Azhar Int J Pharm Med Sci 2025; Vol 5 (2):206-217.
Research Article

Open Access

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Evaluation of The Expression of LncRNA PAX8-AS1 and mRNA LAIR-2 in Chronic Hepatitis C virus (CHC) Among Egyptian Patients

Aya Mohamed Ahmed Ibrahim [1], Abeer I. Abd El-Fattah [2], Olfat Gamil Shaker [3] and Ahmed A. Youssef [1,4]

[1] Department of Biochemistry, Faculty of Pharmacy, Egyptian Russian University, Badr City, Cairo, Egypt.
[2] Biochemistry and Molecular Biology Department, Faculty of Pharmacy (Girls). Al-Azhar University.
[3] Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University.
[4] Department of Pharmacy, KUT University College, AL KUT, Wasit, 52001, Iraq

*Corresponding author: Aya M Ibrahim, E-mail: Aya-mohamed@eru.edu.eg Tel:00201021562493

Article history: Received 20-12- 2024 **Revised** 22-01-2025 **Accepted** 05-05-2025

Abstract: Leukocyte associated immunoglobulin-like receptor-2 (LAIR-2) is an immunological checkpoint strongly produced on immune cells and responsible for the delivery of inhibitory signals. LAIR-2 may regulate the increase in biological activity from the interaction between collagen and LAIR-1. Long non-coding RNAs (lncRNAs) may facilitate novel strategies for the treatment of immune pathologies in chronic hepatitis C (CHC). The paired box gene 8, antisense AS1 (PAX8-AS1) may maintain tissue-specific stem cells by inhibiting differentiation and apoptosis. To investigate the impact of LAIR-2 and PAX8-AS1 expression and their diagnostic biomarkers as hepatitis C virus (HCV) may suggest an exhausted immune state in HCV patients**.** This study included 90 participants, 30 controls and 60 HCV patients. LAIR-2 and PAX8-as1 were evaluated in patients with CHC disease by Real Time-q Polymerase Chain R (RT-q PCR). The present research showed that Pax8-AS1 had decreased expression levels in blood leukocytes. LAIR-2 levels were higher in HCV ($P < 0.0001$) compared to healthy control. PAX8-AS1 and LAIR-2 correlated positively (r and p = 0.23 and 0.05). LAIR-2 negatively correlated with HCV hematological laboratory tests and albumin ($P< 0.001$). However, LAIR-2 positively correlated with Aspartate Transaminase (AST), Alkaline phosphatase (ALP), and total and direct bilirubin ($P<0.001$). Pax8-AS1 and LAIR-2 showed remarkable diagnostic efficacy in HCV, with sensitivity and specificity values of (81.7%, 96.7%) and (91.7%, 96.7%) respectively, according to a receiver operating characteristic curve (ROC) study ($P<0.001$). The current findings suggest the diagnostic role of LAIR2 and PAX8-AS1 in HCV, as well as a potential strategy for developing immunological therapy.

Keywords: Long non-coding RNAs; T cell exhaustion; Immunological checkpoint; diagnosis markers; RT-q PCR.

1. INTRODUCTION

Chronic Hepatitis C is a significant worldwide health issue, affecting around 71 million persons. Several clinical and experimental studies have shown that HCV can directly cause hepatocarcinogenesis through its RNAs or proteins and indirectly through chronic liver inflammation **[1, 2].** The Hepatitis C virus is primarily a bloodborne pathogen, with an estimated worldwide frequency of 2.5%. Significant differences have been observed in Egypt and Pakistan. Pakistan has the greatest frequency of anti-HCV in the general population[3]**.** Worldwide, approximately 79% of CHC individuals were unconcerned about their diagnosis in 2019**[4].** The majority of CHC is asymptomatic; however, it may result in severe complications, including cirrhosis or hepatocellular carcinoma (HCC). Direct-acting antiviral treatments (DAAs) are highly efficacious and well-tolerated by approximately 20% of individuals developing HCC**[5].**

Cite this article: Ibrahim, A., [1], Abd El-Fattah, A., Shaker, O., Youssef, A. Evaluation of The Expression of lncRNA PAX8-AS1 and mRNA LAIR-2 in Chronic Hepatitis C virus (CHC) among Egyptian Patients. Azhar International Journal of Pharmaceutical and Medical Sciences, 2025: *Vol 5 (2):206-217*. doi: 10.21608/aijpms.2025.346193.1304.
DOI : 10.21608/aijpms.2025.346193.1304.

