Carpal Tunnel Syndrome and Calcium Deposit in Surgically Transacted Transverse Carpal Ligament

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Abstract

A diagnosis of carpal tunnel syndrome (CTS) in a human often contains more than one test. Calcification of the carpal transverse ligaments (TCL) is one of the common reasons why patients seek CTS surgery. However, determination of calcium (Ca) concentration in TCL has not been studied, and results of environmental toxicity studies assessing the relationship between Ca, and elemental deposition in TCL are inconsistent. The purpose of this paper was to verify this hypothesis by conducting a chemical analysis of a portion of the released TCL to assess whether there is a relationship between CTS and Ca, measured as the total concentration of Ca, and to measure the precipitation of elements, the most closely related elements associated with Ca and toxic are cadmium and lead. Surgical release of TCL was performed on forty patients, and total concentrations of Ca, Cd and Pb in the extracted portion of TCL were digested and determined using inductively coupled plasma mass spectrometry (ICP-MS) and the possibility of using X-ray spectroscopy (XRF) for direct elemental analysis. Ca mineralization revealed in some TCLs. In assessing patients’ environmental pollution, it was observed that the Cd and Pb concentrations were significant with the higher Ca concentration, and XRF was useful for direct detection of the elements in samples of human body. In conclusion, these results indicate that TCL mineralization by Ca does not characterize CTS, which has important concerns in improving patients’ therapeutic strategies, and Cd and Pb concentrations varied due to different factors.

Keywords: Carpal tunnel syndrome, cadmium, calcium, lead, ICP-MS.

Introduction

CTS surgery in Iraq is the most common operation. Usually, there is no standard method for diagnosing CTS, a physical examination (electrophysiology test) is used. The incidence of CTS increases with frequent use of the hand, in addition to other factors such as gender, age and weight. Calcified in the TCL is known to cause acute CTS diagnosed by the standard method (X-rays), and calcic deposition in the wrist of people with CTS has been reported. The clinical link of TCL crystals with calcium was not understood in CTS patients. Since the X-ray examination of TCL cannot diagnose whether or not calcified crystals are responsible for CTS, instead, it will be possible to measure the Ca concentration in TCL as CPPD has been studied in the synovium and cartilage. In respect of ecotoxicological studies, human exposure to nephrotoxicants (such as Cd and Pb) deposit in tissues. Most epidemiological studies of human exposure to Cd have shown that the main target organs for Cd toxicity are the liver and kidneys because they contain the highest concentration of the protein metallothionine, which binds with Cd and concentrates it. Those epidemiological studies contribute to evaluating pollution in a region. Importantly, ecotoxicological studies are the most effective way to verify the association of measured elements in the human body with other diseases when patients are living in contaminated areas. Therefore, the current
study assumed that the measurement of Cd and Pb in the TCL fraction could be correlated with the deposing of Ca in the TCL. The cause of the association between calcifications and Cd or Pb is due to the immobilization of Cd and Pb with calcium phosphate \((\text{Ca}^{2+}\text{PO}_{4}^{{3-}})\) as \(\text{Cd}_2\text{(PO}_4)_3\) (solubility constant, \(K_{sp} = 2.53 \times 10^{-33}\)) and \(\text{PbHPO}_4\) (solubility constant, \(K_{sp} = 10^{-23.8}\)), respectively [8], which means the disproportionation of Ca with Pb and Cd [9]. TCLs were obtained from forty patients who have been suffering from CTS. This study aimed to evaluate whether patients with CTS were associated with CPPD (diagnosed by measuring Ca in TCL), and the relationship between measured concentrations of Cd and Pb in TCL and Ca released distinguish CTS patients from healthy controls. For comparative analysis, XRF analysis was performed, and the prospective user of XRF for analyzing elements within the human body was discussed in terms of using XRF for direct determination of the elements within the human organism in the medicine. To our knowledge, this is the new analysis of XRF performed on a human to date [10-11].

**Methods**

**Carpal tunnel release surgery**

A day-case surgery on forty patients who suffered from CTS was operated to relieve the symptoms of CTS. The operations were performed under the surgeon and local anesthesia using acid-washed and sterilised surgical tools. Control samples of TCL were obtained from volunteers \((n = 3)\), ultrasound guide and a true-cut biopsy needle 20 gauge was inserted to take a true-cut biopsy from the medial hamate-pisiform side of TCL.

