ORIGINAL ARTICLE

Correlation of hepatitis C RNA and serum alanine aminotransferase in hepatitis B and C seronegative healthy blood donors

Natasha Ali, Bushra Moiz, Tariq Moatter[1], Shiraz Ahmed[1], Salman Naseem Adil, Mohammad Khurshid[2]

Sections of Hematology and [1]Molecular Pathology, Department of Pathology and Microbiology, [2] Dean Medical College, the Aga Khan University, Karachi, Pakistan.

Address for correspondence:
Dr. Bushra Moiz, Section of Hematology, Department of Pathology and Microbiology, The Aga Khan University Hospital, P.O Box 3500, Stadium Road, Karachi – 74800, Pakistan. E-mail: bushra.moiz@aku.edu

ABSTRACT

Introduction: Historically, serum alanine transaminase (ALT) has been used as a surrogate marker in the detection of hepatitis viruses in blood donors. With the availability of newer sensitive technologies for the detection of seroconversion, the value of ALT becomes questionable but continues to be used for this purpose with subsequent discarding of ALT elevated blood units. **Objective:** The present study aims to evaluate the significance and cost effectiveness of ALT as a surrogate marker for hepatitis C virus infection in healthy asymptomatic blood donors who were serologically negative. **Materials and Methods:** The study was conducted at clinical laboratory of a tertiary care hospital for a period of one year from November 2006 to October 2007. All donors were screened serologically for hepatitis B, C and HIV I and II, syphilis and malaria and those tested positive were excluded from further evaluation. Gender-wise reference ranges and minimal and markedly raised results for ALT (described respectively as one and two folds increase above reference range) were defined and, accordingly, donors were grouped into three. Two hundred seronegative blood donors were randomly selected from all three groups of ALT results and tested for hepatitis C nucleic acid through Amplicor® HCV RNA test. The cost of discarding an ALT –only elevated blood unit was also assessed. During the study period, 25117 subjects donated blood. Eight hundred and **Results:** seventy two donors (3.4%) were positive for one or more serological tests. ALT of all donors ranged from 0-1501 U/L (Mean ± SD; 33.4 ± 25.45U/L). The donors seronegative for all disease markers were 24245 (96.6%). Of these, 21164 (87.2%) donors had their ALT within reference range while 2874 (11.8%) and 207 (0.8%) of donors had minimal and markedly elevated results. Thus, 621 blood bags (red cells, platelets and plasma) costing $ 39200.0 were discarded based on ALT results alone. Of 200 seronegative donors evaluated for hepatitis C nucleic acid, only one within markedly elevated ALT levels was found to be positive. The present work did not support a positive association between hepatitis C virus nucleic acid and elevated ALT in healthy serologically negative blood donors. **Conclusion:** We did not find serum ALT testing in donors as cost effective strategy for detection of hepatitis C virus ribonucleic acid. As the number of samples tested by us was small we suggest further work to evaluate the value of ALT levels in serologically negative donors in association with hepatitis C antigen and NAT testing to elucidate the true burden of disease in geographical regions where hepatitis C is endemic and voluntary blood donation is sparse.

KEY WORDS: Hepatitis C, serum alanine aminotransferase, HCV RNA

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INTRODUCTION

Serum Alanine Amino Transferase (ALT) is the most frequently utilized screening test in routine evaluation of liver damage.[1] However, ALT has a low specificity for diagnosis of hepatic diseases since it might be raised in a number of conditions not related to liver such as obesity, alcohol consumption, hypercholesterolemia, hypertriglyceridemia, drugs, hemochromatosis and alpha-1 antitrypsin deficiency.[2-6]

