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Evaluation of Lymphocyte Activation Gene 3 Activity with Hepatitis C Virus Infection in Beta Thalassemia Major Patients

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ABSTRACT

Background: *An infection with the hepatitis C virus can be contracted directly through transfusions or indirectly through contaminated materials (unsafe injections and surgical procedures). The hepatitis C virus (HCV) is the cause of hepatitis C. When the virus gets beyond the host's innate and adaptive immune systems, 60–80% of people with acute hepatitis C go on to acquire the chronic form. Seven significant clades, known as genotypes 1–7, have been identified in the revised HCV categorization based on phylogenetic analysis of several nearly full-length genomes. 86 subcategories have been identified from these genotypes.* ***Purpose:*** *The goal is to study the association of immunological checkpoint activity of lymphocyte activation gene 3 (LAG3) in thalassemia patients infected with the Hepatitis C virus.* ***Methods****: 200 participants that was cross-sectional. 69 patients positive HCV and 131 negative participants their ages range between (2-49) years. The samples were collected using fresh blood and serum samples. The sample consisted of 106 females and 94 males in both the patient. Samples were gathered between November 1, 2024, and the end of January 2025. from Center of Al-Batoul Hospital in Wasit, Kut Governorate, Iraq. The level of serum HCV (IgG-IgM) was employing an enzyme-linked immunosorbent assay (ELISA) to measure it. The enzyme-linked immunosorbent assay (ELISA) was also used to evaluate immune checkpoint LAG3.* ***Results****: There is no statistically significant correlation between LAG-3 levels and any of the hematological parameters (WBC, lymphocytes, platelets, or hemoglobin) in the study, and LAG-3 levels do not substantially correlate with either age or sex (p >0.05). P-values are more than 0.05. There is no discernible relationship between HCV-specific humoral immune responses (IgG or IgM) and LAG-3 expression. P-values are more than 0.05. When comparing HCV-positive and HCV-negative patients, LAG-3 levels are noticeably higher in the former. The P-value < 0.001 indicates a highly significant, Although ROC analysis showed a significant association with HCV status (AUC = 0.662), its low specificity limits its standalone diagnostic utility.* ***Conclusion:*** *LAG-3 levels were significantly higher in HCV-positive thalassemia patients, but showed no association with hematological, demographic, or humoral immune parameters. Although linked to HCV status, its low specificity limits its diagnostic value.*

Keywords. **Hepatitis C Virus, LAG3, Thalassemia.**

Article Information

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INTRODUCTION

Moscases of hepatocellular carcinoma, cirrhosis, and other severe liver diseases are primarily caused by chronic hepatitis. At least 400,000 people die from HCV disease each year, and the World Health Organization (WHO) estimates that the disease affects 71 million people globally (1). The hepatitis C virus (HCV) genotypes are classified into several major types, and together they comprise about 86 identified subtypes. About 9.6 kb of single-strand uncapped RNA with positive polarity and a single long open reading frame (ORF) surrounded by 3′ and 5′ untranslated regions (UTRs) make up the HCV genome(2). Although the positive-strand RNA viral genome's translation is guided by an internal ribosome entry site (IRES) in the 5′ UTR, both highly structured UTRs (3).

HCV RNA replication is believed to take place in close proximity to modified cytoplasmic host cell membranes, which produce unique organelle-like structures known as the membranous web in the case of HCV, as is the case with Like all other positive-strand RNA viruses, HCV can be transmitted through several routes, including sharing injection supplies, receiving unscreened blood or blood products, and injecting drugs(4) (5).

