The 1st International Conference on Sciences and Arts (ICMSA 2017)

المؤتمر الدولي الاول للعلوم والاداب

3 مايو 2017 – اربيل — العراق

http://sriweb.org/erbil/

Investigation of hazardous chemicals exposure on some liver function enzymes using serum and saliva of Iraqi's students

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Abstract. To investigate the potential relevance between health problems and working environment conditions in chemical labs, liver enzymes were examined in both sera and saliva of student volunteers, during the last 15 days of September at which they probably exposed to various chemicals over three months in the Iraqi Chemistry Department. The results showed that there was no significant difference in serum activity for both AST and ALP, however, a significant increase in serum ALT level of Z = -2.355 (p < 0.019), before (median = 4.2 U/l) to after (median = 6.8 U/l) was noticed. Furthermore, the values obtained from salivary AST, ALT and ALP showed highly significant increase of Z= -3.045 (p < 0.002; the median increased from 15 U/l to 31 U/l), Z= -2.512 (p < 0.012; the median increased from 2.7 U/l to 5.0 U/l) and Z=-2.982 (p < 0.003; the median increased from 8.51

U/l to 22.1 U/l) respectively. It can be concluded that the increment of the salivary liver enzymes might explain the slight and early signs of liver dysfunction. Also, the significance correlation obtained between serum and salivary ALP, r = 0.699 (p < 0.005), may be used for pre-clinical diagnosis and monitor various liver dysfunctions caused by exposure to the toxic chemical substances.

Keywords: Aminotransferase enzymes, chemical exposure, salivary enzymes, liver dysfunction, longitudinal study.

1 INTRODUCTION

1.1 Exposure assessments

Different types of variable risk agents (e.g lifestyle, work environment and their interaction) and non-variable risk agents (e.g sex, age, genes, etc) may be directly/indirectly associated with the prevalence of some diseases (Hong, Pedersen, Brismar, & de Faire, 1997; Kim, Won, Ko, Heo, & Chung, 2012).

Exposure assessments can be defined as an analytical method that is used to assess the range and probability of the real or likely exposure of individuals to the origin of chemical danger (Agency, E. (2001) . These assessments based on a certain exposure data or determined from a given compound or metabolites in bio-samples, caused by a disturbance in functions of the body, hence may indicate the presence of diseases (Heinzow & McLean, 1994; Hulka & Wilcosky, 1988).

The susceptibility of some biochemical parameters to chemical exposure is notable not only for determining the cause and effect but also investigating the connection mechanisms between the chemical exposure and negative public health (Dufour et al., 2000a; Klaassen, 1996). Biomonitoring data, which can be used for checking, following up and therapy, have a significant role in characterizing new chemical hazardous of potentially exposed people (Luo, Kuo, Cheng, & Chang, 2001; National Research Council (US) 1991; Thornton, McCally, & Houlihan, 2002).

1.2 ALT, AST and ALP enzymes

Liver is the major site where the degradation of chemicals and toxic substances are occurring, therefore the accumulation of various chemical materials could lead to liver dysfunction (Franco, Fonte, & Candura, 1986). It is the largest vital organ, which has two functions: metabolism of the main molecules (e.g.: carbohydrate, protein, lipid) and the elimination and detoxification of by-products (Giannini, Testa, & Savarino, 2005).

The function of ALT (alanine aminotransferase; EC 2.6.1.2) and AST (aspartate aminotransferase; EC 2.6.1.1), which are considered as disease assessment indexes of the liver, is transferring the α-amino groups from certain amino acids to α-keto group of

ketoglutarate, concomitantly formation of oxaloacetate and pyruvate, which are replenished the Krebs's cycle intermediates (Dufour et al., 2000a; Vanderlinde, 1986). ALP (alkaline phosphatase; EC 3.1.3.1) is an enzyme existing in the cells that lining the biliary ducts of the liver, bone, placenta and other tissues. It hydrolyzes phosphate esters with the subsequent releases of inorganic phosphate (Li, Xu, Fan, Wang, & Xiao, 2014). It has several isoenzymes formed by various cells types within alveolar bone and/or salivary glands (Chapple, Garner, Saxby, Moscrop, & Matthews, 1999). The main roles of ALP are anti-inflammatory and anti-thrombotic, also involved in immunological process (Dufour et al., 2000b). It is well known that the inflammation of the hepatocytes plasma membrane is accompanied with the releasing of ALP in circulation (Li et al., 2014).

