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**PJBS**

ISSN 1028-8880

# **Pakistan Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Alanine Transaminase Level in a Healthy Population in Morocco

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**Abstract:** A little is known about the prevalence of elevated alanine transaminase in a Moroccan healthy population. Our aim was to search for the upper limit of normal alanine transaminase in the blood donors and then to apply the upper limit of normal alanine found in the population so as to assess the prevalence of subjects with abnormal transaminase level. We then, investigated for factors associated with increased level of transaminase in our population. This study was carried out on 14071 blood donors, (74.1% of men and 25.9% female) aged between 18 to 60 years, randomly chosen. Serum transaminase activity was measured using on IEMS Reader, Labsystems. Hepatitis B and C were performed by ELISA. The upper limit of normal transaminase found were 64 for men and 52 for women. Consequently, 2.08% blood donors had an abnormal level of transaminase. Follow up results revealed that drug was the first cause of elevated transaminase in our cohort followed by diet and alcohol consumption. One seroconversion for hepatitis C was identified. In conclusion, this study showed that even though there is an evident lack of efficiency in using alanine aminotransferase testing qualifying blood donors in our country, preventing viral potential transmission through transfusions was possible.

**Key words:** Alanine transaminase, blood donors, upper limit of normal ALT (ULN), etiology, Morocco

### INTRODUCTION

Alanine transaminase or alanine aminotransferase (ALT) is a hepatic enzyme that catalyzes the two parts of the alanine cycle (Dieusaert, 1999). This enzyme is found principally in the liver but is also found in smaller amount in the kidneys, heart, muscles and pancreas (Vincent-Viry, 1990).

Many factors have been associated with elevation of ALT as high alcohol consumption, hepatitis B or C infection, body mass index, drugs intake, diabetes, triglyceride etc. (Tabassum *et al.*, 2000; Pervez, 2000; Oguntibeju and Banjoko, 2003; Kumar *et al.*, 2004; Nasiri and Hosseinimehr, 2006; Prati *et al.*, 2002; Vassault and Fontaine, 2006; Prakash *et al.*, 2008; Meybodi *et al.*, 2008; Ciangura, 2010; Morisco *et al.*, 2010; Kobayashi *et al.*, 2011).

After introduction of specific tests for the detection of hepatitis B, the ALT test was intended to detect potentially infected blood donors with non-A non-B hepatitis (now known to be mainly infection with hepatitis C) (Desforges *et al.*, 1995).

In Some countries, screening blood donations for alanine-aminotransferase levels is still mandatory

(Zaier *et al.*, 2002; Ghaffour, 2009) despite this practice being controversial, and donors with an higher level of ALT are excluded from blood donation, which decrease the donation acceptance rate (Blajchman *et al.*, 1995; Schreiber *et al.*, 1996; Mele *et al.*, 1995).

The upper limit of normal (ULN) alanine transaminase can vary from a population to another and difference can have practical consequences. Most clinical laboratories use 40 UI L<sup>-1</sup> as an upper limit of normal for ALT level. This latter value was set in the 1950s and has stayed almost unchanged (Kaplan, 2002). This is why several studies have recently suggested that the ULN of ALT should be addressed more carefully (Prati *et al.*, 2006; Jamali *et al.*, 2008a,b). A little is known about the prevalence of elevated ALT in Morocco.

In order to determine the ULN in our country, we investigated the level of ALT in healthy population of the capital city of Rabat and surrounding regions. We further identified the prevalence of the elevated ALT level. Finally, we looked for the causes and etiology that could elevate the ALT level in our population. Furthermore, we discussed the relation between elevated ALT and the hepatitis B and C serology.

## MATERIALS AND METHODS

This study was carried out on 14071 blood donors (74.1% of men and 25.9% female) aged between 18 to 60 years, randomly chosen. All tests were done in the Centre Regional de Transfusion Sanguine (CRTS) of Rabat.

The participants were part of a study for identifying the causes of elevated serum ALT level and consented to be part of the study.

Blood samples were collected on citrate and were centrifuged within 10 min of collection. Samples with haemolysis traces were eliminated. The search for markers (of hepatitis B and C were performed by Murex Enzyme Linked Immuno-Sorbent Assay (ELISA). All measurements of serum ALT activity were performed by kinetic method using flat-bottomed microplate techniques on IEMS Reader Labsystems.