Cluster of differentiation 8+ (CD8+) T cell responses primarily determine the progression and management of HCV infection. Robust CD8+ T cell reactions are related to eliminating viruses during acute illnesses, but the lack of these responses, due to viral evasion and T cell exhaustion, occurs in CHC [6]. The long-term effects of impaired T cell responses in CHC are unclear; however, they may be crucial for the clinical progression of the virus, re-infection, treatment-induced viral clearance, and vaccine development. Fibrosis and cirrhosis are the primary outcomes of chronic hepatitis C, which is also linked to molecular and genomic variations[7].

Virological and immunological factors influence the clinical outcome of HCV infection and the course of the natural disease. CHC is known to cause B cell activation often, resulting from humoral and cellular immunological responses[8]. The pathogenic mechanisms of CHC and the immune response's role in liver injury are being studied. The complex liver milieu contains several immune cell types that produce cytokines that can eradicate viruses. Thus, pathogen-host immune system interaction may affect infection outcomes [9]. The chronic stage complicates immune cells' involvement, as changes in their functioning contribute to liver injury[10].

Following the eradication of the virus, the expression of CD8+ T cells is subsequently downregulated. On the other hand, persistently infected patients, including those with CHC, consistently express these molecules in virus-specific T cells [11]. Increased inhibitory checkpoint molecule expression is indicative of T-cell exhaustion in CHC. The expression of these molecules on the surface membrane of T cells is one mechanism that negatively regulates the frequency and duration of effector T cells[11, 12].

The LAIR family is a small group of inhibitory receptors recognizing collagens and containing immunoreceptor-tyrosine-based-inhibition-motifs. Human peripheral blood leukocytes express LAIR-1, a transmembrane glycoprotein. CD4+ T-lymphocytes primarily produce LAIR-2, a released receptor that regulates the inhibitory potential of LAIR-1[13]. LAIR-2 is a soluble homologue of LAIR-1[14]. Researchers have linked both LAIRs to several inflammatory responses and autoimmune diseases. Finding high expression of LAIR-2 in the synovial fluid (SF)of people with rheumatoid arthritis (RA) is important because it shows that LAIR-2 is involved in systemic autoimmunity [15]. Cholangiocarcinoma (CCA) patients had a lot of LAIR2 on their T cells and may indicate that the immune system of CCA patients has been exhausted[16].

Infected cells can potentially disrupt the regulation of cellular Long non-coding RNAs (lncRNAs) lncRNAs and generate viral or chimeric lncRNAs composed of both viral and cellular sequences. The expression of viral lncRNAs has been observed in various viruses[17]. Another group of lncRNAs upregulated by HCV infection but not by other viruses or the antiviral response are carcinogenic [18]. Therefore, these lncRNAs may stimulate cell division and other carcinogenic processes, potentially contributing to the development of HCC in individuals diagnosed with HCV [19].

The lncRNA-PAX8-AS1 located on chromosome 2q13, upstream of PAX8, may regulate it [20]. Downregulation of PAX8-AS1-N suggested a bad prognosis for breast cancer patients [21] and in individuals with subclinical and clinical hypothyroidism relative to healthy controls [22]. The present study investigated mRNA LAIR-2 and PAX8-AS1 expression levels and their potential as HCV diagnostic biomarkers, which may suggest an exhausted immune state in HCV.