1.3 The significance of saliva sample

Whole saliva is a mixed fluid containing (97-9.5) % water, minerals, electrolytes, enzymes, growth factors, immunoglobulins, mucin and other glycoproteins that are synthesized by the acinar cells (Vining & McGinley, 1986). Blood and urine specimens were used for medical control and routine tracking, while saliva comparatively less applied as biological sample for clinical analysis (Amann, 2005; Cao & Duan, 2006). Saliva is considered as a non-communicable fluid and a low-cost storage samples comparing with other biological fluids (e.g.: blood, urine and spinal fluid). Therefore, saliva analysis has been progressed in clinical diagnosis. Recently, diagnosis and control of different biomarkers by using saliva in a newborn or aged individual are more accessible (Chiappin, Antonelli, Gatti, & De Palo, 2007).

1.4 Aims of the study

Malaguarnera *et al.*, 2012 reported that different chemical solvents (e.g. trichloroethylene, toluene, dimethylformamide, tetrachloroethylene, carbon tetrachloride, xylene, chloroform, dimethylacetamide) are suspected to be responsible for the occupational liver toxicity. These solvents are currently used in chemical education laboratories, thence, the main target of this study is the evaluation of possible links between working environment conditions and health impairment. To this aim, longitudinal study was designed, sera and saliva samples from volunteered students (Department of Chemistry) were examined before and after three months of exposed to different hazardous chemical materials during the practical study of undergraduate chemical laboratories. In order to study the possible effects of these chemicals on the liver function in these two fluids of the student volunteers, ALT, AST, and ALP activities were measured. Also, comparative and correlation analysis of the measured parameters in serum and saliva samples were carried out.

2 MATERIALS AND METHODS

2.1 Study subjects

Fourteen students (from Department of Chemistry) were involved in this study. The goal, objective, and method of the study were explained to students, and they were willing to take part in this project. Ethics committee of Baghdad University / College of Science approved the study protocol of this project.

2.2 Questionnaire criteria

Informations were obtained from each individual (e.g., age, smoking, medication, current and past diseases, place of residence and after-university work if possible), in order to minimize external factors that may interfere with internal factors (probable chemical exposure). The mean age of the students was 22 years (range 22-24).

2.3 Sample collection

Samples (blood and saliva) were collected, both before and after the first semester. Serum was separated and kept frozen to be used later for enzymes biochemical analysis. Serum ALP, ALT & AST activities were measured using RANDOX kits. After rinsing the mouth, non-stimulant saliva (1 to 5 ml) was collected, centrifuged for 10 minutes at 2000 $\times$g then the supernatant was frozen for further use. Salivary ALP, ALT and AST activities were measured using kits for diagnostic routine, which was modified for saliva (sample volume of saliva was 150 microliters was used instead of 50 microliters for serum sample).

2.4 Statistical analysis

The SPSS package was used to examine if there is any significant differences between values for liver parameters and matched pair analysis was performed with the Wilcoxon non-parametric signed rank test. The sign test compares the same populations which subjected to more than one condition, and uses the standard normal distributed z-value to test of significance.

Wilcoxon test can easily be rescaled by measuring the effect size, which must be calculated manually, using the following formula: $r = z/\sqrt{n}$ (r: effect size; z: z is the test statistic output by SPSS; n: is the total number of observations that were made).

Pearson's (r) correlation coefficient, as well, was applied to check the correlation among the studied variables.

2 RESULTS

A Wilcoxon test was conducted to evaluate whether the chemical exposure has any effect on serum and salivary AST, ALT and ALP activities. The results showed that three months exposure to possible chemical hazardous, did not elicit statistically significant changes in serum AST and ALP activities being of Z = -1.479 (p = 0.139) and Z = -1.099 (p = 0.363),

respectively. Whilst, there was a significant increase from (median = 4.2 U/l) to (median = 6.8 U/l) in the levels of serum for ALT (Z = -2.355, p < 0.019) and the increase was medium (r = - 0.445) as represented in Table 1.

Table 1: Liver function enzymes in serum before and after exposure

	N	Before exposure median (range)	After exposure median (range)	p value	z statistic	r effect size
AST(U/l)	14	10.00 (4.0 – 24.5)	12.00 (5.0 – 19.0)	0.139	-1.479	- 0.279
ALT(U/l)	14	4.20 (0.6 – 7.5)	6.80 (3.2 - 11.2)	0.019*	-2.355	- 0.445
ALP(U/l)	14	41.53 (30.1 - 61.4)	29.55 (9.6 – 74.7)	0.363	-1.099	- 0.207

*Statistically significant $p < 0.05$

The outcomes, presented in Table 2, indicated that the presence of significant increases in all salivary AST, ALT and ALP activities of Z= -3.045 (p < 0.002; with large effect size = -0.575), Z= -2.512 (p < 0.012; with medium effect size = -0.475) and Z=-2.982 (p < 0.003; with large effect size = -0.563) respectively.

