



Alanine Aminotransferase Levels in Nile Delta Citizens Infected with Chronic Hepatitis C Virus

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ABSTRACT

Approximately 30% of patients with chronic hepatitis C have persistently normal serum alanine aminotransferase (ALT) levels. In most of the previous studies, the follow-up period of ALT levels did not exceed 12 months. Our objective was to redefine the terms persistently normal alanine aminotransferase (PNALT), persistently elevated ALT (PEALT), and fluctuating ALT (FLUXALT) and to determine the proportion of each group among Nile Delta patients. 348 patients infected with chronic hepatitis C were included, HCV infection was proved by a polymerase chain reaction. We studied an average of 19 ALT measurements for each participant between 2004 and 2007 with 2-month interval between successive measurements. We defined a patient as having PNALT, PEALT, or FLUXALT when all the 19 ALT levels were normal (<40 IU/L), elevated (>40 IU/L), or did not fit either of the above two categories, respectively, during the 36-month follow-up period. 73 patients had PNALT, 157 had PEALT, and 118 had FLUXALT ($P<0.001$). There were no significant differences regarding age, sex and body mass index. Patients with PNALT were more likely to have an Ishak fibrosis scores of < 2 while those with PEALT of 2 to 6, the third group with FLUXALT of 1 to 5 ($P<0.001$). 21% of patients infected with chronic hepatitis C had PNALT, and 45% had PEALT, while 34% had FLUXALT. PEALT were significantly more likely to have higher degrees of fibrosis than FLUXALT patients and consequently both groups had higher fibrosis scores than PNALT patients.

Key words: HCV, ALT, PCR, Ishak fibrosis score

Kronik Hepatit C Virüs Enfeksiyonlu Nil Delta Vatandaşlarında Alanin Aminotransferaz Düzeyleri

ÖZET

Kronik hepatit C hastalarının yaklaşık% 30'u devamlı normal serum alanin aminotransferaz (ALT) seviyelerine sahiptir. Geçmiş çalışmaların çoğunda, ALT düzeylerinin takip süresi 12 ay geçmemiştir. Amacımız açısından sürekli normal alanin aminotransferaz (PNALT), sürekli yüksek ALT (PEALT) ve dalgalı ALT (FLUXALT)'yi yeniden tanımlamak ve Nil Deltası hastalarda her grubun sıklığını belirlemektir. Kronik hepatit C ile enfekte 348 hasta dahil edildi, HCV enfeksiyonu polimeraz zincir reaksiyonu ile kanıtlandı. 2004-2007 yılları arasında 2 aylık aralıklı ardışık ölçümlerle, her katılımcının ortalama 19 ALT ölçümünü çalıştık. Hastaları 36 aylık takip döneminde 19 ALT ölçümünün hepsi normal olanlar (<40 IU/L), artanlar (>40 IU/L), veya bu iki kategoriye uymayanlar olarak PNALT, PEALT ve FLUXALT olarak tanımlandılar. 73 hastanın PNALT, 157'nin PEALT ve 118'in FLUXALT vardı ($p < 0.001$). Yaş, cinsiyet ve vücut kitle indeksi açısından anlamlı fark saptanmadı. PNALT hastalar ikinin altında bir Ishak fibrozis skorları olması daha muhtemel iken, PEALT'lıların 2 ila 6, FLUXALT'lı üçüncü grubun 1 ile 5 arasındaydı ($p < 0.001$). Kronik hepatit C ile enfekte hastaların% 21 PNALT vardı ve FLUXALT % 34, PEALT % 45 di. PEALT'ın yüksek derecede fibrozis olma ihtimalleri FLUXALT hastalarından anlamlı daha fazlaydı ve sonuç olarak her iki grupta PNALT hastalarından daha yüksek fibrozis skorlarına sahipti.