LAG-3 is a cell surface inhibitory receptor that has a variety of biological actions in addition to effector and T cell activation(6). As an inhibitory immune checkpoint molecule similar to PD-1 and CTLA-4, LAG-3 regulates immunity and has recently come to light as a possible target for boosting anti-cancer immune responses (7) (8). LAG-3 (CD223) plays a regulatory role in immune function, cell proliferation, homeostasis, and cytokine secretion suppression. It was initially identified by Triebel et al. in 1990 as a transmembrane protein consisting of 498 amino acids, encoded by the LAG3 gene, and possessing four extracellular immunoglobulin-like domains (13)The gene is next to the human chromosome 12 CD4 gene. About 20% of the amino acid sequences of LAG-3 and CD4 are similar. The similarities between LAG-3 and CD4 have resulted in their class II extracellular folding patterns on antigen-presenting cells (APCs), but with a higher affinity than CD4, which inhibited T-cell activation and cellular proliferation(9). One of the most prevalent genetic diseases in the world, thalassemia is a hereditary, autosomal recessive condition of hemoglobin (Hb) synthesis that is characterized by a number of molecular abnormalities (10). Mutations in globin genes cause the two main kinds of thalassemia, α- and β-, to produce the α-globin and β-globin chains of adult Hb, respectively, insufficiently or inadequately(11). Hemoglobinopathies are defects in the synthesis or structure of hemoglobin proteins. An estimated 5% of people worldwide are thought to carry at least one variation allele of thalassemia, the most prevalent hemoglobinopathy. There are two types of thalassemia: beta and alpha. Beta thalassemia carriers may exhibit hypochromic microcytic anemia, which is linked to a high HbA2 level, or they may appear as silent carriers with normal hematological parameters(12). Individuals with major and intermedia beta thalassemia, on the other hand, require transfusions either frequently or infrequently; these conditions are referred to as transfusion-dependent thalassemia and non-transfusion-dependent thalassemia, respectively. The pathogenesis, clinical, and laboratory characteristics of thalassemia(13) (14). The study primarily focuses on the management and follow-up, of the condition.

METHODS

Study design: Across-sectional study

Timeline: a period from November 1, 2024, and the end of January 2025.

Specimen collection: 200 people (69 with positive HCV and 131 with negative HCV) participated in a cross-sectional study. In both groups, 106 females and 96 males were included in the samples. Every patient underwent a number of laboratory tests, including the hematological tests CBC by automated analyzer and serum HCV and LAG3 by enzyme-linked immunosorben assay (ELISA).

ELISA, or enzyme-linked immunosorbent test

For the Immune Checkpoint (Lymphocyte-activation gene3) and Hepatitis C Three milliliters of blood were collected in sterile gel tubes, allowed to clot at room temperature, and centrifuged for 10 minutes at 2500 r.p.m to separate the serum. The obtained serum was stored at –20°C until analysis The microtiter plate was pre-coated with the target antigen. Samples and positive/negative controls were added and incubated to allow antibodies to bind to the antigen. After washing away unbound antibodies, an HRP-conjugated detection antibody was added and incubated, followed by

another washing step. TMB substrate was then added to develop color, and the reaction was stopped with an acidic stop solution, producing a yellow color measured at 450 nm. The optical density of the samples was compared with the controls to determine the presence of HCV IgG.

RESULTS

The immune checkpoints LAG3 in patients with beta thalassemia major and their correlation to age of patients are shown in **(table 1**). Regarding LAG3, the median level (interquartile range) was 4.29 (9.63) and the range was between 0.00 -25.20; there was no significant correlation to age of patients (p>0.05). The immunological checkpoints LAG3 in beta thalassemia major patients and how they relate to the patients' sex are displayed in (**table 2),** Regarding LAG3, the median level (interquartile range) was 4.29 (9.63) and the range was between 0.00 -25.20; there was no significant correlation to sex of patients (p >0.05). Correlations of LAG3 to hematological characteristics of enrolled patients are outlined in **(table 3**). The level of LAG3 showed no significant correlation to WBC count of enrolled patients (r = 0.044 and p = 0.718). Added to that, the level of LAG3 showed no significant correlation to lymphocyte count of enrolled patients (r = 0.092 and p = 0.456). Moreover, the level of LAG3 showed no significant correlation to platelet count of enrolled patients (r = 0.097 and p = 0.429). Furthermore, the level of LAG3 showed no significant correlation to hemoglobin level of enrolled patients (r = 0.098 and p = 0.421). Correlation of LAG3 to HCV IgG and IgM levels are outlined in (**table 4**). The level of LAG3 showed no significant correlation to HCV IgG level of enrolled patients (r = 0.141 and p = 0.249). Moreover, the level of LAG3 showed no significant correlation to HCV IgG level of enrolled patients (r = 0.039 and p = 0.751). Comparison LAG3 between patients with positive HCV and patients with negative HCV is shown in **(table 5).** Those with positive HCV had a noticeably greater level of LAG3 compare to patients with negative HCV, 10.02 versus 3.12, respectively (p < 0.001), Regarding the diagnostic potential of LAG3, the Youden Index indicated an optimum cutoff value of The cutoff value of LAG3 was >2.209 that has a significant p-value (<0.001); but the area under curve (AUC) was <0.7 (0.662) indicating poor diagnostic potential with high sensitivity of 85.5 %, low specificity 41.2 % and low accuracy level 66.2 % as shown in (table 6).

