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Elevated Alanine Aminotransferase in Blood Donors: Role of Different Factors and Multiple Viral Infections

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Many different aetiological agents stimulate alanine aminotransferase (ALT) production. Viral markers and other aetiologies were investigated in 2166 individuals, randomly selected from 10 000 consecutive blood donors. Elevation of ALT was found in 10.8% of subjects. Grouping donors according to ALT level and correlating with, respectively, hepatitis B core antibody (HBcAb), cytomegalovirus antibody alone, or associated with HBcAb, showed similar findings (high ALT 11.1%, normal 11.6%; high 85.4%, normal 81.4%; high 10.2%, normal 11.0%, respectively). Hepatitis C virus (HCV) antibody was found to be significantly associated with elevated ALT levels (high 1.7%, normal 0.26%). Other causes of ALT elevation were alcohol abuse (17%), obesity (25%) and dyslipidaemia (38%), but in 11% there was no obvious aetiology. Although HCV is a rare cause of elevated ALT in blood donors, it seems to be the only virus, among those tested, to account for liver damage. This may be due to the non-protective role of HCV antibody, the low specificity of ALT, or the pathogenic role of uninvestigated viruses.

KEY WORDS: Alanine aminotransferase elevation; Blood donors; HCV; HBV; Hepatitis; Viruses

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Alanine aminotransferase and viral infections

INTRODUCTION

Blood donors are excluded from further donation if elevated serum alanine aminotransferase (ALT) concentrations are identified. This is because the donor is thought to be a likely carrier of putative viruses responsible for transfusion-associated hepatitis.[1-4] The introduction of a hepatitis C virus (HCV) enzyme-linked immunosorbent assay (ELISA) for the screening of Italian blood donors[5] has expanded our knowledge of the epidemiology of HCV infection, and has prompted us to analyse the role of HCV and other hepatotropic viruses as risk factors for elevating ALT levels and subsequent liver damage.

The aim of this study was to identify the potential causes of ALT elevation in the normal adult population, focusing on the effect of known hepatitis viruses on the biochemical signs of liver injury.

PATIENTS AND METHODS

PATIENTS

A single-centre, retrospective study was performed to investigate blood samples from 2166 randomly selected donors from 10 000 consecutive blood donations received by the Italian Red Cross in Rome, Italy. A 3-month study duration was chosen to avoid retesting the same donor. Exclusion criteria were those already used by the Italian Red Cross for blood donation: at-risk sexual activity; intravenous drug addiction; male homosexual activity; history of hepatitis or jaundice, syphilis or malaria; use of drugs; alcohol abuse (> 50 g ethanol/day); receipt of blood transfusion, implication in post-transfusion hepatitis; positive for *Treponema pallidum* haemagglutination assay, hepatitis B surface antigen (HBsAg); history of elevated ALT levels; human immunodeficiency virus (HIV) positive; or sex with any person with any of the exclusion criteria.

ENZYME ASSAY

A blood specimen collected from each donor was assayed for ALT within 24 h using an automated spectrophotometric method.[1] Serum was separated by centrifugation (800 ***g***) the morning after blood collection, and stored at 4°C for no longer than 24 h; all assays were performed at the National Blood Transfusion Centre. Normal levels of serum ALT using this method are ≤ 40 IU/l.

VIRAL MARKER ASSAY

Frozen blood samples (stored at –20°C for no longer than 7 days) were tested for: HBsAg (*AUSZYME* Monoclonal, Abbott Diagnostic); hepatitis B core antibody (HBcAb) (*CORZYME*, Abbott Diagnostic); hepatitis B surface antibody (HBsAb) (*AUSAB* enzyme immunoassay, Abbott Diagnostic); HCV antibody (HCV 3.0 ELISA, Ortho Diagnostic; HCV enzyme immunoassay 3.0, Abbott Diagnostic; and Chiron recombinant immunoblot assay [RIBA] HCV 3.0 SIA, Ortho Diagnostic); HIV antibody (HIV-1/-2 enzyme immunoassay Recombinant 3, Abbott Diagnostic); human T-cell lymphotropic virus type I/II (HTLV-I/-II) antibody (HTLV-I/-II ELISA, Diagnostic Biotechnology PTE) and cytomegalovirus (CMV) antibody (CMV AB enzyme immunoassay, Abbott Diagnostic). All blood samples shown to be positive for HCV by ELISA were reassayed with HCV enzyme immunoassay 3.0 (Abbott Diagnostic) and confirmed by RIBA (HCV 3.0 SIA, Ortho Diagnostic).

