An Investigation of Salivary Biomarkers in Acute Leukemia Patients

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Abstract

Background: Leukemia is a fatal disease. A proliferation of immature bone marrow-derived cells is called acute leukemia that may also involve solid organs or peripheral blood. A sampling source for clinical diagnosis is saliva which has been used and it is a promising approach as collecting saliva is relatively easy and non-invasive. Over the past two decades, using saliva as biomarker, specifically for early cancer detection has attracted much research interest.

Objective: To estimate the role of some salivary components as biomarkers in patients with acute leukemia.

Patients and Methods: A total of 60 individuals with age range 19-56 years, 30 healthy individuals compared with 30 patients with acute leukemia in order to investigate the following salivary parameters: zinc (Zn), chromium (Cr), copper (Cu), iron (Fe), superoxide dismutase (SOD), and lactate dehydrogenase (LDH) enzymes.

Results: The mean salivary trace elements: Cu, Cr, Fe, and Zn concentrations were significantly raised in patients with acute leukemia when they compared with controls ($p<0.005$, $p<0.05$, $p< 0.001$, and $p<0.001$ respectively). Also the mean salivary enzymes activities of SOD and LDH were raised significantly in patients with acute leukemia when they compared with healthy subject ($p< 0.05$ and $p<0.001$).

Conclusion: altered levels in salivary components in this research may be used as a diagnostic tool, especially when a concurrent analysis for significantly raised markers is carried out. This is because salivary diagnosis involves a non-invasive method, and may thus represent an effective alternative to serum testing.

Key words: Superoxide dismutase, lactate dehydrogenase, trace elements, and acute Leukemia.

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Introduction

A malignant disorder influncing all groups types of ages is acute leukaemia. The aggregation of blast cells in the bone marrow, is defined as acute leukemia. The failure results in bone marrow, reflected by peripheral blood cytopenias and circulating blast cells. The etiology is unclear, but internal and external factors related with breakdown of DNA can predispose to acute leukaemia [1][2]. Nowadays, the prevalence of blood cancers, such as lymphoma and leukemia, have been raising in human societies .These diseases are neoplasms of immune systems and blood forming and are diagnosed with various clinical and pathological symptoms. The etiology view showed a wide range of chemical, physical,

genetic, and other environmental factors have been released to be related with these diseases [3]. A sampling source for clinical diagnosis is saliva which has been used and it is similar to blood in various biological aspects. Saliva provides advantages over serum for its uninvasive nature, easy to transport and storage, safe handling and cost-effectiveness [4][5].

A fluid located in the mouth is saliva, which secreted by the major salivary glands, which a 99.5% is consist of water, and a 0.5% is consist of inorganic and organic components, trace elements, etc. Mean daily saliva production of a healthy person's ranges from 1 to 1.5 L. The salivary metals have lenty important roles such as influencing of many enzymes activities, manufacturing bone and teeth, preventing tooth decay, neutralizing mouth environment, etc [6].

The biomarker is defined as an indicator of pathogenic processes, normal biological, or pharmacological responses to a therapeutic or other health care intervention. Several salivary constitutes and gingival crevicular fuid have been characterized as biomarkers [7]. The salivary constitutes levels acting as biomarkers have been indicated to be sensitive as serum levels. Use of saliva in estimating the biomarkers for early diagnosis of cancer risk is potential [8]. Collecting blood for assessment is an invasive procedure and has a potential risk of transmission disease through needle stick injuries. A growing number of researchers are finding that saliva delivered an easily available, non-invasive diagnostic of disease and clinical situations [9]. The important roles of trace elements is by regularizing the immune system, nerve contraction, mitochondrial activity and etc. The studies have been reported the relationship of too many metal levels in blood with diseases, such as gastrointestinal cancer, leukaemia, lung cancer, and etc. [10]. Till now the mechanism of the trace elements for causing cancer in human body is unknown from the outlook of cancer diagnosis and prevention. However, investigations on the association between the causes of cancer and the elements levels are still of great importance [11]. The activity LDH levels exhibit positive correlation with tissue inflammation during gingivitis disease and tissue destruction [7].

High levels of iron and copper have been reported in the liver and spleen of patients with cancer of the urinary tract, respiratory tract, and thorax, and the copper content of benign tumors of the esophagus, intestine and bronchi, are said to be lowered than in cancers. An association between Cr and carcinogenesis have been pointed out to lung cancer. A high incidence of lung cancer has been explained as an occupational disease among workers related in the chromate production process in Germany and the United States. Zinc is a constitute of SOD, an enzyme that removes free radicals, and it is also necessary for stimulation of DNA repair enzymes, zinc influenced and protects against carcinogénesis [12]. Superoxide dismutase is believed to play a very important role in defending Living cells against toxic oxygen derivatives[13].