Anahtar kelimeler: HCV, ALT, PCR, ishak fibrozis skoru

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INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with about 170 million people infected worldwide. Most (70-80%) HCV infections persist after inoculation and about 30% of individuals with persistent infection develop chronic liver disease, making this disease a significant cause of morbidity and mortality (1). The severity of disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (2). It has been estimated that approximately one-third of patients with chronic HCV infection have persistently normal alanine aminotransferase (PNALT) levels, however, there is no precise definition of the normal ALT till now (3). The 2002 National Institutes of Health Consensus Conference on HCV did not define accurately what constitutes a "normal" ALT level (4). Many investigators have defined PNALT as > three normal ALT measurements over a 6-12-month period of time in persons infected with HCV. But, in studies with short-time duration of follow-up with small number of patients, there may be false higher percentages of patients with PNALT or those with persistently elevated ALT (PEALT) subtracted from the percentage of patients with fluctuating ALT (FLUXALT) (5-8). Until recently, the benefits of antiviral therapy of HCV-infected patients with persistently normal serum alanine aminotransferase (ALT) levels were of uncertain value and should not be undertaken routinely (9). The aims of this study were: first, to compare demographic data as well as viral load between patients with PNALT, PEALT and FLUXALT, second, to correlate mean ALT level with the histopathological changes, especially, in PNALT group patients, as well as PEALT and FLUXALT groups, and third, to do a follow-up of patients with a greater number of ALT measurements throughout a longer duration in a trial to redefine the terms PNALT, PEALT, and FLUXALT.

MATERIAL AND METHODS

HCV screening

Mansoura University Hospital laboratories provide free testing for anti-HCV to persons attending all Mansoura University hospitals and clinics from Nile Delta governorates. All patients positive for anti-HCV performed by enzyme-linked immunosorbent assay (ELISA), and confirmed by a positive HCV RNA by polymerase chain reaction (PCR) assay were enrolled in this study.

Upon enrollment, a patient medical history, physical ex-

amination, and laboratory evaluations were performed on each participant. Exclusion criteria were the treatment for HCV infection during the 36-month follow-up period and the positivity for hepatitis B surface antigen. At the end of the follow-up period, and because of the availability of the governmental support for free antiviral therapy, participants who met the criteria outlined by "The Egyptian Committee for the Control of Viral Hepatitis" were offered antiviral therapy.

Laboratory testing

ALT testing was performed by automated biochemistry analyzer (Cobas Integra 400, Roche diagnostics). A normal ALT level was defined as < 40 IU/L. Testing for anti-HCV was performed by ELISA using Sorine Biomedica kits (Sorine Biomedica Diagnostics Division, Italy) (10). Persons with an anti-HCV antibody-positive specimens had further testing for the detection of HCV-RNA by qualitative polymerase chain reaction (PCR), Cobas amplicore, Roche Diagnostics.

Histopathological examination

All patients were subjected to a valid liver biopsy at the time of inclusion. All liver biopsies were reviewed by one experienced hepatopathologist who was blinded to the clinical and laboratory data. Biopsies were scored for fibrosis using the Knodell scoring system and the modified scoring system of Ishak (11).

Ethical approval

A written informed consent was obtained from each participant. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by the approval of the "Mansoura University Ethical Committee".

Study population

In this study we tried to put a new identification for PNALT, PEALT, and FLUXALT. Between June 1, 2004 and December 31, 2004, 450 patients were enrolled, with a follow-up period of thirty six 36 months. At the end of follow-up period, 348 persons had a full data, (102 persons were lost to follow-up). ALT level measurement was done at a time interval of 2 months during the three 3-year follow-up period. During the three year follow-up period, patients were classified as having PNALT if all 19 ALT levels were normal (<40 IU/L), PEALT if all 19 ALT levels were elevated (>40 IU/L), or FLUXALT for persons who did not fit into either of the above two categories.