Serum HCV RNA was assessed by the reverse transcription polymerase chain reaction (RT-PCR) using a commercial kit (HCV Amplicor, Roche).

STATISTICAL ANALYSIS

Donors were divided into two groups according to whether ALT levels were elevated or normal. The two-tailed statistical significance of differences in viral markers between ALT-normal and ALT-elevated subjects was determined using the χ^2 or Fischer's exact test. Student's *t*-test was performed to compare the means of continuous variables.[6]

FOLLOW-UP SUBJECT ASSESSMENT

Donors found to have elevated ALT concentrations were physically evaluated and invited to follow a low-fat, 1500-kcal diet for 1 month. Thereafter, blood samples were obtained and ALT re-evaluated. In cases of persistent ALT elevation, in order to evaluate liver damage other laboratory tests were performed (e.g. aspartate aminotransferase [AST], prothrombin time, serum alkaline phosphatase, creatine phosphokinase, bilirubin, protein electrophoresis, triglycerides, cholesterol, glucose, blood count [red and white blood cells], platelets, serum iron concentration, ferritin, and liver ultrasonography). Lactate dehydrogenase and γ-glutamyl were assayed: no abnormalities were detected. Albumin and globulins were determined by serum electrophoresis.

If ultrasonography revealed abnormal findings, a liver scintigraphy was performed to enable diagnosis of the severity of disease,[7] by the presence of intrahepatic shunt. In order to establish an aetiology, antibodies against other viruses (Epstein-Barr virus [EBV], herpes simplex virus [HSV], parvovirus B19 [PVB19]) and autoantibodies (anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, anti-liver–kidney antibody) were tested.

Liver biopsy was not included in the study protocol, but biopsy was proposed to individuals who were followed up at our Outpatients' Department after completion of the study.

RESULTS

Of the 2166 donors randomly selected (age range 19 – 60 years, mean ± SD age 37 ± 7), approximately half donated blood regularly and all came from central Italy. In 1932 (89.2%), serum ALT concentrations were normal; the remaining 234 (10.8%) had elevated levels.

ALANINE AMINOTRANSFERASE ASSAY

Distribution of ALT concentrations was not normal; the curve (as shown in Fig. 1) was slightly skewed to the right, with a small second peak beyond the normal range (> 40 IU/l). The subdivision of the population into two groups based on the presence or absence of any viral marker demonstrated that a putative second population cannot be identified in this way, as confirmed by comparing the means of the different groups (Fig. 2).

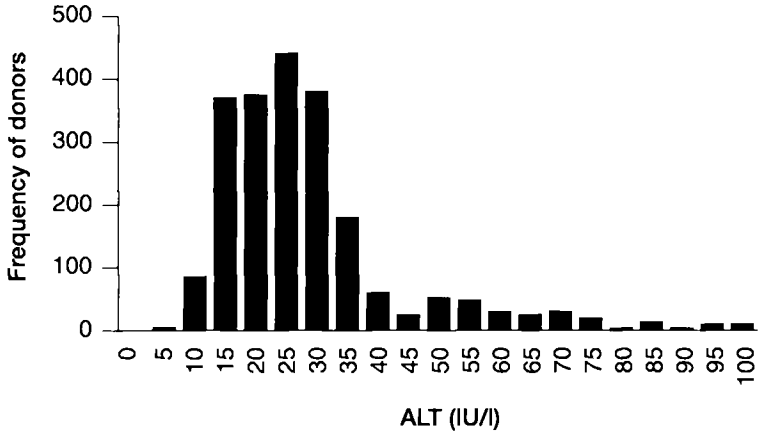
PRESENCE OF VIRAL MARKERS

The respective overall prevalence of HBsAg and HCV antibodies was 4/2166 (0.18%) and 9/2166 (0.42%), whereas HBcAb and HBsAb were found in 250/2166 (11.5%) and 217/2166 (10.0%) subjects, respectively; 215 (9.9%) donors were positive for both HBcAb and HBsAb. Only one donor (0.05%) was HIV antibody positive, and two individuals (0.09%) were reactive to HTLV-I/-II antibody. Cytomegalovirus antibody was found in 1693/2069 donors (81.8%).