This study aims to estimate the role of some salivary components e.g. SOD, LDH Cu, Cr, Fe, and Zn as biomarkers in patients with acute leukemia.

Patients and Methods

Subjects and Samples

Sixty salivary samples were collected randomly. Two groups of individual were being included with acute leukemia patients and healthy groups. The acute leukemia patient groups; included 30 salivary samples (17 AML, and 13 ALL) with age range 19-57 years (16 males and 14 females) who were

selected from newly diagnosed acute leukemia patients admitted to Nanakaly Hospital in Erbil city early diagnosed and the samples were collected before treatment, while the second groups included the control group consisted of 30 controls of healthy persons with age range 18-59 years (18 males and 12 females). Before treatments, 10 ml of un-stimulated salivary samples would be taken between 10:00-11:00 am from each participants, then centrifuged and stored in a disposal tube without anticoagulant and were be preserved in an ice-box then were be transferred to laboratory to determine the following enzymes: lactate dehydrogenase (LDH), and superoxide dismutase (SOD) levels were assayed enzymatically using commercial reagents (kits, Randox Laboratories Ltd, Crumlin, UK) using Bio-Tek instruments, Milan, Italy [6], while trace elements parameters: copper (Cu), chromium (Cr), iron (Fe), and zinc (Zn) concentrations were examined by using 1275 A A Varian, atomic absorption spectrophotometer. The statistical analyses were runned with SPSS 13.0 [14].

Results

The salivary enzyme levels of LDH and SOD have been shown in Figure 1, while the results of the salivary trace elements levels: Cu, Cr, Fe, and Zn have been shown in Figure 2. The results showed that the levels of salivary levels of LDH ($p < 0.001$), SOD ($p < 0.05$), Cu ($p < 0.005$), Zn ($p < 0.001$), Fe ($p < 0.001$), and Cr ($p < 0.05$) were increased significantly in patient with acute leukemia as compared with control groups (Figure1,2).

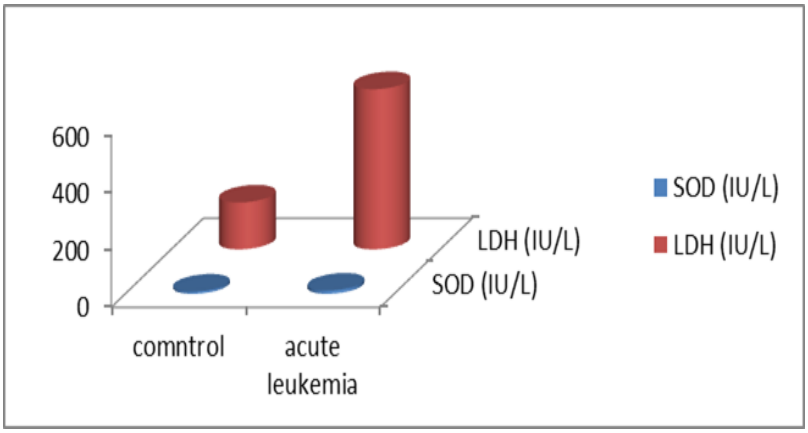

Figure (1): Salivary levels of SOD and LDH in control and acute leukemia patients groups. (Values are expressed in mean ± SD)

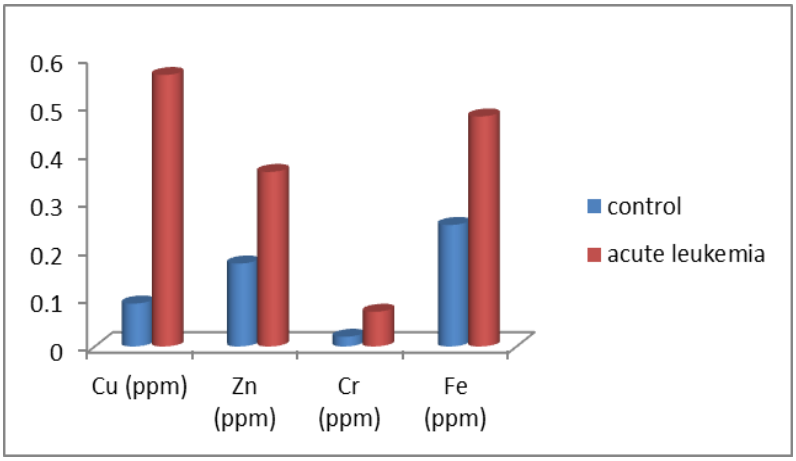

Figure (2): Salivary levels of trace elements in control and acute leukemia patients groups (Values are expressed in mean ± SD)

