



Effects of Occupational Toluene and Trichloroethylene Exposure on Liver Enzymes

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Abstract—Toluene and trichloroethylene are organic solvents which are widely used in industrial settings. Occupational exposure of these solvents may induce hepatic injury. In this study we determined the hepatic enzymes alanine transaminase (ALT) and aspartate transaminase (AST) of the workers who are occupationally exposed to toluene (n=152), trichloroethylene (n=125), or both (n=77); group 1, group 2 and group 3 respectively. Comparison with a control group (n=158) whom are not occupationally exposed to any organic solvent was done. Regarding AST and ALT we observed a significant increase in solvent exposed (toluene, trichloroethylene (TCE), toluene and TCE) groups when compared to non-exposed control group ($p < 0.001$). The median values of AST and ALT for solvent exposed groups did not exceed reference range. ALT levels were above reference levels in 5.3% of group 1, 7.2% of group 2 and 5.2% of group 3. Also AST levels were above reference levels in 5.9% of Group 1, 4% of Group 2 and 3.9% of Group 3. In all of the exposed groups, the percentage of above the reference level of both enzymes, were significantly higher than the control group. None of the controls had above the threshold enzyme levels. Exposed groups all had a ratio of ALT to AST (ALT/AST) higher than 1; 1.14, 1.2, 1.09 for group 1, group 2, and group 3 respectively. According to our results toluene and TCE does not cause acute hepatotoxicity but some elevation of ALT and AST is present. ALT and AST of occupationally solvent exposed workers should be routinely monitored and necessary precautions should be taken.

Index words: alanine transaminase; aspartate transaminase; toluene; trichloroethylene; occupational exposure.



I.Introduction:

Acute and subacute hepatocellular injuries are the most commonly recognized occupational liver disorders. They include a spectrum of liver injury ranging from acute injury following a single massive hepatotoxic exposure, as occurs in accidental poisoning, to repeated hepatotoxic exposures over days to weeks. Although numerous suspected and known animal hepatotoxins, primarily organic solvents, are widely used throughout industry today, there is a limited amount of human data on the effects of such exposures.

The liver is the largest visceral organ. Its mass of parenchymal cells, portal tracts and abundant blood supply is evidence to its crucial role as the body's main metabolic factory. The liver is particularly vulnerable to the effects of organic solvents if already reeling under the effects of regular and excessive dosing with ethyl alcohol. Pre-existent liver disease may enhance the effects of a new toxic insult. Furthermore, it is worth noting that hepatic enzyme induction may alter the liver's ability to handle certain toxins, and the organ itself is occasionally subject to hypersensitivity reactions.

Organic solvents are used in various industrial processes such as paint manufacturing, spray painting, shoe making, degreasing, metal processing, auto manufacturing, and aeronautical

maintenance and manufacturing, as well as in various chemical storage facilities. Exposure to hepatotoxic solvents can occur in 1) occupational setting through either daily inhalation or skin absorption of solvents, 2) residential setting during either accidental or intentional ingestion in food or as a toxic contaminant of food, and/or exposure to toxic agents such as in the form of glue sniffing, 3) environmental setting, commonly residential, usually through groundwater contamination. Ingestion of the water, bathing in the water with skin contact and absorption, and volatilization of the solvents through heated bathing or showering water, are possible routes .

The hepatotoxic effects of some of the solvents were recognized as early as 1887. The earliest solvent studied in the context of liver injury has been carbon tetrachloride and has been described in 1967 [1]. Since some of the solvents cause chronic health effects, including liver injuries, it may take decades to study and document such events. The most famous example is vinyl chloride which was once thought to be safe and has been used for many years until it was found to cause liver tumors.

Several tests including serum aspartate aminotransferase, serum alanine aminotransferase, γ -glutamyltransferase, and alkaline phosphatase can serve as sensitive indicators of liver injury



[2]. A recent cohort study found that there was a positive association between serum aminotransferase concentration, even within normal range, and mortality from liver disease, suggesting that moderately increased aminotransferase activity is significant in predicting liver disease [3].

Toluene is a clear, colorless, volatile hydrocarbon that is principally used in gasoline blending, but also as a solvent for paints, lacquers, thinner, adhesives and many other products. Most exposures to toluene occur by inhalation; however, it may also be absorbed through ingestion and to a lesser degree through skin. Toluene is a highly lipophilic molecule. It is distributed quickly to highly perfused tissues such as brain and liver, with accumulation in tissues with high lipid content. High concentration of toluene exposure involves either toluene containing solvent abuse or occupational exposure to toluene. Solvent abusers are generally exposed to higher levels of toluene than are occupational exposures. Acute high concentration of toluene can cause headache, fatigue, dizziness, nausea and loss of consciousness. If exposure continues, toluene causes death from respiratory failure or arrhythmias [4].