VIRAL MARKERS ASSOCIATED WITH ALANINE AMINOTRANSFERASE LEVELS

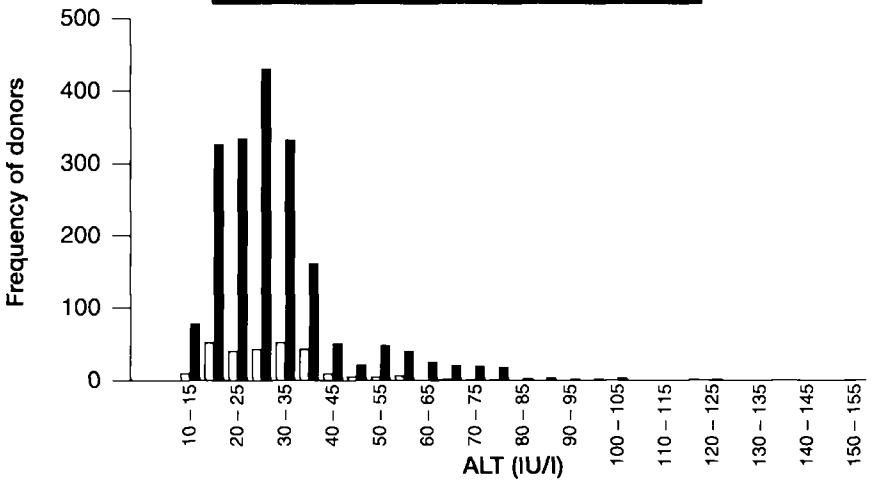
In donors found to be positive for HBcAb, 26/234 (11.1%) had elevated ALT concentrations and 224/1932 (11.6%) donors showed normal ALT (not significant).

FIGURE 1



Distribution of ALT levels in 2166 volunteer donors of the Italian Red Cross Centre in Rome. Normal values for the spectrophotometric method are < 40 IU/l. Mean ALT level ± SD is 26.5 ± 21.5 IU/l.

FIGURE 2



Distribution of ALT levels in donors with (white bars) and without (black bars) signs of HCV and/or HBV (HBcAb) infection. Comparison of the mean between groups does not show a significant difference (27.7 ± 20.7 IU/l versus 26.3 ± 21.6 IU/l).

There was also no significant difference between ALT-elevated and ALT-normal groups in donors positive for HBsAb (922/234 [9.4%] versus 195/1932 [10.1%]) or positive for both HBsAb and HBcAb (22/234 [9.4%] versus 193/1932 [10.0%]). The prevalence of CMV antibodies was similar in individuals with elevated ALT (193/226 [85.4%]) and normal ALT (1500/1843 [81.8%]). Individuals who were HBsAg positive were all in the normal ALT range.

Antibodies to HCV were found in 5/1932 (0.26%) donors with normal ALT; however, in donors with elevated ALT, a significant association with the presence of HCV antibodies was found (4/234 [1.7%], odds ratio 6.7, $P = 0.007$).

The most frequent viral association was hepatitis B virus (HBV) (HBcAb used for identification in assay) with CMV, but even in this case the presence of viral markers was not related to ALT elevation (high ALT, 23/226 [10.2%] versus low ALT, 202/1843 [11.0%]).

Among the 234 donors with elevated ALT, 205 (87.6%) had no serological evidence of HBV or HCV infection, whereas 32/226 (14.2%) were negative for all investigated viruses (CMV included). Elevated ALT concentrations were detected in 29 (12.8%) donors who were HBV and/or HCV positive: 25 were anti-HBcAb positive and HBsAg negative, one was anti-HBcAb positive and anti-HCV positive, whereas three were only anti-HCV positive.

HCV RNA

Of the nine anti-HCV positive donors, HCV RNA was detected in three subjects with elevated ALT, and in one subject with normal ALT ($P > 0.05$). All donors with serum HCV RNA were found to be positive for at least one of the four tested antibodies to HCV recombinant peptides (Table 1). A significant relationship was found ($P < 0.05$).

AETIOLOGY IN FOLLOW-UP PATIENTS

Among the 205 individuals with elevated ALT who were successfully followed up, 74 (36.1%) normalized after 1 month on a low-fat, low-calorie diet, whereas in 131 (64.0%) elevated ALT levels were sustained. Of the 131 subjects with sustained ALT elevation, 51 withdrew from the study.