Table 1. Baseline characteristics of study population

Characteristic	Group 1 (n:73)	Group 2 (n:157)	Group 3 (n:118)	p value
Age at consent, years	41.8±1.8	42.5±2	41.39±2.1	0.13
Male sex, n (%)	35 (48)	97 (62)	61 (52)	0.1 [#]
BMI, kg/m ²	28.5 + 2.8	29.12 + 1.7	28.9 + 3.2	0.43

- All values are expressed as the mean + standard deviation unless otherwise stated. The test used is one way Anova. Abbreviation: BMI, body mass index.

- [#]P values were calculated with Chi-square (x2) test. P value is considered significant if < 0.05.

Statistical analysis

The statistical analysis of data done by using excel program and SPSS program statistical package for social science version 10. To test the normality of data distribution, K-S (Kolmogorov-Smirnov) test was done only significant data revealed to be nonparametric. N.B: all tested data revealed to be parametric. The description of the data done in form of mean (+/-) SD for quantitative data. The analysis of the data was done to test statistical significant difference between groups. One way Anova test to compare more than two groups, followed by Post Hoc test LSD (least significant difference) for inter groups comparisons, if subtraction between two groups mean gives > LSD so there is a statistically significant difference between these two groups. For quantitative data, student's t-test was used to compare between two groups. Chi square test was used for qualitative data. Correlation coefficient was done to detect association between variables. N.B: P is significant if ≤ 0.05 at a confidence interval of 95% (12).

RESULTS

Patient population

Starting from June 1, 2004 and throughout seven months till December 31, 2004, 450 Egyptian patients from Nile Delta region with HCV RNA - proved HCV infection were enrolled in this study, with a follow-up period of thirty six 36 months. At the end of follow-up period, 348 patients had a full data.

Demographic data

There were no significant differences among the three groups with regard to age, sex and body mass index (Table 1).

ALT levels in the three groups

We found that of those HCV RNA-positive 348 patients with 19 ALT measurements available during the follow-up period; 73 (21%) had PNALT, 157 (45%) had PEALT, and 118 (34%) had FLUXALT (p< 0.001, Table 2).

Table 2. Comparisons of level of ALT, level of viremia and Ishak fibrosis score among the three groups.

Characteristic	Group 1 (n:73)	Group 2 (n:157)	Group3 (n:118)	p value
ALT, IU/L	26.23±3.88	81.42±9.6	55.88±7.3	<0.001 ^{##}
Level of viremia, n (%)				
Mild	22 (30.1)	49 (31.2)	33 (28)	
Moderate	22 (30.1)	51 (32.5)	36 (30.5)	0.930 [#]
High	29 (39.8)	57 (36.3)	49 (41.5)	
Ishak fibrosis score, n (%)				
0/6	25 (34.2)	NA	NA	
1/6	31 (42.5)	NA	5 (4.2)	
2/6	17 (23.3)	3 (1.9)	41 (34.7)	
3/6	NA	10 (6.4)	54 (45.8)	< 0.001 [#]
4/6	NA	50 (31.8)	14 (11.9)	
5/6	NA	63 (40.1)	4 (3.4)	
6/6	NA	31 (19.7)	NA	

- All values are expressed as the mean + standard deviation unless otherwise stated. Mild viremia, less than 100.000 IU/ml; moderate viremia, between 100.000 till less than 800.000 IU/ml; high viremia, 800.000 IU/ml or more. Abbreviations: ALT, alanine aminotransferase; IU/L, international unit per liter; BMI, body mass index; NA, not available.

- ^{##}LSD (least significant difference) : 25.53.

- [#]P values were calculated with Chi-square (x2) test. P value is considered significant if < 0.05.

Table 3. Correlation between level of viremia and Ishak fibrosis score

	Group 1 (n:73)	Group 2 (n:157)	Group 3 (n:118)	Total
<i>r</i>	0.001	0.045	0.121	0.007
<i>P</i>	0.99	0.57	0.194	0.89

-Spearman correlation co-efficiency is used. There is a non significant positive correlation between the level of viremia and Ishak fibrosis score.