Historically, ALT has been used as a surrogate marker for screening of hepatitis C infections in blood donors.[7] Subsequently, with the introduction of second or third generation ELISA and chemi-lumiscence techniques for the detection of hepatitis C antibodies, there was a marked reduction in the incidence of transfusion induced hepatitis C in the recipients of blood.[8] This led to reconsideration of the value of testing donated blood for elevated aminotransferase. Currently, American association of blood banks (AABB) and FDA do not require ALT to be tested in blood donation.[9] Moreover, the serological gap or window period for this infection has further closed down with introduction of hepatitis C antigen and NAT testing.[10,11] However, many countries continue to use this test as a mandatory part of screening blood with subsequent discarding of all such donations with elevated ALT.[12] This practice has changed since the introduction of better screening

procedures but in Pakistan ALT is still being used as a surrogate marker for testing blood donors. Moreover, donors with increased catalytic activity of ALT are excluded from future blood donations, even if their anti Hepatitis C virus status is non reactive.[13]

There is a need to re-look the value of ALT in screening blood products in geographical locations where most of the donations are non-voluntary with an upsurge tendency in incidence of hepatitis C. Pakistan observes only 25% of donations as voluntary according to WHO report but individual local data represent even lower rate of 7.1%.[14] The low rate of voluntary donations coupled with an increasing incidence of hepatitis C in healthy blood donors in Pakistan ranging from 1.1 to1.8 % is alarming.[15, 16]

Under these conditions it is expected that a number of asymptomatic donors would be suffering from early hepatitis C at the time of blood donation. Failure to diagnose early infection may be secondary to undetectable levels of hepatitis C virus antibodies during initial phase of infection. This is further compounded by different sensitivities and specificities of various commercially available reagents which are being used for detection of anti-HCV.[17] Secondly, polymerase chain reaction which can be successfully employed for recognition of hepatitis C virus ribonucleic acid is neither practical nor cost effective for developing countries. Because of these obstacles, ALT testing may serve as a probable indicator of continuous viral replication reflecting a possible chronic hepatic involvement as well as potential infectivity.[18] Hence, we hypothesized that serum ALT levels might indicate an early hepatitis C infection in an asymptomatic blood donor lacking seroconversion. ALT test costs $2.5 and expenses of discarding three blood products (red cells, platelets and plasma) from a donor on the isolated basis of elevated ALT is approximately $ 63 in our clinical laboratory.

The study aims to correlate the association of hepatitis C virus ribonucleic acid with serum alanine transaminase in healthy seronegative blood donors and to evaluate the cost effectiveness of ALT testing as a surrogate marker for hepatitis C virus infection. It hoped to clarify the stigma involved with elevated ALT in an otherwise healthy blood donor.

MATERIALS AND METHODS

After approval from institutional ethical review committee, we enrolled healthy non commercial blood donors between the ages of 17-65 years (according to Sindh blood transfusion authority) with their informed consents. The study was conducted prospectively at clinical laboratory of our hospital during a period of one year from November 2006 to October 2007. The donors were scanned to assess suitability for donation using an in house questionnaire. We collected 5ml of blood in gel tube (BD vacutainer) after collection of blood bag from each donor. Tubes were centrifuged for 5 minutes at 2500g. Separated sera were transferred to eppendorf tubes and transported to molecular research lab of our university on the same day where samples were kept frozen at -80°C till their testing within 6 weeks. All donations were tested simultaneously in our blood bank for mandatory screening tests (anti- HIV I and II, anti HCV, HBsAg, syphilis and malaria). Viral serology was performed via third generation CA (Vitros Eci, Johnson and Johnson, Ortho Clinical and Diagnostic, NY, USA) while VDRL (VDRL Carbon antigen Plasmatec Laboratory products – RPR kit) and ICT malaria tests (Now malaria® Binax incorporated, USA) were done manually for screening syphilis and malaria respectively. No confirmatory test was done on a serologically positive sample because of financial issues. However, each donor was communicated through phone advising him for further testing for confirmation of his reactive serology results. Each donation was also tested for serum ALT by kinetic method at a reaction wavelength of 340nm at a temperature of 25°C (Beckman Synchron® CX7, USA). As per institutional policy, all such blood bags which were reactive for one or more disease markers and or two times the upper limit of ALT were incinerated.