Among the 80 subjects who continued in the study, one donor each had anti-CMV immunoglobulin class M (IgM), anti-EBV Epstein-Barr nuclear antigen, anti-PVB19 IgM, and high creatinine phosphokinase level. Sixteen individuals (20.0%) had fatty liver, as detected by ultrasonography. In six this was associated with obesity and dyslipidaemia, and was associated with alcohol abuse in three subjects; there was no apparent cause in seven subjects. In 50 subjects (62.5%) hypertriglyceridaemia and/or hypercholesterolaemia was detected, which was associated with hyperglycaemia in two cases. In 13 cases, metabolic abnormalities were associated with obesity only, seven cases were related to alcohol abuse and 30 were isolated findings in obese patients. Ten of 80 donors had no identifiable cause of ALT alteration.

The 76 patients without signs of viral disease and myopathy (the single patient with raised creatine phosphokinase) were analysed according to the predominant clinical feature (Fig. 3). Twenty-five per cent of the patients were obese; more than 25% were above ideal body weight; 38% presented with dyslipidaemia as a major clinical finding; 17% had a medical history of alcohol abuse; 9% had a sole finding of a bright ultrasound pattern, usually associated with fatty liver of unknown origin; in 11% the cause of ALT elevation was of unknown origin.

TABLE 1

Biochemical and serological data of nine donors found to be anti-HCV positive by ELISA. Normal ALT values are ≤ 40 IU/l. 5-1-1, c100-3, c33c and c22-3 are recombinant antigens encoded from the nonstructural region 1 through 4 of HCV genome; the NS5 region encodes a polymerase involved in the replication of HCV RNA. Recombinant immunoblot assay positivity or negativity for the different bands is expressed according to a semiquantitative arbitrary scale

Donor	ALT (IU/l)	ELISA[a]	5-1-1 c100-3	c33c	c22-3	NS5	HCV RNA	HBsAg	HBsAb (μg/ml)	HBcAb	CMV antibody
3031096	208	pos/pos	+++	+	+	+	pos	neg		neg	pos
7016712	45	pos/pos		+			neg	neg		neg	pos
1064067	38	pos/pos	+	+	+	+	pos	neg	571	pos	pos
9108320	22	pos/pos			+		neg	neg		neg	neg
1064529	30	pos/low		+			neg	neg	601	pos	pos
0207818	15	pos/pos		+ – –			neg	neg		neg	pos
5040686	17	pos/pos	+ – –	+ – –	+	+	neg	neg		neg	neg
5040901	76	pos/pos	+	+	+	+	pos	neg	8	pos	
9029333	63	pos/pos	+	+	+	+	pos	neg		neg	pos

[a]As revealed by Abbott immunoenzymatic test and repeated by Ortho immunoenzymatic test.

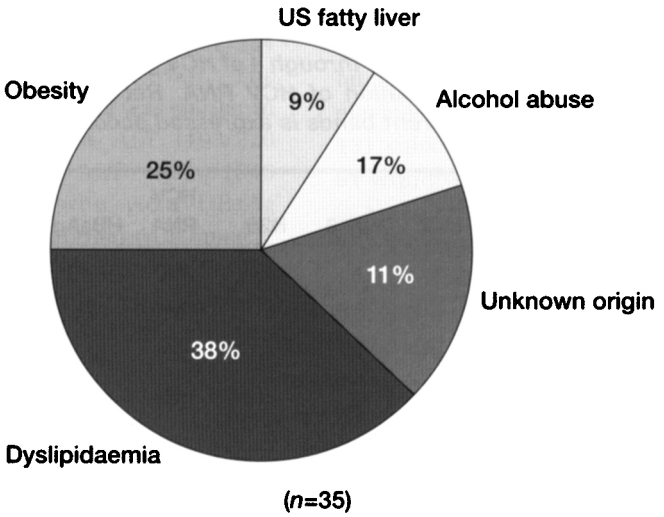
DISCUSSION

In 1983, ALT screening was introduced in international blood banks as a surrogate test for non-A, non-B hepatitis.[8] In the intervening years, which have included the discovery of the principal agent of post-transfusion hepatitis, the test continues to be routinely performed. The present study confirms the importance of ALT, although it is not a consistent marker for infectious hepatitis.

Only a few cases of elevated ALT may be due to HCV (or to HBV or CMV) and there are other factors that can influence ALT levels. Obesity and related diseases (e.g. diabetes, liver steatosis) alcohol or drug abuse, myopathies, autoimmune diseases and metabolic liver diseases may all result in elevated ALT levels.[1,9] Commonly, two-thirds of donors with elevated ALT levels have been found to have an intermittent or persistent ALT elevation, and most were obese and/or were alcohol abusers.