- *P* value is considered significant if < 0.05

The relationship between ALT and the level of viremia

Also it was shown that no statistically significant relationship was detected among the three groups as regards HCV RNA level of viremia ($p: 0.930$, Table 2).

The relationship between ALT and Ishak fibrosis score

Liver biopsies were performed on all patients in this study including those of PNALT group.

Patients with PNALT were more likely to have an Ishak fibrosis scores of < 2 while those with PEALT of 2 to 6, the third group with FLUXALT of 1 to 5 ($p < 0.001$, Table 2, Figure 1, 2 and 3).

The relationship between the level of viremia and Ishak fibrosis score

There was a non significant positive correlation between the level of viremia and Ishak fibrosis score among the three groups ($p: 0.89$, Table 3).

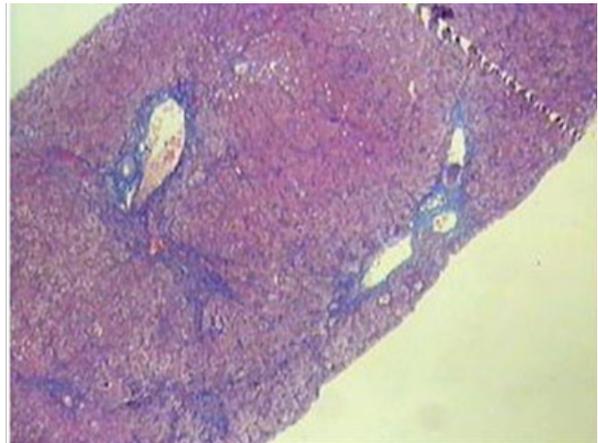


Figure 1. Ishak 2; portal tract expansion by fibrosis (Masson trichrome x100).

DISCUSSION

The upper limit of normal for serum alanine aminotransferase (ALT) has long been considered to be 40 IU/L regardless of sex or body mass index (BMI) (13). To our knowledge, this is one of few prospective studies that were carried out to study the level of ALT among HCV-infected individuals. We found that 21% of Egyptian patients from Nile Delta region with chronic HCV infection had PNALT. Several works have reported that PNALT patients constituted around 30% of HCV cases (14-16).

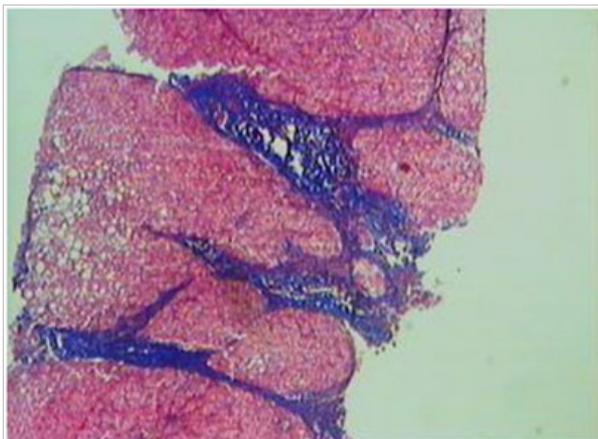


Figure 2. Ishak 4; marked portal-portal bridging fibrosis (Masson trichrome x100).

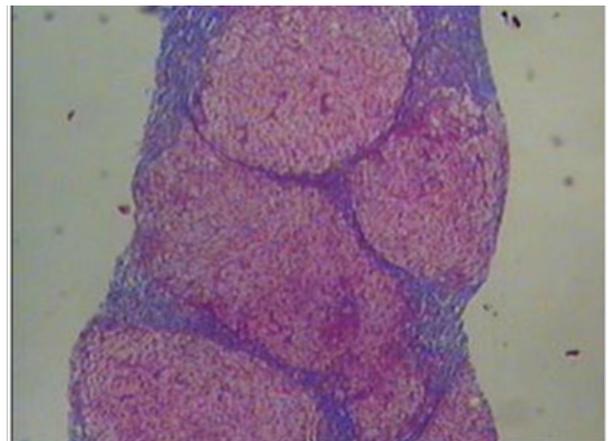


Figure 3. Ishak 6; loss of normal architecture with regenerating nodules surrounded by fibrous tissue (Masson trichrome x100).