**Determination of Ca, Cd and Pb concentrations in TCL using ICP-MS and XRF**

Determination of Ca, Cd, and Pb concentrations in TCL was performed after *aqua regia* digestion, according to Egger et al. [12]. The *aqua regia* solution was obtained by mixing one volume of nitric acid (>68%) with three volumes of hydrochloric acid (<37%), and the development of the golden colour was observed after a few minutes. The three wet sample ligaments (30 mg) from each patient was transferred to a Falcone tube (50 mL), and the digestion of the samples was done by adding 10 mL of the *aqua regia* and left for 24 h. After digestion, the volume to 50 mL was completed with 0.2% of nitric acid (v/v), and indium was added into samples with final concentration 0.5 mM for using as an internal standard prior analyses. The concentrations Ca, Cd, and Pb were analyzed using ICP-MS (Thermo Scientific, X Series 2). ICP-MS was validated by evaluating some analytical parameters such as linearity, LOD, LOQ, precision, and accuracy. Yttrium at a concentration of 0.5 mM was used as an external standard during the measurement. As for carpal ligament, there is no Certificate Reference Material (CRM) marked, therefore, a spike recovery test was performed. The spike recovery test was carried out by spiking the ddH₂O (1 mL) with nominal concentrations of Ca, Cd, and Pb (50 mM, \(n = 3\) samples), then followed by *aqua regia* digestion. Meanwhile, *aqua regia* digestion was carried out with the same ddH₂O (1 mL) before being spiked with Ca, Cd and Pb. Results of the Ca, Cd, and Pb spike test for the ddH₂O (accuracies: 95 ± 0.02 %, 99 ± 0.01 %, and 93 ± 0.03 %, respectively. In the ICP-MS analysis, the procedural blanks (ddH₂O or *aqua regia*) were used to correct the measured concentrations of elements in samples of ddH₂O or TCL, respectively.XRF spectrometry (NITON XL31EXRF) was used along with the calibration of biological certified reference material (bovine liver, NIST1577C, Sigma - Aldrich), and the duration of the reading was 120 s. The measurement was performed in triplicate, and in each analysis, the position and orientation of the cup were changed. The accuracy percentage values were estimated from the recorded and certified values of standard reference material, bone ash (SRM 1400,Sigma- Aldrich). The recovery % of XRF was Pb (98 ± 0.3 %).

**Statistical Analysis**

A one-way ANOVA followed by Tukey *post hoc* was performed on the data of concentrations to evaluate whether the concentrations in the TCL were different between 40 patients or XRF and ICP-MS techniques. Correlation tests (Pearson) were performed on the data of the measured concentrations in the TCLs to determine if Cd or Pb concentrations were affected by the content of Ca in each patient.