Previous studies reported that in about 20% of individuals with persistently elevated ALT, there was no apparent explanation for the elevation other than non-A, non-B hepatitis.[1] This observation, however, is not consistent with the findings of our study. In the present study, 87.6% of donors with elevated ALT levels were found to have no viruses, other than CMV, and HCV was found in only 1.7% of donors with elevated ALT. Our findings are also inconsistent with Katkov *et al.*,[8] who found anti-HCV antibodies in 17% of donors with elevated ALT levels. Nevertheless, the high prevalence of HCV antibodies in that study may be explained by the lower specificity of the test (second generation RIBA) and by a

FIGURE 3



Causes of alanine aminotransferase elevation in 76 blood donors without signs of viral infections or muscular disorders (one donor with high creatinine phosphokinase level). The chart was drawn according to the predominant clinical feature of the pre-donation medical-examination. A single patient may display more than a single variable as, often, obesity, increased ethanol intake and dyslipidaemia are associated. Nineteen patients were obese, 29 presented with hypercholesterolaemia and high serum triglyceride levels, 13 were alcohol abusers (i.e. an intake > 50 g ethanol/day), seven had a fatty liver without associated risk factor and eight individuals had raised ALT of unknown origin.

possible selection bias (exclusion of HBsAg positive).

A recent study carried out in donors with elevated ALT, and who proved to be anti-HCV negative by ELISA, demonstrated a high prevalence of HBV DNA-positive cases in the absence of HBsAg and HBcAb;[10] this could explain another group of donors with elevated ALT, but who are not HCV-positive.

Finally, other viruses such as EBV, HSV, or PVB19, may be responsible for ALT elevation and liver damage.[11,12] The role of very prevalent viruses with minor effects on the liver, such as CMV and EBV, is of potential importance. The demonstration of a CD8 proliferative memory many years after HBV exposure[13,14] and the association of CMV with HBV, HCV with HBV, PVB19 with HCV, and of multiple viral infections in drug abusers found to result in liver damage, requires re-interpretation of old data.

Obesity and alcohol abuse are frequently associated with ALT elevation, as has been shown in this study. Of the 80 patients who were followed up, 19/80 (23.8%) and 10/80 (12.5%) donors, respectively, were found to be obese or alcohol abusers. In 30/80 (37.5%) donors, dyslipidaemia was associated with ALT

elevation, but there was no other apparent pathological condition (e.g. obesity or alcohol abuse. It is possible that these patients had a fatty liver that was not diagnosed by ultrasonography or scintigraphy, but at biopsy, as found in 64% of cases in another study.[15] The small percentage of alcohol abuse (17%) found in our study could be due to misdiagnosis or to the strict criteria of enrolment used by the Italian Red Cross, and may illustrate that alcohol is not the major cause of chronic liver disease in Italy.

In this study, the prevalence of HCV antibodies detected by ELISA and confirmed by RIBA (0.42%) was lower than that previously reported in blood donors from Italy overall (0.87%), central Italy (0.68%) and Sardinia (1.45%).[5,10] This may be due to a difference in sensitivity and specificity of the previous generation of diagnostic tests and to the different flow-chart system used in diagnosing HCV infection. In our centre, each sample was tested with two commercial kits and confirmed by RIBA. The total number of samples shown to be HCV antibody positive, at least once using the ELISA method only, was 15/2166 (0.69%). This prevalence was similar to what we found in 1993 (271/39768, 0.68%) (National Blood Transfusion Centre databases, Italian Red Cross, unpublished data). In that study, however, only 167 samples (0.42%) were confirmed by RIBA.

In conclusion, the ALT level is a good screening test for post-transfusion hepatitis, but the implication of this increase is often unclear, and the role of risk factors other than HCV and HBV remains to be established. In particular, the co-activation of hepatotropic and common viruses needs to be investigated. There is extensive evidence that HCV, CMV and, probably, HBV remain in the body in a hidden reservoir, although not necessarily in the liver, and that an intervening virus may activate the pathogenic mechanism of a silent one.[16]

Finally, as mentioned above, many non-infectious causes of increased ALT levels exist; therefore, a diagnostic algorithm for the classification of donors and safety in transfusion is necessary.[17] In many cases, however, only hepatic biopsy enables the identification of asymptomatic patients with non-alcoholic fatty liver[18] or metabolic liver diseases.

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