This overestimation of the proportion of HCV-infected individuals may be at the expense of patients with fluctuating levels of ALT (FLUXALT). A salient aspect of our study is the adoption of 19 ALT measurements taken 2 month apart over a 3-year period, this, to our opinion, has minimized or even nullified any overestimation. Also we reported that 34% of patients with chronic HCV had fluctuating levels of ALT. We suggest that the previous studies, with fewer ALT measurements and shorter follow-up time, may added a proportion of FLUXALT cases to PNALT, this may cause false sense of disease stability over a short period of time such as 3-6 months with negligence of regular follow-up which may carry the hazard of disease progression with loss of the chance of intake of a specific antiviral therapy in this sector of FLUXALT individuals who were considered as PNALT. On the other hand, there may be an overestimation from another point of view, as we considered the upper limit of normal for serum alanine aminotransferase (ALT) to be 40 IU/L, while there are two studies, Italian and Korean, which suggested that healthy serum ALT values should be adjusted to 30 IU/L for men and 19 IU/L for women (13,17). The 40 IU/L concentration was determined principally from studies performed before the introduction of anti-hepatitis C virus (HCV) testing, and prior to development of the concept of nonalcoholic fatty liver disease (NAFLD) (18,19). So, we recommend a large population-based study that considers age, sex, body mass index (BMI), nonalcoholic fatty liver disease (NAFLD), and other metabolic parameters for the assessment of serum ALT concentrations in healthy Egyptian individuals and to determine the factors affecting ALT levels in these populations.

We found no statistically significant predictive value of age, sex or body mass index, but we found that a higher proportion of HCV-infected patients with PNALT were females, a finding supported by previous studies (6,20,21). Also we could not get a significant relationship between the level of viremia in relation to ALT category, an observation shared by other studies (20-22).

One of the novel aspects provided by this work is that it demonstrates the histopathological criteria in all patients enrolled especially those of PNALT group. We observed that most of PNALT group have an Ishak fibrosis score of 1/6 (42.5%) followed by 0/6 score (34.2%) with the minority of 2/6 score (23.3%), these findings go hand-in-hand with many other studies (23-30). this suggests that chronic hepatitis C with PNALT may have

a mild degree of fibrosis with a slow disease progression at the level of histopathological examination. Bruce et al. (21) concluded that PNALT cases represent only 6% of their chronic hepatitis C series, but their study included only 208 HCV RNA-positive persons, also they could not comment on histopathological findings in such group of patients because liver biopsy was done in only one patient (21). Some other studies suggest that 9-20% of chronic HCV-infected persons with PNALT have moderate to severe fibrosis (6-8,20,31), these differences could be explained by the unclear definition of PNALT, also many patients included in these studies may be misclassified as having PNALT while they are truly, FLUXALT with fewer ALT measurements and shorter timeframe. The correlation between ALT levels and the progression of fibrosis still lacks a confirmation by other studies with serial liver biopsies.

In conclusion, close follow-up of ALT with short time intervals, not exceeding two months, with a longer timeframe may be beneficial for accurate diagnosis of ALT status, also, it is better for persons infected with chronic hepatitis C virus with PNALT to have a histopathological diagnosis of a liver biopsy, Thus, they will be accurately diagnosed, and they may be considered, according to both serological and histopathological diagnosis, for the specific antiviral therapy. We recommend a more detailed study on the correlation between HCV level of viremia and histopathological changes in relation to ALT category. Thus, the decision to initiate therapy with interferon and ribavirin should be based on a combination of factors independent of ALT levels including the amount of fibrosis on liver biopsy, hepatitis C viral load, patient age and motivation, and co-morbid illness, and the presence of other complicating conditions.

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