TCE is a colorless, nonflammable liquid with a sweetish odor resembling chloroform. In the past, TCE was used as an extractant in food-

processing, but was discontinued. TCE was used as a general anesthetic in humans for many years beginning in the 1930s. It has also been used as inhaled patient controlled analgesic agent, mainly for the treatment of trigeminal neuralgia. TCE is a powerful fat solvent and therefore is widely used as a degreasing agent. It has also been used, in degreasing, processing, fabrication of metals and in some of the food processing industries. It has been identified as a common contaminant of air and degrades very slowly in ground water and therefore is commonly found as a groundwater and drinking water contaminant. The liver has clearly been shown to be a target organ for TCE in experimental animals. The data in humans, although limited, clearly suggests a toxic effect on human livers. Many case reports described TCE induced hepatitis and liver necrosis [5].

In industrial settings exposure to multiple solvents are common. Multiple exposures may potentiate the hepatotoxic effect of these solvents. The aim of this study is to evaluate and compare the serum ALT and AST levels of toluene-exposed, TCE-exposed and combined toluene-TCE exposed industrial workers.

II. Materials and Methods:

Study Population

512 male workers aged between 30-45 years were included in this study. Laboratory results of

industrial workers who came for periodic evaluation to Ankara Occupational Diseases hospital between 1/1/2013 and 12/31/2013 were reviewed and 3 different groups were determined. Those with high urinary levels of hippuric acid (>1400 µg/L) were placed in toluene exposed group (group 1, n=152), while those with high

urinary levels of TCA (>10 µg/L) were placed in TCE exposed group (group 2, n=125). Those with both high urinary levels of hippuric acid and TCA were placed in multiple exposure group (group 3, n=77), random workers with normal urinary hippuric acid and TCA levels, with no known

TABLE 1. AST,ALT and Hematological parameters of control and exposed groups. Values are expressed as median (min-max) For statistical evaluation of ALT, AST, RBC, Hb, WBC and Htc, Comparison of groups are made using Kruskal-Wallis and with Bonferroni correction if between group comparisons are made. ^aThe comparison of percentages were done with Fisher exact test, P<0,005 was considered significant. *Significant difference from the control group,** Significant difference from group 1, ***Significant difference from control group 2.

| Parameters | Control group | Group 1 (Toluen) | Group 2 (TCE) | Group3 (Toluen+TCE)) | P |
|--|-------------------|------------------------------|-------------------|-------------------------|---------------------|
| | (n=158) | (n=152) | (n=125) | (n=77) | |
| AST(U/L) | 17 (11-28) | 21 (8-46)* | 20 (10-64)* | 20 (10-64)* | <0,001 |
| ALT (U/L) | 14(10-17) | 22 (5-85)* | 23 (7-87)* | 23,10(7-132)* | <0,001 |
| % high AST(n) | %0(0) | %5,9(9)* | %4(5)* | %3,9(3)* | <0,034 ^a |
| % high ALT(n) | %0(0) | %5,3(8)* | %7,2(9)* | %5,2(4)* | <0,014 ^a |
| % Both high AST,ALT (n) | %0(0) | %2,6(4) | %2,4(3) | %2,6(2) | >0,05 ^a |
| ALT/AST ratio | 0,833 (0,50-1,31) | 1.05 (0,43-2,13)* | 1.2 (0,35-2,23)* | 1 (0,38-2,17)* | <0,001 |
| RBC (10 ⁶ /mm ³) | 5,03(3,94-7,11) | 5,13(4-5,96) | 5,15(4,01-6,67)* | 5,04(4,08-6,64) | 0,041 |
| Hb (g/dL) | 15,3 (9,20-17,5) | 15,6 (12,8-17,8)* | 15,7 (12,5-17,7)* | 15,3 (13-17,1) | <0,04 |
| WBC (/mm ³) | 7,15 (4-14,3) | 7,2 (3,9-14,8) | 7,7 (4,4-15,4) | 6,9 (4,1-15,4) | 0,092 |
| HCT (%) | 45 (28,5-53) | 45,9 (36,7-54,9) | 46,2 (36,5-53,5)* | 44,8 (37,50-49,7) | 0,012 |
| TCA (µg/L) | 4,1 (0,7-6,80) | 4,4 (1-6,96) | 12,4 (10-81,8)*** | 14,8 (10-204)*** | <0,001 |
| Hippuric acid (µg/L) | 196,27(22-305) | 2081,02(1588,33-5844,71)**** | 283,5(23,1-436) | 1908,72(1403-5428)**** | <0,001 |
| Age | 38 (19-68) | 43 (21-64)* | 41 (19-58) | 39 (20-62)** | <0,28 |

active disease were taken as controls. The urines were collected at the end of working shift.