**Questionnaire:** Donors with elevated level of ALT and hepatitis B and C negative were contacted by phone and/or regular mail 6 months after the first analysis for medical follow-up and serological monitoring, to check if there is any eventual persistence of the abnormal ALT level. A questionnaire was presented to the returned blood donors to monitor for the probable factors of higher level of ALT. The questionnaires discussed issues related to diet, hepatotoxic medication, alcohol and finally any eventually causes that could elevate the ALT level.

**Statistical method:** Chi-square ( $\chi^2$ ) analysis was performed to evaluate the relation between ALT level, age and gender.

## RESULTS

Characteristics of the 14071 of blood donors studied are given in Table 1. The upper limit of normal ALT was found 64 and 52 for men and women, respectively. Most of the samples (13777) had a level of ALT below the ULN. Consequently, the prevalence found was 2.08% (294/14071).

**ALT level and age:** Table 2 shows that, depending on the age bracket, the percentage of blood donors with an abnormal level of ALT vary from 2.02 to 2.24%. Statistical analysis using Chi-square ( $\chi^2$ ) test showed no significant differences between the age and the level of ALT ( $p < 0.90$ ).

**ALT level and gender:** Out of the total tested, 71 women (1.94%) and 223 men (2.13%) had an abnormal level of ALT. Chi-square results showed no significant

Table 1: Characteristics of blood donors studied

Factor	No.	%
<b>Sex</b>		
Female	3648	25.90
Male	10423	74.10
<b>Age (years)</b>		
18-30	6087	43.26
31-40	4615	32.80
41-50	2767	19.66
51-60	602	4.28
Total	14071	100.00

Table 2: Characteristics of blood donors with abnormal ALT

Factor	No.	%
<b>Sex</b>		
Female	71	1.94
Male	223	2.13
<b>Age (years)</b>		
18-30	123	2.02
31-40	96	2.08
41-50	62	2.24
51-60	13	2.15
Total	294	2.08

Table 3: Prevalence of hepatitis B and C in the population studied

Sex	Total tested No.	Hepatitis B (+)		Hepatitis C(+)	
		No.	%	No.	%
Male	10423	302	2.89	72	0.69
Female	3648	79	2.16	33	0.90
Total	14071	381	2.70	105	0.74

Table 4: Hepatitis status and ALT

ALT	Hepatitis status	No.	%
Abnormal ALT	HBs-Ag (-) and HCV-Ab (-)	266	90.48
	HBs-Ag (+) and HCV-Ab (-)	8	2.72
	HBs-Ag (-) and HCV-Ab (+)	20	6.80
	HBs-Ag (+) and HCV-Ab (+)	0	0.00
Total		294	100.00
Normal ALT	HBs-Ag (-) and HCV-Ab (-)	13319	96.68
	HBs-Ag (+) and HCV-Ab (-)	373	2.70
	HBs-Ag (-) and HCV-Ab (+)	85	0.62
	HBs-Ag (+) and HCV-Ab (+)	0	0.00
Total		13777	100.00

differences between the gender and the level of ALT at  $p < 0.50$ , as shown in Table 2.

**Hepatitis B and C in the studied sample:** The prevalence of hepatitis C in our sample was 0.74% (105/14071) and the hepatitis B prevalence was 2.70% (381/14071). The proportion of infected female with HCV was a slightly increased compared to infected male, 0.90 and 0.69%, respectively. The opposite scheme was observed when we compared the proportion of blood donors infected with hepatitis B (male more infected than female) (Table 3).

Table 4 showed that in the 294 samples with abnormal level of ALT, hepatitis C was found in 20 subjects (6.80%), at a higher proportion compared to the proportion of 8 donors (2.72%) with hepatitis B. Beside,

Table 5: Gender and status of hepatitis B and C in the 294 blood donors with elevated ALT

	Male No. (%)	Female No. (%)	Total No. (%)
HBS (+)	6 (2.04)	2 (0.68)	8 (2.72)
HCV(+)	15 (5.10)	5 (1.70)	20 (6.80)
Total	21 (7.14)	7 (2.38)	28 (9.52)

Table 6: Etiology for elevated ALT

Etiology	No.	%
Drugs	45	51.72
Diet	21	24.14
Alcoholism	12	13.80
Cause not identified	9	10.34
Total	87	100.00

266 (90.48%) blood donors with abnormal ALT were negative simultaneously for hepatitis B and hepatitis C. On the other hand, the prevalence of blood donors with hepatitis B and normal level of ALT was found in 373 (2.70 %). The percentage of donors with normal level of ALT and hepatitis C was 0.62% (85/13777).