2. METHODS

2.1 Subjects

This research included 90 participants: 30 controls and 60 HCV patients enrolled from the outpatient clinic and inpateint in the gastrointestinal unit of Cairo University's Department of Hepatology, Kasr Al-Ainy Hospital, from October 2023 until July 2024. Laboratory characteristics of HCV patients were identified. A liver pathological examination was conducted to ascertain the extent of hepatic fibrosis (stage) and the severity of inflammatory conditions (activity) using the METAVIR scoring system. The fibrosis score can determine the degree of inflammation in the liver: F0: Liver fibrosis is absent; (2) F1: Portal fibrosis is present without septa; (3) F2: Portal fibrosis has a limited number of septa; (4) F3: Multiple septa are present without cirrhosis[23]. The inflammation was classified from A(0 to 3) based on the degree of necrotic tissue and immune system invasion[24].

All enrolled patients performed an intensive medical history assessment and clinical evaluation. Liver function assessments were conducted. Additionally, a complete blood count was evaluated.

Patients above the age of fifteen from each gender were included in the study. Additionally, individuals identified with malignancies in other locations, a history of chemotherapy or radiation for hepatocellular carcinoma, alcoholism, any autoimmune liver disorders, hepatitis B virus infection, or severe nonalcoholic liver illnesses linked to metabolic syndrome were eliminated. HCV (17 females and 43 men, aged 55 to 63 years) confirmed liver function tests.

2.2. Sample

Two vacutainers were used to separate six ml of blood specimens from patients. The initial three ml of whole blood were collected using EDTA vacutainer tubes for a complete blood count (CBC). All vacutainer tubes were kept at -80°C until they were required. Serums were isolated from the clotted whole blood by centrifuging the remaining three mL of blood at 4000 rpm for 10 minutes, followed by 30 minutes of storage in yellow gel vacutainer containers at ambient temperature. The initial aliquoted sera were employed to execute RNA extraction. The serum was kept at -80°C until they were needed.

2.2.1.The expression levels of mRNA LAIR-2 and lncRNA PAX8-as1 were assessed using RT-qPCR.

Initially, a volume of 200µL 200 ml of serum was used to separate leukocytes and extract whole genome RNA from blood samples using the QIAzol lysis reagent, followed by the miRNeasy extraction martial (Qiagen, Valencia, CA), according to the manufacturer's instructions. The Nanodrop microvolume spectrophotometer (Thermo Fisher, USA) was used to evaluate the isolated RNA's quantity and purity.

In a 20 µL reaction volume, 0.1 µg of total RNA was reversed using the RT2 First Strand reagent (Qiagen, Valencia, CA). 50 µL of RNase-free water and ccDNA were mixed and kept at −20 °C until the analysis. GAPDH (Catalogue No: 249900, specific number: QT00079247), LncRNA-PAX8-AS1 (Specific number: LPH21217A-200, Catalogue Number; 330701), LAIR-2 (Catalogue Number; 249900), and SYBR Green PCR (Thermo Fisher Scientific, USA) were employed in RT-qPCR with ready-made primers from Qiagen Valencia, CA. To generate a 20 µL reaction mixture, combine 2.5 µL of diluted cDNA template, 5µL of RNase-free water, 10 µL Master Mix, and 2 µL of primers. The following conditions were used to produce RT-qPCR: Rotor-Gene Q system (Qiagen): initial denaturation period at 95 ℃ / 10 minutes, denaturation 15 seconds (S) at 95 °C, annealing at 60 °C/ 60 S for 40 cycles, and final extension30 seconds at 60 ℃ for 40 cycles. PCR products of the investigated genes were analyzed using a melting curve analysis to validate their diagnostic accuracy. Gene expression was quantified to the internal control using $2^{-\Delta\Delta Ct}$, and the fold change was determined compared to the control group.

2.2.2. Routine examinations

An automated blood cell counter (Cell Dyne-2,700, Abbott Lab) was used to obtain a thorough blood profile, with measurements of serum bilirubin (DAIOMOD diagnostic kits), liver enzymes including ALT and AST (using human kits), and serum albumin (Biosystem kits). Renal function tests, including creatinine and urea levels, are performed via Reactivos GPL. All examinations were conducted on each specimen.