ALT levels of all donors were evaluated with respect to age and gender. Accordingly, the donors were grouped into three: A: donors within reference range, B: minimally elevated and group C with markedly raised levels. Reference ranges for ALT established in our lab were 3-33U/L for females and 0-55U/L for males. Markedly elevated ALT was defined as results greater than twice the upper limit of reference range (≥110 U/l for males and ≥66 U/l for females). ALT within these two limits was considered as minimal elevations (56-109 U/l for males and 34-65 U/l for females).

Within these three groups, 200 seronegative blood donors were randomly selected and tested for hepatitis C virus ribonucleic acid utilizing AMPLICOR® HCV test (version 2.0 New Jersey, United States of America) which has an analytic sensitivity of 50 IU/ml. The test was used according to manufacturer's instructions: Briefly, it is a qualitative *in-vitro* diagnostic test which permits simultaneous transcription and polymerase chain reaction amplification of hepatitis C virus and internal control ribonucleic acid. The detection of amplified deoxyribonucleic acid was performed using target specific oligonucleotides (KY78 and KY80) to define a sequence of 244 nucleotides within the highly conserved 5'UTR of the hepatitis C virus genome. All tests were run in duplicate including positive and negative controls in each batch and the test was completed within a single working day. The samples were read calorimetrically at 450nm and ribonucleic acid virus was detected if absorbance was >1.0. A value of less than 0.3 was considered negative. Intermediate values (0.3-0.8) were taken as inconclusive.

Statistical Analysis

Descriptive analysis was computed with SPSS version 16.0 statistical software (SPSS, Chicago, IL, USA) for various variables. ALT and anti-HCV results were compared through bivariate analysis (Kendall's correlation test) and the level of significance was 0.01.

RESULTS

During the study period, 25117 subjects donated blood. There

Table 1: Demographics, biochemical and seropostivity of donors with respect to donor type and gender (*n* = 25117)

Donor type	*n (%)*	*Age (Y) Mean ±SD*	*HBsAg n (%)*	*Anti-HCV n (%)*	*Anti-HIV n (%)*	*VDRL n (%)*	*Malaria n (%)*	*ALT >2 X reference range*
Exchange	23248 (92.5)	26±7	331 (1.4)	442 (1.7)	20 (0.1)	87 (0.3)	3 (0.01)	212 (0.9)
Voluntary	1869 (7.4)	30±11	12 (0.6)	16 (0.8)	5 (0.3)	0	0	30 (1.6)
Total	25117(100)	26±83	343 (1.3)	458 (1.8)	25 (0.1)	87 (0.3)	3 (0.01)	242(0.9)

were 23685 (94.2%) males and 1432 (5.6%) females with age ranging from 17 to 65 years (median ± SD 27.7 ± 7.621). The exchange or replacement donors were 23248 (92.5%) while 1869 (7.4%) were voluntary donations. Interestingly, only 3.6% females donated blood as replacement donors in sharp contrast to 31.1% as voluntary contributors. There were 872 donors (3.4%) who had one or more abnormal screening results. A total of 908 abnormal tests were detected [Table 1]. This grid shows that proportions of donors with reactive tests were more in exchange vs. voluntary donations excepting anti-HIV Test.

About 86.7% donors (n = 21795 had ALT within reference range while minimal and markedly elevated ALT were seen in 12.2% (n = 3077) and 0.9% (n = 245) subjects. Slight and strong ALT elevations were more frequent in females (16% and 3.49%) then men (12.08% and 0.82%) with a significant P - value of 0.000 (data not shown). Elevated ALT results were more frequent in males aging 27-46 years. A decrease in this tendency was noted in younger (<26 years) and older (>47 years) group. Such a uniform pattern was not seen in females. Interestingly, within each age group, more proportion of females had elevated ALT than males except age group of 27-36 years (data not shown).