In the group with abnormal level of ALT, 6 (2.04%) were male and were bearing a hepatitis B and 15 (5.10%) had a hepatitis C. On the other hand, 2 (0.68%) of the female had hepatitis B and 5 (1.70%) had hepatitis C. Overall, the proportion of blood donors with serology positive for either hepatitis B or C and an elevated ALT level was 21 (7.14%) and 7 (2.38%) for male and female, respectively (Table 5).

**Follow-up:** The 266 blood donors with elevated level of ALT and were negative for hepatitis B and C, were contacted 6 months after the first analysis for medical follow-up and serological monitoring, to check if there is any eventual persistence of the abnormal ALT level and the hepatitis B and C status.

Out of the 266, only 87 came back for control and fill out the questionnaire. The results found was that drugs was the first cause of elevated ALT in our cohort with 51.72% (45/87). Followed by nature of diet 24.14% (21/87) and alcohol consumption in 13.80 (12/87). For 9 donors (10.34%), we couldn't find any probable explanation (through the exploration of the questionnaires) of the persistent elevation of ALT (Table 6).

**Laboratory assessments:** ALT monitoring for the returned 87 blood donors, revealed that 78 (89.65%) subjects had their ALT level normalized and had kept the same serological status as 6 months earlier (HBs-Ag (-) and HCV-Ab (-). On the other hand, all the other 9 blood donors (10.35%) kept an abnormal ALT level. The hepatitis analysis showed that one of these 9 donors, who used to be negative for hepatitis 6 months earlier, had become HCV(+). The other 8 donors stayed negative for hepatitis B and C.

## DISCUSSION

In this study, we found that the ULN was 64 and 52 in male and women, respectively. The prevalence Abnormal ALT in our population 2.08% (294/14071). The threshold value found was consistent with those found among blood donors from neighbouring countries (Zaier *et al.*, 2002; Ghaffour, 2009), but was bellow what was reported from other countries (Brinkmann *et al.*, 2003; Pourshams *et al.*, 2005).

No statistically difference was found between the ALT level and the age. A slight increase, though, was found in the 41-50 years bracket, in both sexes, compared to the other age brackets. Similar results were observed elsewhere in the age group 40-64 years old (Zhang *et al.*, 2011).

In our population, the frequency of increased ALT by gender was higher in males compared to females (2.13/1.94), even though no statistically difference was found. Similar results have been found in other populations of blood donors (Mohamadnejad *et al.*, 2003; Liu *et al.*, 2005). The reason for the observed difference between male and female blood donors is probably due to a physiological and hormonal status and sexual dimorphism; witch might affect ALT levels more in men than in women.

Analysis of the Table 3 showed that males are somehow more likely than female subjects to acquire hepatitis B during life. In the contrary, we noticed a slight difference when it comes to hepatitis C, This brings us back to say that an influence of gender on the acquisition of an anomaly during the life span of the two sex. These differences are explained by the mode of contamination and various risk factors in both sexes (heterosexual, dental, beauty care, use of injecting drug, etc) (Allard and Parent, 2008). The determination of the level of ALT and the screening of hepatitis B antigens and C antibodies are complementary for the qualification of blood donation. Indeed 90.48% (266/294) of blood donors with elevated ALT level do not possess marker for Hepatitis B nor for Hepatitis C, this might be explained by the existence of other etiology, as drugs, diet habits, alcohol consumption, physical exercise, other viruses, etc. (Valla, 2003). The association between the presence of HBs-Ag or HCV-Ab and abnormal level of ALT was found respectively in 2.72 and 6.80% of our blood donors. On the other hand, donors positive for B or C and normal level of ALT where 2.70 and 0.62%, respectively. Our results demonstrated that the prevalence of hepatitis C is more linked to the level of ALT (6.80% when abnormal ALT, compared to 0.62% when Normal ALT). By opposition, the presence of hepatitis B is independent

to the level of ALT (2.72 and 2.70% for blood donors with abnormal and normal level ALT, respectively). Identical findings were found in the research done by Pan and Zhang (2005) and by Ito *et al.* (2004).