Discussion

There are some body fluids which can be used for diagnosis such as saliva, serum, urine, cerebrospinal fluid etc. [8]. In recent years, saliva has been indicated as a bio-fluid that can be used to determine for markers of various disease processes according to its easy of availability and non-invasive means of collection [15]. Using saliva as diagnostics tools are more accessible, presents less risk of infection to the patient, less expensive and accurate, health care worker and cross infection. With all these above revealed addition of saliva advantages can serve as diagnostic appliance as compared to serum. Antioxidant enzyme are responsible for the quenching of free radicals e.g. SOD which are liberated through the chemical reactions of the many metabolic path ways. Different iso-enzymes of SOD are described. The cytoplasmic enzyme is copper-zinc dependent, mitochondrial enzyme is manganese dependent. It is a non-hemeprotein. The defect in SODs gene can be found in patients with a myotrophic lacteral sclerosis. These enzymes are spread in human body in different amounts. SOD work in conjugation with two enzymes that eliminate OH in human cells e.g. catalase, and glutathione peroxidase. The enzyme activity levels of SOD are changed to verity diseased states showes either raising or reduction in their activity, this phenomenon was found to be noticeable in leukemias [16], and in this study the salivary levels of SOD elevated significantly $P<0.05$ (Figure 1) as other study supported it e.g. The highest serum SOD levels were seen in untreated leukemia patients or during the early time period of the treatment [17].

The Cu, Zn SOD also is significantly increased in acute lymphoblastic leukemia. [18]. Alteration of oxidative stress metabolism are commonly related within tumor cells among patients, which is high with disease severity degree and the accumulation of chromosomal aberrations [19], also The increased leukocyte SOD activity may be due to change gene expression in hematopoietic cells. It could be explained that the activity of leukocyte SOD reflects oxidative stress in CML patients, and SOD could be used as an indicator for stress oxidation associated to disease progression [20].

Lactate dehydrogenase is an enzyme that transfers hydrogen and it is one of the reactions in the metabolic of anaerobic glycolysis. The LDH can be used to indicate cell breakdown or death, LDH activities levels is abnormal in a plenty disorders [9]. In the present study showed significant ($P<0.001$) elevation in salivary LDH (Figure 1), and this may originate from various sources, which excreted from the gingiva, oral epithelium, and cellular and other debris and also it is agreed with the study which serum and saliva enzymes showed increasing in enzymes levels e.g. LDH and alkaline phosphotase in acute leukemia and oral squamous cell carcinoma significantly [21]. It is belived that changing of oral epithelium in pathological conditions e.g. cancer diseases may result in changing of salivary LDH activeties levels [22].

Trace elements are essential for physiological processes and numerous metabolic in the body. Therefore, any disturbance in the normal range levels of trace elements may influence biological processes and have been related with plenty diseases including: cancer, heart autoimmune, neurological disorders and renal failure [23]. On the basis of different studies, the elevation in copper or copper/zinc ratio leads to elevate lipid peroxidation, damage of the antioxidant system, and production of OH radicals

through raise in malondialdehyde, which attacks the DNA and causes mutations that lead to cancer, while copper is a constitute of the nine growth factors, particularly the endothelial growth factor. The factor of endothelial growth causes tumor progression and angiogenesis upon increase in sera copper levels [3].

In the current paper salivary copper levels in acute leukemia patients were raised when compared with healthy group ($p < 0.005$). The current results were in line with the results of [3][24] and [25] who indicated that patients with AML, and ALL have higher levels of serum copper than those in control.

Higher concentrations of serum Cr, Fe, Hg, Rb and Zn but low concentrations of Se were found in patients with acute leukemia [26] as similar in the current study which increased in salivary levels for Zn ($p < 0.001$), Fe ($p < 0.001$), and Cr ($p < 0.05$). By supporting another study high levels of Cu, and Fe seen in leukemic lymphocytes in children [27]. Also other researchers showed that serum Se and Zn levels were lowered in leukemia patients, while serum Cu and Cu−Zn SOD concentration was higher in leukemia patients [28]. In another hand rising in serum levels of Cu, Cr, and Zn were shown in colon cancer patients [29].

In conclusion, this study the alteration of salivary levels may be used as a non-invasive diagnostic tool alternative to serum testing the enzymes (SOD and LDH) and trace elements (Cu, Zn, Fe and Cr) were significantly altered in patients with acute leukemia.

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