Only minority of the donors with elevated ALT levels was positive for anti HCV [Table 2]. The anti hepatitis C virus reactivity was almost four fold increased with midrange ALT and 13 times with markedly elevated ALT levels. Statistical analysis using Kendall's 2- tailed correlation was applied on ALT levels and anti-HCV results of all donors. The results showed lack of significant correlation between the two variables (P - *value*=0.000). However, proportion of hepatitis C virus seroconversion was highest among donors with normal ALT (63.3%) compared to minimal (29.7%) and marked elevated ALT levels (6.9%). Based on these results, specificity of ALT for positive hepatitis C virus serology can neither be supported nor refuted as hepatitis C virus ribonucleic acid was not evaluated in any of the donors showing seroconversion.

Table 2: Association of anti-HCV positivity with various ALT ranges in 25101 donors

ALT status	*Anti-HCV results*			
	Negative	*Positive (%)*	*Total*	*P value*
Normal	21494	290 (1.33)	21784	0.000
Minimally elevated	2939	136 (4.42)	3075	
Markedly elevated	210	32 (13.22)	242	
Total	24643	458 (1.85)	25101	

We found 24245 (96.5%) donors as seronegative for all disease markers [Figure 1]. Of these, ALT ranged from 0-1501 U/L (mean ± SD; 33.4 ± 25.45U/L). This shows that of 24245 donors, 21164 (87.2%) donors had their ALT within reference range while 2874 (11.8%) and 207(0.8%) of donors had respectively once and twice or more the reference range of ALT. We discarded all blood bags with levels of ALT twice the reference range. Thus 621 blood products (including red cells, plasma and platelets from 207 blood donors) costing $ 39200.0 were discarded based on ALT results alone.

Polymerase chain reaction for detection of hepatitis C virus ribonucleic acid was performed in 200 randomly selected seronegative donors from groups A, B and C [Figure 1]. This depicts that out of 200 seronegative blood donors, one subject in group C was positive for HCV RNA [Table 3]. This subject was a 34-year-old-male with serum ALT of 191U/L. His repeat sample also tested positive for hepatitis C virus nucleic acid. We did not perform viral load of hepatitis C virus ribonucleic acid because of financial restrains or hepatitis C antigen for the unavailability of the test. The subject was contacted through phone and informed of his hepatitis C virus status and was further advised for a hepatologist consultation.

DISCUSSION

The significance of routine ALT testing for prevention of post transfusion hepatitis C in voluntary blood donors has been denied.[19] However, the same cannot be applicable to regions where most of the blood units collected are from exchange or first-time donors. In developing countries, ALT testing continues to be employed as a surrogate marker for hepatitis C virus infection based on the assumption that recent hepatitis C virus infection can be detected earlier by ALT than by anti- HCV. The

Table 3: Association of HCV RNA with ALT levels in 200 seronegative donors

Groups	*N*	*Gender*	*Age Y (mean ± SD)*	*Serum ALT U/L range (mean ± SD)*	*HCV PCR results*
A	88	79 males	29.4 ± 8.2	10-55 (27.30 ± 10.631)	Non-reactive
		9 females	32.7 ± 9.9	11-33 (21.56 ± 7.020)	Non-reactive
B	12	9 males	27.0 ± 6.5	60-97 (76.67 ± 12.796)	Non-reactive
		3 females	29.7 ± 5.0	35-44 (39.67 ± 4.509)	Non-reactive
C	100	98 males	28.5 ± 7.2	100-245 (146.80 ± 32.076)	1 reactive
		2 females	23, 28	110,122	Non-reactive