Interpretation of the 87 questionnaires, allowed us to identify the main causes of the abnormal levels of ALT in our hepatitis free population. Hepatotoxic drugs seemed to be the first cause of the elevated ALT level (45/87), as stated by Megarbane *et al.* (2007). Many other studies, in human and in animals, have indicated the elevation of ALT caused by the administration of drugs (Umar *et al.*, 2008; Konca *et al.*, 2009; Mosallanejad *et al.*, 2011).

A close look into the questionnaire results showed that the most intake drugs were antibiotic, followed by vitamin C and paracetamol (data not shown). Despite the benefit of medication, hepatotoxicity is highly probable, especially when there is a coadministration of many drugs at the same time (Jain and Kaplowitz, 2010). The second cause found to be linked to the elevated ALT level was the diet habits (21/87).

Ruhl and Everhart (2003) had documented that diet could be one of the causes of elevated ALT (Assal *et al.*, 2007; Ruhl and Everhart, 2003). In addition, Kechagias *et al.* (2008) had found that high fat and carbohydrates foods could cause inflammation and damage to the liver cells and their membranes. The 3rd cause (12/87) of elevated ALT level found in our cohort was the excess alcohol consumption. As predicted, alcohol intake increases the level of ALT in the blood stream, especially in the heavy drinkers who exceed 80 g of alcohol a day (Ebuehi and Asonye, 2007).

For the other 8 blood donors without any specified cause of elevated ALT level might be, many speculations could explain this abnormality, as the eventual contamination with other viruses CMV, EBV, TT virus, VHGV (Khammassi *et al.*, 2009; Tarrass *et al.*, 2006; Mastouri *et al.*, 2005). Other etiologies could also explain the elevation ALT level, particularly, physical exercise, body mass index, drugs intake.

Many researches around the world were done on the cause of the elevated level of ALT. Morisco *et al.* (2010) had found that high body index was the first cause of the elevated ALT level in Italian blood donors. Beside, Jamali *et al.* (2008a) had found that 79.6% of the population studied in Iran had no obvious aetiology. Liu *et al.* (2005) found that hypertriglyceridemia and hyperuricemia were significantly related to elevated serum ALT levels in Taiwan. Zaier *et al.* (2002) in Tunisia, on the other hand, demonstrated that the main cause of elevated ALT in men was smoking.

The comparison of the results found in this research with studies from different countries showed us that the causes of the increased ALT level vary from one country

to another. This is due to several factors closely related to the studied population, including geographic and demographic variations, age and sex and their life style.

Serological monitoring for the 87 blood donors, who returned 6 months later, showed that most of them (86) kept the same serological status (negative for both hepatitis viruses, B and C). But for one female donor with an abnormal level of ALT and who used to be negative for hepatitis B and C become 6 months later positive for HCV. From a blood banker perspective, this could be as an important finding, to prevent such seroconversion in the blood units produced by blood centres. A deep investigation demonstrated that this blood donor had done some dentistry interventions before donating blood. This surgical intervention could be the responsible for the HCV seroconversion.

Finally, this study showed that even though there is an evident lack of efficiency in using alanine aminotransferase testing qualifying blood donors in our country, preventing viral potential transmission through transfusions was possible.

## CONCLUSION

In conclusion, this paper showed for the first time the upper limit of normal level of ALT in a Moroccan blood donors' population (64 for men and 52 for women), the prevalence found was 2.08%. On the other hand, in virus-free blood donors, we demonstrated that drugs followed by diet were the main causes of elevated ALT in the population studied. Beside, we found that the presence of hepatitis C correlate more with the abnormal level of ALT.

**Funding and resources:** This research had been funded by the Centre National de Transfusion Sanguine, Morocco. A.L., A.A. and R.A. were supported by the Ministry of Health. A.S. was supported by Ibn Tofail University, Kenitra.

## ACKNOWLEDGMENT

First of all, we are very grateful to all blood donors who participated in this study. We also would like to thank Dr Mohamed Benajiba for encouraging the progress of this research. Many thanks to the serology laboratory team (H. Othmani, F.E. El Akkari, B. Adouani and R. El Moussaouiti) for their precious advices and continuous support. A.L. set up and carried out experiments, followed blood donors and wrote the paper; A.A. designed the research; A.S. conducted the analysis of the data; R.A. oriented the study and validated the manuscript.

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