2.3. Statistical analysis

Statistics were analysed using GraphPad Prism 9.5.1 (Ukrainian colleagues and against the Russian invasion) and SPSS 25 (Chicago, IL, USA). The statistics were shown as the median value (25%-75%) and means ± standard deviation (SD) or as percentages, as applicable. The Anderson-Darling test and D'Agostino & Pearson tests were used to assess normality. Normal data and non-normally distributed data were analyzed by unpaired student's t-tests/the Mann–Whitney U analysis. Qualitative data were compared using the chi-square or Fisher's exact test. ROC curve analysis calculated AUC to determine the parameters' diagnostic and prognostic accuracy. An AUC between 0.7 and 0.89 indicates prospective or promising discrimination, whereas AUC above 0.9 indicates great discrimination. Spearman's rho coefficient assessed parameter correlation. Two logistic regression analyses were conducted to determine risk variables for HCV. A stepwise forward multivariate analysis was performed using important predictor factors identified in the univariate study. Gender and age have been adjusted for confounding. The significance level of all assays was $P < 0.05$ with a 95% confidence interval (CI).

3. RESULTS

3.1. Demographical, clinical and virological patient features.

Table 1. Illustrated demographic, clinical and virological patient features. In HCV patients vs. healthy controls, age was significantly different ($p < 0.0001$). Sex was significant ($p = 0.001$), with 71% of HCV patients being male. Total leukocyte count (TLC), hemoglobin, total bilirubin, INR, and albumin were not significantly different in HCV patients vs. healthy controls. However, HCV patients presented a remarkably significant decrease in platelet count, creatinine and urea and a remarkably increased AST, ALT, ALP, direct bilirubin and $P < 0.05$ compared to healthy controls. HCV cases included 36.66% were diagnosed with viral load 0.276(0.027-0.863).

3.2. Fold change of PAX8-AS1 and LAIR-2 serum expression between the studied groups.

HCV patients presented a remarkably increased in serum LAIR-2 levels, with a median fold change of 7.6 ($p < 0.0001$), when compared with healthy controls. HCV patients had significantly lower serum PAX8-AS1 expression ($p < 0.0001$) in comparison to healthy subjects (Fig 1, A and B).

3.3. Correlation between the studied parameters' expression levels and the studied group's clinical and laboratory characteristics.

In the HCV group, PAX8-AS1 and LAIR-2 correlated positively (r and p = 0.23 and 0.05), no correlation PAX8-AS1 with liver activity, liver fibrosis and viral load was detected (PAX8-AS1 vs viral load: (r and p = −0.23 and 0.2); PAX8-AS1 vs liver activity: (r and p = 0.22 and 0.2); PAX8-AS1 vs liver fibrosis: (r and p = 0.22 and 0.23) and LAIR-2 no correlation with liver activity, liver fibrosis and viral load was detected (LAIR-2 vs viral load: (r and p = −0.34 and 0.06); LAIR-2 vs. liver activity: (r and p = 0.09 and 0.6; LAIR-2 vs. liver fibrosis: (r and p = 0.17 and 0.3) (Figure 2) and (Table 2). Furthermore, the results showed a significant positively correlated between LAIR-2 and laboratory characteristics in individuals with HCV, e.g., AST (r and p = 0.3 and 0.025); ALP (r and p = 0.3 and 0.02); BIL -T (r and p = 0.5 and 0.000); BIL D (r and p = 0.3 and 0.019) of HCV group, respectively. Additionally, a substantial negative significant correlation was identified among LAIR-2 against albumin (r and p = -0.4 and 0.02); hemoglobin (r and p = −0.250 and 0.05); platelet count (r and p = −0.274 and 0.03); leucocytes count (r and p = −0.413 and 0.001); respectively.

3.4. The diagnostic accuracy of the biomarkers in the context of the studied group

Serum levels of PAX8-AS1 and LAIR2 were higher in HCV diagnoses than in controls (AUC = 0.928 and 0.943, respectively) (Table 3) and (Figure 3).