Urinary hippuric acid and TCA determination

Hippuric acid in urine was determined with Agilent 1200 series HPLC using a commercial kit (Eureka, Italy). Spectrophotometric method was used for urine TCA levels,.

Serum ALT and AST determination

Serum Alanine Transaminase (ALT) and Aspartate Transaminase (AST) analysis were performed with Konelab Prime 60i auto-analyzer. Normal range is 10-35 U/L for AST and 10-45 U/L for ALT.

Whole blood cell count

Red blood cell (RBC) numbers , hemoglobin (Hb), white blood cell (WBC) numbers and hematocrit (htc) percentages were determined by Beckman Coulter LH780.

III. Statistical analysis

Results are given as median (minimum-maximum). Acquired data did not show normal distribution. Comparison of groups are made using Kruskal-Wallis and with Bonferroni correction if between group comparisons were made. Percentages were compared using Fisher's exact test. $P < 0,05$ was considered as statistically significant.

IV. Results:

With regard to the levels of liver enzymes AST and ALT, we observed a significant difference between solvent exposed (toluen, TCE, toluen and TCE) groups and non-exposed control group ($p < 0.001$) (Table 1).

Although the mean values of AST and ALT for solvent exposed groups (Group1 ,2 and 3) did not exceed reference range, ALT levels were above reference levels in 5.3% of group 1, 7.2% of Group 2 and 5.2% of Group 3 . Also AST levels were above reference levels in 5.9% of Group 1, 4% of Group 2 and 3,9 % of Group 3. In all of the exposed groups, the percentage of above the reference level of either enzymes were significantly higher than the control group. None of the controls had above the threshold enzyme levels.

In all the exposed groups ratio of ALT to AST (ALT/AST) median was higher or equal to 1; 1.05, 1.2, 1.0 for group 1, group 2, and group 3 respectively.

RBC and Hb levels also were found to be different from control group ($p < 0.001$) for solvent exposed groups.

V. Discussion:

The liver is the main organ responsible for the metabolism of drugs and toxic chemicals, and so is the primary target organ for many organic solvents. Organic solvents used in different industrial processes may be associated with



hepatotoxicity. Several factors contribute to liver toxicity; among those are: nutritional condition, genetic factors, interaction with medications in

use, alcohol abuse and interaction, and age. Very little is known about the frequency of liver injury by solvents. It is still difficult to assess the damage from exposure due to difficulties in controlling the workplace [6]. Acute and subacute hepatocellular injuries are the most commonly recognized occupational liver disorders. [7].

After the occupational exposure history has been registered, common laboratory tests are usually the first line diagnostic tools when deleterious solvent effects are suspected. For the screening of latent liver disease, alcohol abuse or adverse liver effects caused by solvent exposure liver enzymes are often determined in routine occupational health examinations. The most widely used tests are those used to detect the aminotransferases—alanine and aspartate—which are associated with hepatocellular injury [8]. In the case of hepatocellular injury to avoid progression, preventing of exposure is obligatory, as there is no specific antidote or treatment. There is some evidence for TCE-induced hepatotoxicity in humans. However, much of this information is limited by the fact that the exposure levels associated with these effects were usually not reported, and the individuals may have been exposed to other substances as well [9]. Nagaya T et al. investigated the early hepatic effects of chronic exposure to low-level TCE in 148 workers (a cross-sectional study) and in 13 workers (a 2-year follow-up study) occupationally exposed to TCE in air. In this study, it was reported that the exposure had no effect on the serum activities of ALT and AST [10]. Likewise other investigators also found that exposed groups had AST and ALT levels within the normal range and not to be significantly different from the control groups [11,12,].

In our study concerning the levels of liver enzymes AST and ALT, a significant difference was found,

between solvent exposed (toluen, TCE , toluen and TCE) groups and non-exposed control group ($p < 0.001$). In a study investigating the harmful effects of toluene inhalation in the liver of male Wistar-albino rats, Ufuk Tas et al, concluded that toluene inhalation significantly increased serum ALT and AST levels. The increase was less than twofold. They also detected oxidative damage in liver tissue (7). In a study with 29 solvent-exposed workers, Ari Kaukiainen et al. reported that ALT and AST levels correlated positively with cumulative solvent exposure in the past 5 years [13].

