<td>19/14±9/79</td>
<td>3/72±0/49</td>
<td>94/5±17/7</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Mean and standard deviation of serum level of zinc, albumin and hs-CRP according to age, sex and HD vintage
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One way analysis of variance shows no significant difference in age group or HD vintage (P value >0.5). Also t test analysis declares no significant difference between sex groups (P value >0.5). The mean level of serum albumin among study subjects was 3.73±0.51 (range 3.2-5.2). Table 1 also shows serum level of albumin based on demographics. One way analysis of variance shows no significant difference in age groups (P value= 0.55) but t test shows there was significant difference based on HD vintage (P value= 0.0008).

Serum levels of hs-CRP in all subjects were 16.78± 9.71 (range 1-52) Table 1 shows the serum level of hs-CRP based on demographics.

One way analysis of variance demonstrates no significant difference between age group and HD vintage (P value > 0.05). t test shows no significant difference in sex groups. Table 2 shows the correlation between zinc, albumin, hs-CRP and age variables in the study population.

Table2. Correlation between serum level of zinc, Hs-CRP, albumin, HD vintage and age

<table>
<thead>
<tr>
<th></th>
<th>Zinc</th>
<th>Hs-CRP</th>
<th>Age</th>
<th>Duration</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zinc</strong></td>
<td>Pearson Correlation</td>
<td>-819**</td>
<td>.000</td>
<td>.025</td>
<td>.176</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>.000</td>
<td>.004</td>
<td>.079</td>
<td>.000</td>
</tr>
<tr>
<td><strong>Hs-CRP</strong></td>
<td>Pearson Correlation</td>
<td>-.025</td>
<td>1</td>
<td>.015</td>
<td>-.106</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>.000</td>
<td>.884</td>
<td>.292</td>
<td>.000</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Pearson Correlation</td>
<td>.015</td>
<td>.845</td>
<td>1</td>
<td>.076</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>.004</td>
<td>.884</td>
<td>.455</td>
<td>.777</td>
</tr>
<tr>
<td><strong>HD vintage</strong></td>
<td>Pearson Correlation</td>
<td>.176</td>
<td>-.106</td>
<td>.076</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>.079</td>
<td>.292</td>
<td>.455</td>
<td>.064</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>Pearson Correlation</td>
<td>-.644*</td>
<td>-.564*</td>
<td>-.029</td>
<td>.186</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>.000</td>
<td>.000</td>
<td>.777</td>
<td>.064</td>
</tr>
</tbody>
</table>

There is a negative correlation between serum level of zinc and hs-CRP (-0.82) which was significant (P value < 0.001). In the other hand, there is a correlation between serum level of zinc and albumin (0.64) which was statistically significant (P value <0.001). There was a negative correlation between serum level of albumin and hs-CRP (-0.564) which was significant (P value <0.01). Otherwise, there was no significant relation between HD vintage and other variations. Also, there was no significant relation between age and other variables. In curves (2-4) the relation between serum level of zinc and hs-CRP, zinc and albumin, hs-CRP and albumin are shown. To evaluate the effect of albumin relation between zinc and hs-CRP, subjects divided in two normal and abnormal groups based on serum albumin. In both groups there was a significant relation between serum level of zinc and hs-CRP (P value 0.05). Based on Pearson test correlation in patients with normal serum albumin was – 0.81 (P value < 0.001) and in patient with abnormal serum albumin was -0.7 (P value < 0.001) which showed in curves 5 and 6.
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Chart 2: correlation between serum level of zinc and Hs-CRP

Chart 1: age distribution of subjects

Chart 4 correlation between serum level of Hs-CRP and albumin

Chart 3: correlation between serum level of zinc albumin

Chart 6: correlation between serum level of zinc and Hs-CRP in subjects with normal albumin level

Chart 5: correlation between serum level of zinc and Hs-CRP in subjects with abnormal albumin level
Discussion

The aim of this study was to evaluate the possible relation between serum level of zinc and hs-CRP in Isfahan HD patients. The result of this study showed that at least 12% oh HD patients have zinc deficiency. Zinc deficiency, also, is confirmed in other studies. Based on our result, there are no significant relation between age, sex and HD vintage, but there was an inverse relation between serum zinc level and serum albumin. It seems, decreased serum zinc is result of zinc elimination during dialysis, decreasing serum albumin and decreased gastrointestinal intake of zinc. In addition, increased by the intracellular metallothionin expression following oxidative stress or increasing of zinc containing proteins induced by pre inflammatory cytokines which in turn cause a decrease of serum zinc. On the other hand that is reported hs-CRP bound to cells and causes activation of the complement system. This means hs-CRP has a potential role in the formation of foam cells and phagocytosis and formation of cardiovascular complications. Numeral agents can cause release of cytokines from monocytes and macrophages in HD patients. These proinflammatory cytokines increase during HD. The coincidence of circulating mononuclear cells with dialysis membrane or Lipopolysaccharides in the dialysis surface of the membrane is a potential source for increases of cytokines. Hs-CRP as a marker of inflammation has a relationship with cardiovascular disease in ESRD patients. The documents also show hs-CRP is related to numerous marker of cardiovascular disease such as lipoprotein (a), fibrinogen and LDL. It seems some part of observed relation between serum zinc and hs-CRP is a consequence of oxidative stress. In other hand oxidative stress can be due to release of reactive oxygen species (ROS) from monocytes and neutrophils. Some studies report relation of hs-CRP with thiorbarbatic acid substances (lipid peroxidation marker). In the other hand, inflammatory related proteins such as hs-CRP, Fibrinogen and anti-trypsin is significantly related to oxidative stress. Based on our results, serum level of hs-CRP was higher than healthy controls. This indicates the presence of chronic inflammation in HD patients. According to inflammation prevalence in ESRD, chronic inflammation is a new risk factor for cardiovascular risk factor in these patients. That is cleared increase of hs-CRP is associated with increase of mortality in HD patients. This study shows a negative correlation between decreases of serum zinc and increases of hs-CRP. So based on our results, serum zinc is related to some inflammatory markers such as hs-CRP and the lower serum zinc has inflammatory effects.

Conclusion

That is observed serum zinc in HD patients is lower than normal and have significantly inverse relation to serum hs-CRP. Considering that hs-CRP causes a increase of cardiovascular effects in HD patients, so zinc supplementation could play important role in suppress of oxidative stress in these patients. Considering decreased absorption of zinc from gastrointestinal track with high fiber foods such as rice, corn grain and Beans should be avoided. An appropriate diet can improve zinc deficiency in these patients.

Acknowledgment

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Reference

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