4. DISCUSSION

Hepatitis C virus (HCV) infection significantly contributes to liver-related morbidity and mortality, presenting a global challenge to healthcare systems. Globally, the annual rate of new HCV infections is 1.5 (1.3–1.8) million. In Egypt, chronic infection with HCV is a significant contributing factor to liver malignancy and is associated with molecular and genomic alterations, as well as fibrosis and cirrhosis[25]. Immune response effectiveness depends on an optimum balance between activating and inhibitory signals [26]. In vitro activation enhances T cells' production of LAIR-2 [27].

The relationship between the lncRNA Pax8-AS1 and HCV remains unclear. The present study assessed serum lncRNAs and mRNAs, PAX8-AS1, LAIR-2, and liver fibrosis progression in patients with CHC as diagnostic biomarkers in chronic HCV. In this research, we detected a significant down regulation of PAX8-AS1, however, a significant upregulation of LAIR-2 in HCV.

The present investigation revealed significant age differences among the groups examined. The predominant age of HCV patients in this study was around 59 years. This result matches a recent World Health Report (WHO) from 2022. In another research, the average age of haemodialysis HCV patients was 52 years. A younger age was strongly correlated with a higher incidence of HCV (mean difference −1.41 years; 95% CI −2.27 to −0.56; p = 0.001; I2 = 96.46%)[28]. HCV frequency is determined by multiplying the viral prevalence among persons aged 15–64 in each nation [29]. Within the age cohort of 50 to 59 years, Genotype 1 was the most prevalent among male patients, constituting 29.05% of 148 individuals30.

Table 1. Demographic and laboratory characteristics of studied group

Variables		Control (*n* = 30)	HCV (*n* = 60)	p-value
Age (years)		37.5(28.75-55.5)	59(55-63)	**0.000**
Sex, *n* (%)	**Male**	11(36.7%)	43(71.7%)	
	Female	19(63.3%)	17(28.3%)	**0.000**
Hemoglobin (g/dL)		12.4 ±1.3	12.6± 2.1	0.57
Platelet count ×10^3/mm^3		222.5(191-305.8)	147(94- 210.75)	**0.000**
TLC × 103/mm3		6.8(4.6-7)	5.3(4.2-6.4)	0.67
AST (U/L)		13.3 (8.83-18.2)	57(43-91.5)	**0.000**
ALT (U/L)		20 (15-25)	54 (39.2- 78.5)	**0.000**
ALP (U/L)		103 (85.3-115)	180 (126-270)	**0.000**
BIL T (mg/dl)		0.8 (0.6-0.9)	0.9 (0.6-1.2)	**0.25**
BIL D (mg/dl)		0.15 (0.1-0.2)	0.4 (0.3-0.6)	**0.000**
S. Albumin (g/dl)		3.86 (3.7-4)	3.7 (3.5-4.1)	0.316
Creatinine (mg/dl)		0.9 (0.7-1)	0.75(0.5-0.9)	**0.039**
Urea (mg/dl)		24.5 (22-30)	19(15.6-29)	**0.033**
INR		1.3(1.2-1.4)	1.2(1-1.5)	0.56
HCV RNA(equivalents/ml) x 10^6		--------	0.276(0.027-0.863)	----------

Data were presented as mean ± SD, median (IQR), percentage. ALP, alkaline phosphatase; AFP, alpha feto protein, ALT, alanine aminotransferase; AST, aspartate aminotransferase HCV: Hepatitis C virus; INR: International normalised ratio; TLC: total leukocyte count; BIL T: total bilirubin; BIL D: direct bilirubin. When applicable, t test or mann whitney u tes.t P values are reported across groups. The bolded p values indicate statistical significance, which is < 0.05.