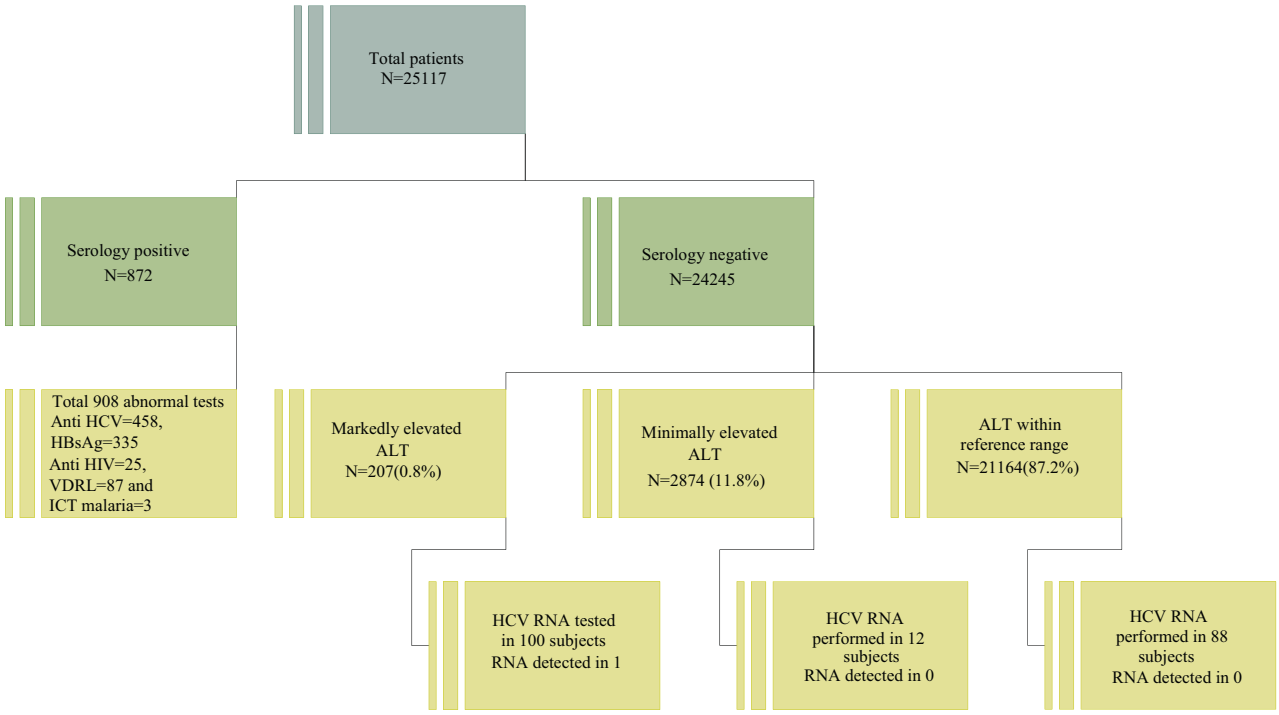

Figure 1: Flow sheet for subjects enrolled in the study

serological screening test, like chemi-lumiscence, may miss a hepatitis C virus infected unit during approximately 5-6 weeks of seroconversion period before hepatitis C virus antibodies are detected.[20] The introduction of newer tests like hepatitis C virus antigen and NAT testing have reportedly shortened the window period to a mean of 35 days and 7-14 days respectively.[10]

The study aims to evaluate the proportion of seronegative ALT elevated donor units with polymerase chain reaction detectable hepatitis C virus nucleic acid. Blood units with ALT within reference range serving as controls for the study were similarly assessed. The results were used to estimate the solo value of ALT with current hepatitis C virus assays for minimizing post transfusion hepatitis C. In principle we should have tested all 207 subjects with markedly elevated ALT for hepatitis C virus nucleic acid, however, our finances restrict us from this maneuver.