Table (1): The immune checkpoints LAG3 in patients with beta thalassemia major and their correlation to age of patients.

Characteristics	Total, *n* = 200	Correlation to age	
		R	***p***
LAG3			
Median (IQR)	4.29 (9.63)	- 0.079	0.521 NS
Range	0.00 -25.20		

Table 2: The immune checkpoints LAG3 in patients with beta thalassemia major and their correlation to sex of patients.

Characteristic	Total *n* = 200	Correlation to sex	
		r	*p*
LAG3			
Median (IQR)	4.29 (9.63)	-0.074	0.547 NS
Range	0.00 -25.20		

Table 3: Correlation of LAG3 to hematological characteristics of enrolled patients.

Characteristics	Mean ±SD	Range	LAG3	
			R	*P*
WBC count	4.94 ±1.97	1.06 -11.30	0.044	0.718 NS
Platelet	257.86 ±98.34	11 -39.7	0.091	0.456 NS
Lymphocyte	23.35 ±8.15	100 -443	0.097	0.429 NS
Hemoglobin	7.66 ±1.32	4.6 -10.2	0.098	0.421 NS

Table 4: Correlation of LAG3 to HCV IgG and IgM levels.

Characteristics	LAG3	
	R	*P*
HCV IgG	0.141	0.249 NS
HCV IgM	0.039	0.751 NS

Table 5: Comparison of LAG3 between patients with positive HCV and patients with negative HCV.

Characteristics	Positive	Negative	*P*
LAG3			
Median (IQR)	10.02 (15.13)	3.12 (9.77)	< 0.001, M ***
Range	0 -25.2	0 -25.2	

Table 6: Analysis of the receiver operating characteristics (ROC) curve demonstrating LAG3's capacity for diagnosis.

Characteristics	LAG3
Cutoff	>2.209
AUC	0.662
95 % CI	0.592 to 0.727
P	<0.001 ***
Sensitivity %	85.5
Specificity %	41.2
Accuracy %	66.2

DISCUSSION

In the present study, no significant correlations were observed between LAG3 expression and the demographic variables of age and sex among the enrolled patients (Table 1 and Table 2). The correlation coefficients for age (r = -0.079, p = 0.521) and sex (r = -0.074, p = 0.547) only show marginally significant negative associations. The lack of statistical significance may be explained by several biological and methodological considerations. Activated T cells are the primary source of LAG3 expression, which is known to regulate both T cell exhaustion and chronic immunological activation. It has been clarified(15). is usually brought on by ongoing antigenic stimulation, as occurs during long-term viral infections, as opposed to static host characteristics like age or sex(16). the immune status of the individual, including ongoing inflammation and antigen burden, may have a more profound impact on LAG3 levels than demographic attributes(17). In chronic HCV infection, the variability of immune responses shaped by disease stage, liver inflammation, and comorbidities may mask the influence of minor demographic effects(18).

In this study, LAG3 expression did not show significant correlations with key hematological parameters, including white blood cell (WBC) hemoglobin levels (r = 0.098, p = 0.421), platelet count (r = 0.097, p = 0.429), lymphocyte count (r = 0.091, p = 0.456), and count (r = 0.044, p = 0.718), as shown in Table 2. Additionally, LAG3 levels were not significantly correlated with either IgG (r = 0.141, p = 0.249) or IgM (r = 0.039, p =0.751)are HCV-specific immunoglobulin's., indicating no clear association between LAG3 and humoral immune responses in the population under investigation These results imply that peripheral LAG3 expression is likely not influenced by general hematological status or baseline immune cell counts in HCV-infected individuals. LAG3 is primarily expressed on activated T cells, particularly CD4+ and CD8+ subsets, and plays a role in regulating T cell exhaustion and chronic immune stimulation. Moreover, the lack of correlation with broad parameters like WBC or lymphocyte counts may reflect the specificity of LAG3 as a marker of functional immune exhaustion rather than general immune cell abundance(15).