Table 2: Liver function enzymes in saliva before and after exposure

	n	Before exposure median (range)	After exposure median (range)	p value	z statistic	r effect size
AST(U/l)	14	15.00 (7.5- 28.0)	31.00 (12.0 – 70.5)	0.002*	-3.045	- 0.575
ALT(U/l)	14	2.7 (1.2- 6.6)	5.00 (2.3 – 8.4)	0.012*	-2.512	- 0.474
ALP(U/l)	14	8.51 (0.9 – 34.5)	22.10 (14.3 – 42.9)	0.003*	-2.982	- 0.563

*Statistically significant $p < 0.05$

The correlation coefficient was computed to determine whether the relationship between liver function enzymes levels in serum and saliva is statistically significant. In Table 3, the data were re-designed with the more correlated parameters (r > 0.4) from the original SPSS report outcomes, while weakly correlated or uncorrelated parameters were not shown.

Discernibly, the variations in the serum liver function enzymes (AST, ALT and ALP) have a significant concurrent relationship, as shown in Table 3. Also, the findings reflect statistically insignificant relationship among salivary AST, ALT and ALP. Where there was a negative relationship between salivary AST versus salivary ALP (r = -0.513). There are a good correlation between serum and salivary liver enzymes, as presented in Table 3. A significant positive correlation was observed between serum ALP (after) versus salivary ALP (after) with r = 0.699 (p < 0.001) and insignificant negative relationship between ALP-serum (before) versus ALP-saliva (before) with r = -0.422 (p > 0.05).

Table 3: Correlation between liver enzymes in serum & saliva, before & after exposure

		Correlation Coefficient (r)	p value	n
ALT serum - after	AST serum - before	0.535[*]	0.049	14
ALP serum - before	ALT serum - before	0.525	0.054	14
ALP serum - after	ALT serum - after	0.729[**]	0.003	14
AST saliva - after	AST saliva - before	0.476	0.085	14
ALT saliva - before	AST saliva - after	0.488	0.077	14
ALP saliva - after	AST saliva - after	-0.513	0.061	14
ALT saliva - after	ALT saliva - before	0.455	0.102	14
ALP saliva - before	AST serum - after	-0.407	0.149	14
AST saliva - before	ALP serum - before	0.480	0.083	14
AST saliva - after	ALP serum - before	0.584[*]	0.028	14
AST saliva - before	ALP serum - after	0.505	0.065	14
ALP saliva - before	ALP serum - before	-0.422	0.133	14
ALP saliva - after	ALP serum - after	0.699[**]	0.005	14

*Statistically significant $p < 0.05$
**Statistically significant $p < 0.01$

3 DISCUSSION

Previous studies showed that exposure to various chemicals, resulting in acceleration the metabolism of the chemical compounds, that lead to produce more toxic products (Brautbar & Williams, 2002, Stiles, 1904). Therefore, exposing to occupational or environmental conditions might cause deleterious and/or complications health effects. Acute chemical exposure and liver toxification have been linked with steatosis, cirrhosis, and mortification of liver. As the potential progression of chronic hepatic toxicity leads to liver failure, and consequently may cause malignancy (Lee, 1993).

Alterations in (AST, ALT and ALP) activities, sensitively and specifically reflect liver dysfunctions. From practical point of view, the measurements of these enzymes activities are relatively more applicable and easier compared with other liver injuries markers which may represent high cost per run and much limitations to be usable in safe laboratory or clinic (Ozer, Ratner, Shaw, Bailey, & Schomaker, 2008). The study findings represent that serum AST and ALT activities were increased after three months of possible chemical exposure (Table 1). These results confirmed with previous studies (Ikai et al., 1995; Kawai, Kawai, & Kawai, 1995), which reported that the chemical exposure could strongly relate with increasing of the liver dysfunction incidents (Luo et al., 2001).

The results presented that the variation in the level of serum (AST and ALP) activities were insignificant, except serum ALT activity which gave significant increasing (p