WHO data shows that only 25% of blood donations in Pakistan are voluntary. Our data also testified the same fact with majority of blood donors being replacement donors Results of positive HBsAg and anti HCV rates at our blood bank are generally comparable with the prevalence rate of the same in Pakistani blood donor population.[21] Hepatitis C virus seroconversion was found to be highest (1.8%) followed by seropostivity for HBsAg (1.3%) with increase frequency in exchange compared to voluntary donors. Similar results have been witnessed by others as well.[22] The frequency of seroconversion for HIV, syphilis and malaria appears to be low.

Majority of the donors (87%) had ALT within reference range while approximately 12% and > 1% had minimal and significantly elevated ALT respectively. We observed greater number of hepatitis C virus seroconverted subjects with markedly elevated than with normal ALT levels [Table 2]. But the proportion of hepatitis C virus positive donors was highest with subjects having normal ALT. This poor correlation of ALT with seroconversion for hepatitis C virus may be due to fact that many of the reactive test results among donors with normal ALT could be false positive. We at present are unable to comment on this issue as no further confirmatory tests were carried on donors with positive serology. It is noticeable that poor correlation of ALT with anti HCV has been reported before.[23]

Since 1985, our clinical laboratories were discarding blood units with strongly elevated ALT. With the availability of polymerase chain reaction technologies, it became desirable to evaluate the true value of ALT in identifying hepatitis C virus in our setting where most of the blood units are replacement or one time donations. Of 200 blood bags that tested negative for any disease marker one polymerase chain reaction positive unit was identified among blood bags with elevated ALT levels. Similarly, Notari EP *et al.*[24] found hepatitis C virus ribonucleic acid in none of his 166 blood donors evaluated with a cut-off of ALT>120 IU/L.

The minimal limit of detection of polymerase chain reaction assay is 50 IU/ml.[25] This demonstrates that viral load in the single subject positive for hepatitis C virus ribonucleic acid (in group C) must be above this limit. It is quite possible that we failed

to depict viremia in blood samples with low viral copies and the actual number of donors at risk for transmitting infection is probably more than what was assessed by us. Moreover, hepatitis C virus ribonucleic acid can also be expected in the other two ALT groups (A and B) because of the same reason.

At our institute, more than 25,000 donors are bled annually. Currently, NAT testing for donor screening is not being employed in our blood bank on routine basis. The situation is grimmer in community based centers or city's outskirts where screening kits may not be available even for serological testing.[26] Considering the high number of blood units with markedly elevated ALT levels (207 or 0.8% of 24245 units), ALT testing does not seem to be the best strategy because only one out of 207 (0.4%) samples seem to be infectious. The cost of losing to so many donations was $ 39,000. Hence the cost effectiveness of ALT testing in detection of hepatitis C infection in window phase is doubtful. We need to consider other sensitive assays like hepatitis C virus antigen or mini-pool NAT testing to reduce the diagnostic gap without losing to so many non infectious blood donations.[10, 11, 27, 28]

Limitations of the Study

The study was fraught with several limitations mainly because of financial restrains.

Firstly, total number of samples included in the study was small and we did not investigate all 207 samples with elevated ALT levels (over two times of the reference level) for hepatitis C virus ribonucleic acid. Secondly, the donors with elevated ALT levels and negative results for hepatitis C virus nucleic acid were not tested for other viruses like CMV or EBV to exclude other reasons for elevated ALT. Moreover, the single donor who was polymerase chain reaction positive was not investigated further for viral load.

In developing counties like ours, donors' serological results are not confirmed through alternate tests and since number of voluntary donations are small; the residual risk of transmission of hepatitis C virus from seronegative donors cannot be computed. It is therefore difficult to estimate level of window phase hepatitis C virus infections in our donor population.

CONCLUSIONS

Although further studies on a large scale are required to prove the relationship of an elevated ALT with viral hepatitis C, our preliminary results failed to show any correlation between these two variables. In developing countries where there is low turnout for voluntary donations and high incidence of hepatitis C, it is important that longitudinal data retained on repeat donors be evaluated to give insight into the frequency of seroconversion to anti HCV in our donor population.

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