In this study, the results clearly show no statistically significant correlation between LAG3 and either. Table 3 displays HCV IgG (r = 0.141, p = 0.249) or IgM (r = 0.039, p = 0.751).

The absence of significant correlation suggests that LAG3 expression is likely independent of the antibody response in the context of HCV infection. This finding is consistent with the established understanding of LAG3 as an inhibitory receptor primarily involved in T-cell function, particularly in regulating effector T-cell activity, promoting T-cell exhaustion, and maintaining peripheral tolerance(19).The expression of LAG3, a key immune checkpoint protein that controls the responses of T cells, was found to be markedly increased among those infected with the hepatitis C virus, As shown in the updated table, patients with positive HCV exhibited a median LAG3 level of 10.02 (IQR: 15.13), which was significantly higher than the 3.12 (IQR: 9.77) observed in HCV-negative individuals. The difference was statistically significant. LAG3 expression and HCV infection status are strongly correlated, according to the Mann-Whitney U test ($p < 0.001$).

The broader range when compared to the HCV-negative group, the HCV-positive group (0–25.2) represents the variation in immunological response among infected people, which may be impacted by host immunity, viral load, and length of illness. According to a prior study conducted, the elevated LAG3 levels suggest a state of T-cell

exhaustion or suppression, a well-documented feature in chronic viral infections. This aligns with the biological function of LAG3, which acts to downregulate T-cell proliferation and cytokine production mechanisms that may help the virus evade immune clearance and persist in the host(20). Across LAG3's potential role as a biomarker of immune suppression in chronic HCV infection, more importantly, they raise the possibility of targeting LAG3 therapeutically to rejuvenate exhausted T cells and restore effective antiviral responses in affected patients(21). The ROC curve analysis of LAG3, as presented in detail in Table 6, offers valuable insights into its potential as a diagnostic biomarker. With a cutoff value of >2.209, LAG3 demonstrates a statistically significant association with the condition studied ($p < 0.001$). However, the clinical utility of LAG3 remains debatable due to the modest AUC of 0.662, which falls below the generally accepted threshold of 0.7 for a fair diagnostic test. From a clinical perspective, the high sensitivity (85.5%) is encouraging, suggesting that LAG3 can correctly identify a large proportion of affected individuals. This feature makes it potentially useful in screening or early detection settings, where failing to identify true positives (false negatives) can have serious consequences. However, the low specificity (41.2%) poses a significant limitation, increasing the likelihood of false positives and potentially leading to over diagnosis, unnecessary anxiety, and additional invasive diagnostic procedures. This trade-off is a common challenge in biomarker development and highlights the importance of balancing sensitivity and specificity (9). Furthermore, the statistical significance observed ($p < 0.001$) does indicate a strong association and justifies further exploration of LAG3's role in disease pathogenesis and progression. However, clinical translation requires not only statistical significance but also clinical relevance, which includes acceptable diagnostic metrics and reproducibility across populations(22).

CONCLUSION

This study examined LAG3 expression in the peripheral blood samples of those who have a persistent infection with the chronic Hepatitis C virus, aiming to understand its relationship with clinical and immunological markers. Results showed a significant elevation of LAG3 levels in HCV-positive individuals compared to uninfected controls, suggesting its involvement in the immune system's reaction to persistent infections. While ROC curve analysis confirmed a statistically significant link between LAG3 and HCV status, its diagnostic performance was limited by a modest AUC and low specificity. These findings highlight LAG3 as a potential marker of immune exhaustion in chronic HCV infection and underscore the need for expanded studies to validate its role in diagnostic panels and targeted immunotherapeutic approaches.

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Statement of permission and conflict of interests

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Ethical approval

Before beginning this study, the University of Kufa's Faculty of Medicine's ethics council approved it. Every participant provided their consent, and the province of Waist's Al-Batool Hospital approved it.

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