In this study we found that median values of AST and ALT for solvent exposed groups (Group1 ,2 and 3) did not exceed the reference range. ALT levels were above reference level in 5.3% of Group 1, 7.2% of Group 2 and 5.2% of Group 3 . Also AST levels were above reference levels in 5.9% of Group 1, 4% of Group 2 and 3,9 % of Group 3. All of these percentages were significantly higher than the control group. Both AST and ALT were concomitantly higher in the 2,6% of group 1, %2,4 of group 2 and 2,6% of group 3, and all was not significantly different than the control group. Our results clearly indicate a cytotoxic mechanism for the solvent exposed workers. The intracellular enzymes ALT and AST are found to be above normal in a significantly higher percentages of the exposed groups. The study conducted by Sancinni et al also found above the threshold percentage for AST and ALT to be higher in the solvent exposed group when compared to the non-exposed group. In their study the percentage above the threshold off both enzymes in the same individual were also higher from the control group [14].

In our study the highest AST level 64 U/L seen in two patients one in TCE exposed group the other in mixed exposure group. The increase is approximately two fold. The highest ALT level is 139 U/L seen in a both TCE and toulen exposed worker. The increase is

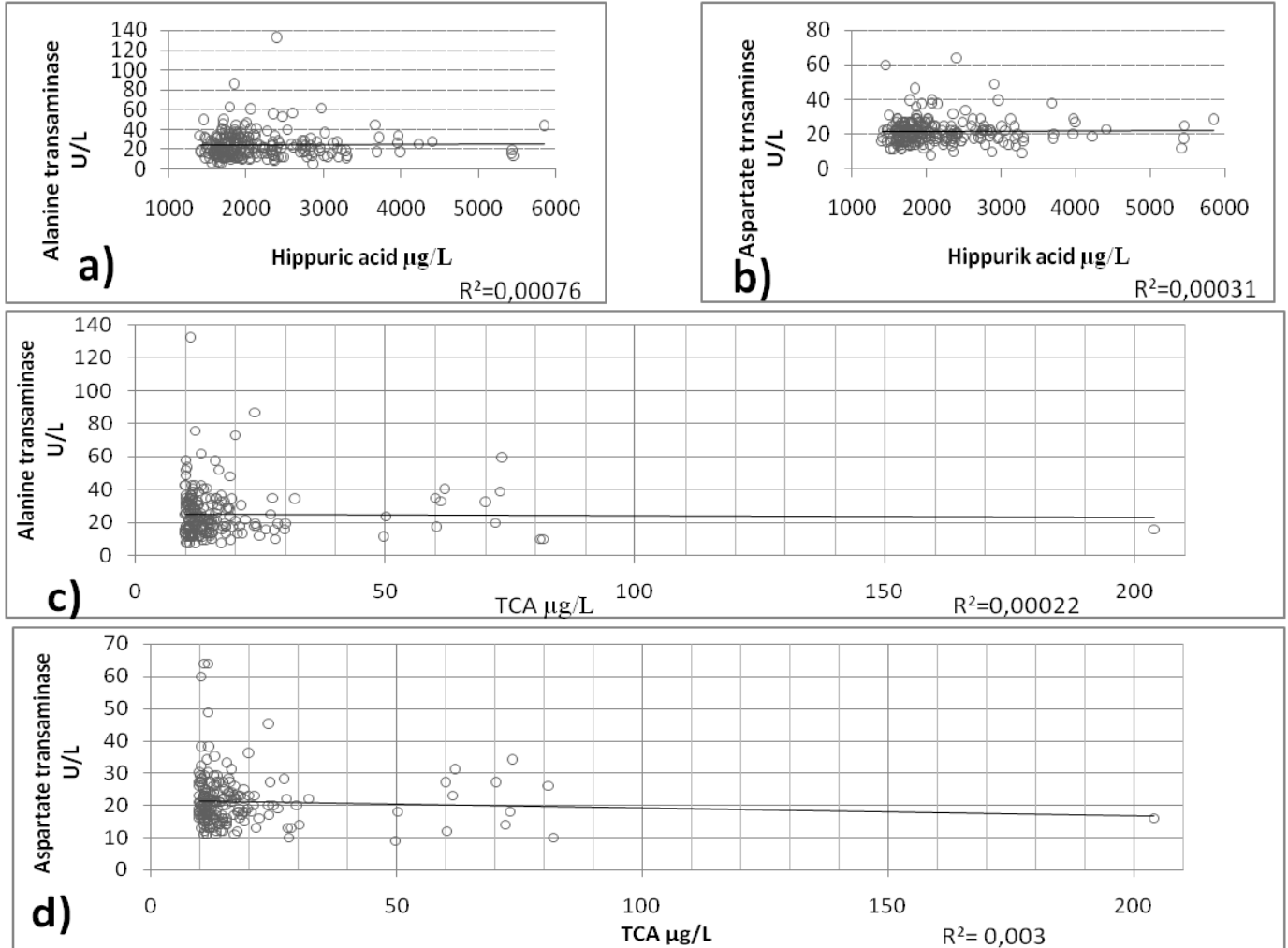


Fig.1a) Hippuric acid levels are plotted against ALT at toulen exposed groups (group1-2), there is no significant correlation, **b)** Hippuric acid levels are plotted against AST at toulen exposed groups (group1-2), **c)** TCA levels are plotted against ALT at TCE exposed groups (group3,4), **d)** TCA levels are plotted against ALT at TCE exposed groups (group3,4), there is no strong correlation(Spearman's correlation).

approximately 3 fold. These levels of AST and ALT are not indicative for acute toxic hepatitis. It is generally accepted that less than 5 fold increase is seen in chronic hepatic diseases [15]. The workers with only AST elevation could have toxicity in tissues other than liver as ALT has not increased. Our present study did not include

parameters that could show toxicity to other tissues. There was no important correlation between urinary TCA and hippuric acid and ALT or AST levels (highest Spearman's correlation coefficient being 0,26 after cross pairings) (Fig 1a,1b,1c,1d).



The median of ALT/AST ratio was less than 1 in the control group. In the exposed groups the mean of this ratio was equal or higher than 1. studies also found higher ALT/AST ratios when compared to control group and claimed that there was a cytotoxic damage caused by the solvents [14]. When all the exposed groups are pooled, the percentage of ALT/AST ratio greater than 1 was 56% (198/354) while in the control group 10% (16/158) had ratios greater than 1. In the control group the highest ALT/AST ratio was 1,31 however in the exposed groups only 31% (110/354) had a ratio greater than 1,31. Contrary to our findings in another study the solvent exposed workers all had ratios greater than 1, with a mean of 1,5 and this ratio was greater than 1,6 in more