< 0.019). However, the normal levels of AST, ALT and ALP activities in serum are 7-40 U/l, 5-35 U/l and 40-160 U/l, respectively. Manifestly, the value of liver enzymes exceeded the upper reference limits resulted in abnormal level, while low value has no clinical significance (Giannini et al., 2005). All students had serum liver enzymes activities which did not exceed the normal levels (as shown in Figure 1- A, B & C), otherwise, ALT activity presented a significant increment after exposure, but the increment still within the normal level. This may be due to the undetectable concentrations of hazardous chemicals exposure to the study individuals and relatively short exposure time. The alteration in ALT level is usually considered as a specific marker for liver injury comparing to AST level. ALT is existed solely in the liver cytoplasm, whilst AST is concentrated in mitochondrial liver tissues (Lanphear, Dietrich, Auinger, & Cox, 2000) as well as presented in myocardial, muscular, renal, neural tissues and others cells (Wroblewski, 1958).
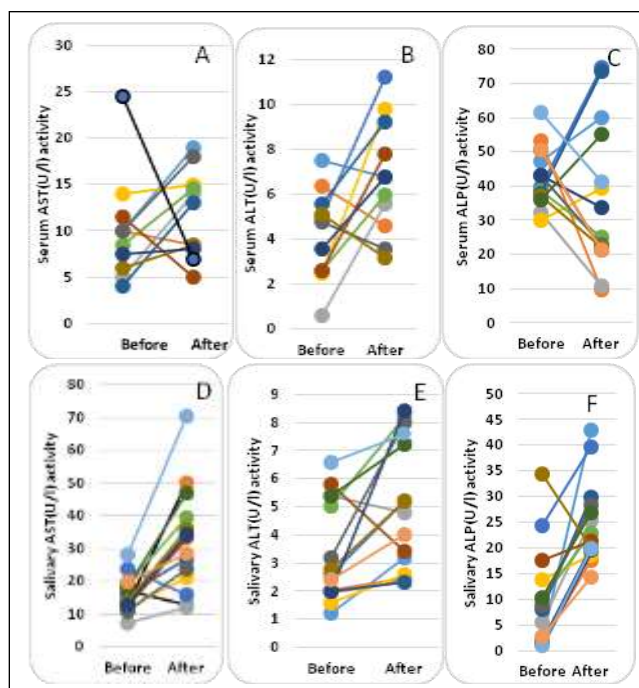

Fig. 1. AST, ALT & ALP activities in serum (A, B & C) and saliva (D, E & F) before and after exposure respectively.

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Exposure to small amounts of chemicals may give a rise to extensive influences on people health. Even though there was no additional exterior exposures, chemicals are cumulating in tissues and organs of human body. The accumulated compounds that are metabolically inactive (Heinzow & McLean, 1994), can contaminate even infants when transferring this accumulated substances to, via utero or a nursing. Biochemical alterations may be produced from chemical exposure, these changes could be at a structural or functional level of a given organelle or organ in the living organism (Heinzow & McLean, 1994). It has been suggested that the major pathological consequences induced by chemical toxicities are cell inflammatory, mitochondrial and cytochrome P450 system impairments, and the overproduction of reactive substances, leading to oxidative stress (G. Malaguarnera et al., 2012). Several researchers (Berson et al., 1998; G. Malaguarnera et al., 2012; M. Malaguarnera et al., 2009) reported that cationic and amphiphilic chemicals can be easily concentrated in mitochondria due to the potential driving force of the mitochondrial membrane. The accumulated chemicals could cause a leak in the electron transfer respiratory chain that resulted in inhibition of fatty acid oxidation, propagations of reactive oxygen species and consequently causing liver injury.

Human saliva has considerable biological roles, which are essential for the oral protection. Several researches confirm the possible role of saliva as an index of oral pathologies. Furthermore, saliva analysis might be used in the detecting, diagnosis and monitoring of various disorders (Al Kawas, Rahim, & Ferguson, 2012; O., R., 2009; H. Hasan, R and Dawood, R., M., 2012). Also, the existence of different metabolites in blood can be reflected in the saliva (Pfaffe, Cooper-White, Beyerlein, Kostner, & Punyadeera, 2011; Xiao & Wong, 2010).

The level of salivary AST and ALT increased significantly, as presented in Table 2, and the elevation after the exposure was observed in all samples except two individuals (Figure 1- D and E). Whereas ALP activity increased significantly in all studied subjects but one individual (Figure 1- F). The significant variations in salivary liver enzymes might indicate liver impairment. Thus, liver enzymes determined in a biological fluid (saliva) of people exposed to potentially hepatotoxic chemicals may have a significant value. The outcomes of present longitudinal study indicated that the group of students that were exposed to the organic solvents for three months, resulted in increasing of the salivary liver enzymes levels which this might be a sign of slight liver dysfunction.

A significant correlation between the level of serum ALP and salivary ALP were suggested. This leads to the possibility of utilizing salivary ALP activity in pre-clinical diagnosis and monitoring of different liver dysfunctions. Thus, the study's results suggest presence of liver impairment indications as an effect of probable chemical exposure. This prediction needs further clinical and pathological studies. Also, identifying chemical agent as toxicants and specifying the amount, concentration or level in concerned laboratories are required to enhance this proposal.

4 CONCLUSION

In conclusion, it is noteworthy that, salivary liver enzymes might be considered to be promising parameters of hepatotoxicity induced by different organic solvents comparing with the conventional serum tests and it can be used as a biological marker of subclinical liver injury. The results of this study might draw attention to increase chemical safety requirements in the educational laboratories. Periodic health checkups to the laboratory staffs and students are necessary because of the potential risks of chemicals exposure.

Acknowledgments

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