**Results and Discussion**

Concentrations of Ca, Cd and Pb in TCL
determined by ICP-MS

The results of the mean concentrations of Ca, Cd, and Pb analyses of carpal ligaments are summarised in Figure 1. Whenever the calcium investigation was performed in the TCL of patients with CTS, Ca determination was initially performed in controlled TCL extracted from healthy people. This control was measured to compare the concentration of Ca in control and patient samples. The mean concentration of Ca in TCL of the volunteer was $37 \pm 0.2 \text{ mg/g}$. The result was consistent with previous studies on Ca contents in human tissues [13]. The measured concentration of Ca in TCLs of patients with CTS, as presented in Figure 1A shows that the Ca concentration has a broad distribution, and the statistical differences were observed. It is possible to note that the concentrations of the Ca are higher in some samples of TCLs than in control samples of TCLs. These results suggest that the detection of Ca in the TCLs of CTS patients is not necessary, because the differences in Ca contents may be caused by several factors, such as age and different disease stages [14]. Therefore, we did not find any indication that Ca concentration caused the patients with CTS. After measuring Ca in TCL, the possible deposition of elements was determined. We focused on Cd and Pb as these elements could be precipitated with calcified crystal in the TCL.
Cd concentrations in TCL patients are reported in Figure-1B. Concentrations were markedly varied significantly, and this difference may be explained by several factors, such as exposure to pollution, age, gender, and smoking habit. The range of Cd content was between $31.4 \pm 1.3$ and $62.5 \pm 3.7$ mg/kg, which showed a good agreement with published data. In comparison, from Figure 1C, it can be seen that Pb concentrations in TCL of CTS patients were lower than Cd concentrations (ranging from $0.12 \pm 0.03$ to $0.73 \pm 0.02$ mg/g), which may be caused by having a $K_{sp}$ of $10^{-23.8}$ of PbHPO$_4$, lower than the $K_{sp}$ of Cd$_3$(PO$_4$)$_2$ ($2.53 \times 10^{-33}$). In addition, the level of exposure to Pb pollution, metabolism process, and age also have an effect on Pb content in the human body [15]. Pb concentration data in human tissue from this study are comparable to Mari et al. [16], who reported that the concentration of Pb in the kidney was 0.18 μg/g. Recently, García et al. [17] have estimated the concentration of Pb in human tissues (liver, kidney, brain, lung, and bone) with a range of 0.08 to 1.0 μg/g. In the next step, we attempted to find the correlation between Ca and Cd or Pb concentrations in the TCL.

**Correlations between Ca and Cd or Pb concentrations**

Relationship between Ca and Cd is illustrated in Figure 2A shows that a moderate positive correlation between the concentration of Cd and Ca content in the TCL (correlation coefficient = 0.564, $p<0.001$), indicating that the contents of Ca in TCLs of CTS patients may have a positive effect on Cd concentrations and enables it to precipitate. The result of the correlation between Pb and Ca suggested that a minor decrease in Pb concentration as a result of increasing Ca concentrations in TCLs of CTS patients may have a positive effect on Cd concentrations and enables it to precipitate. The result of the correlation between Pb and Ca suggested that a minor decrease in Pb concentration as a result of increasing Ca concentrations in TCLs of CTS patients may have a positive effect on Cd concentrations and enables it to precipitate. The result of the correlation between Pb and Ca suggested that a minor decrease in Pb concentration as a result of increasing Ca concentrations in TCLs of CTS patients may have a positive effect on Cd concentrations and enables it to precipitate. The result of the correlation between Pb and Ca suggested that a minor decrease in Pb concentration as a result of increasing Ca concentrations in TCLs of CTS patients may have a positive effect on Cd concentrations and enables it to precipitate.
Comparative of two techniques for analysis of Ca, Cd and Pb concentrations in TCLs

XRF analysed directed measurement of Ca, Cd, and Pb concentrations in TCLs. Figure 3 shows the comparison in the measuring of Ca, Cd, and Pb concentrations in TCLs by XRF and ICP-MS, and differences in some samples show concentrations are more significant (\(p<0.05\)) in using the XRF technique. Detection of Ca, Cd, and Pb concentrations in TCLs by XRF is the main finding. Vanhoof et al. [19] stated that measurement with XRF technology gives a higher concentration of elements than ICP-MS. These results were due to samples from an analysis by ICP-MS being digested by acid to release the component, and sometimes digesting acid is not sufficient to release elements [20].
Figure 3: Comparison of (A) Ca, (B) Cd, and (C) Pb concentrations in TCLs of 40 patients with CTS measured by XRF (red column) and ICP-MS (blue column) techniques. Error bars are standard error of the mean, and a different letter indicates a significant difference in the concentrations between the techniques.

Conclusions

The results obtained above were answered the question of this study, and the contents of Ca in TCLs of forty patients with CTS were not higher than the contents of Ca in TCLs of the control samples, indicating that the contents of Ca in TCL are not connected with CTS. The possible deposing of Pb and Cd with Ca content in the TCL was investigated, and the results showed no correlation between them as other factors could control this and more studies are needed. The novel use of XRF for direct analyses of elements in the TCL was
reported in this study. In this study, the determination was performed from a living human body, which enabled more diagnosis studied could be conducted. The assessing patients’ environmental pollution indicated the concentrations Cd and Pb concentrations and more studies for determining theme concentrations in other parts in their body.

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