than half of the exposed workers [16]. From a medical surveillance study of 289 printing factory employees exposed primarily to toluene, Guzelian P et al. identified eight workers who had persistently abnormal serum transaminase values. All eight had mild elevations (less than 2 to 3 folds) of serum transaminases (ALT) and (AST). However, there was a marked increase in the ratio of ALT/AST (mean = 1.61) they proposed an increased ALT/AST ratio may represent a convenient, previously unrecognized indicator of solvent induced hepatotoxicity [17].

The difference between exposed groups and control group was statistically significant. Other

According to our results ALT/AST ratio does not appear to be a sensitive marker for solvent exposure.

Although in the normal range the elevation of ALT and AST in the exposed groups may have serious consequences as shown by Hyeon Chang Kim et al. In a large prospective cohort study found even in the normal range, as ALT and AST increases, the likelihood of death from liver disease also increases [3]. In our practice, industrial workers that can be exposed to solvents are routinely monitored for AST and ALT and even slight elevations are noted and appropriate precautions are advised.

In our study the highest urinary hippuric acid level was 5844 µg/L and there were 4 workers over the 5000 µg/L range and all of them had normal AST and ALT levels. For TCA the highest level was 204 and there was seven workers above 70 µg/L and six of them had normal ALT and AST levels while only one had slight ALT elevation. Of course urinary levels of solvent metabolites only show the extent of the last exposure and doesn't give any information about the chronicity of the exposure. We included



workers that were working at the same industrial unit over 2 years but the records we were able to reach did not imply the exact duration. It was surprising that in the mixed exposure group the AST and ALT levels or percentage of above normal was not higher either than the TCE or toluene exposed groups (for ALT $p=0,218$ for group 1 and $p=0,077$ for group 2, for AST $p=0,209$ for group 1, $p=1,00$ for group 2 (Kruskall-Wallis with Bonferroni correction). The cytotoxic effect does not seem to be additive.

As has been in the majority of the studies we also measured the function of ALT and AST in the serum and this can be a limitation, as there is little information on the effect of solvents on this function. AST and ALT are both intracellular enzymes released after cellular injury. If the function is effected by the insult the true amount may not be detected as seen in alcoholic patients [16].

Although our results indicate that solvents toluene and TCE does not cause any acute hepatotoxicity even in high levels of exposure, it is wise to monitor AST and ALT levels and take necessary precautions. It is clearly known that the main target of solvents are neuronal tissue and according to our results the hepatic enzymes AST and ALT are not indicative for the extent of

exposure. Biomarkers aside ALT and AST are necessary to predict toxic exposure to the solvents.

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