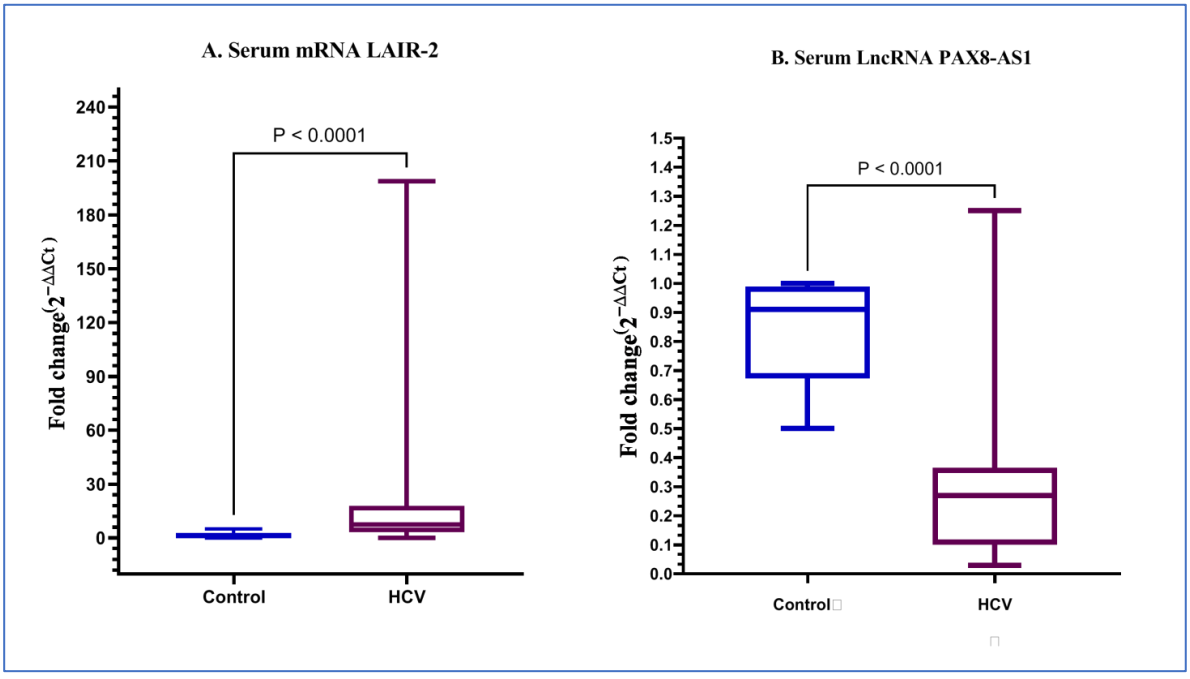

Figure 1. Fold change of PAX8-AS1 and LAIR-2 serum expression between the examined groups. Comparing HCV patients to healthy controls (A, B). Control (n=30) and HCV (n=60). The box blot shows the 25–75% percentiles, the median, and the 10–90% percentiles. The bolded p values indicate statistical significance, which is less than 0.05.

Table2. Correlation between the studied parameters' expression levels and the studied group's clinical and laboratory characteristics.

			PAX8-AS1	LAIR-2	HCVRNAQuant	Liver Activity	Liver Fibrosis
Spearman correlation	Hb	Correlation Coefficient	-0.097	-0.250	-0.047	-0.098	0.082
		P-value	0.459	0.05	0.804	0.608	0.668
	TLC	Correlation Coefficient	-0.035	-.274*	-0.110	-0.094	-0.037
		P-value	0.791	.034	0.562	0.620	0.844
	PLT	Correlation Coefficient	-0.109	-0.413**	0.027	-0.004	0.204
		P-value	0.409	0.001	0.886	0.983	0.279
	AST	Correlation Coefficient	-0.127	0.3	-0.029	-0.293	-0.237
		P-value	0.332	.025	0.878	0.116	0.208
	ALT	Correlation Coefficient	-0.147	0.065	0.142	-0.041	-0.223
		P-value	0.263	0.621	0.453	0.830	0.237
	ALP	Correlation Coefficient	0.100	0.3	-0.314	-0.036	0.078
		P-value	0.449	0.022	0.091	0.849	0.681
	BIL T	Correlation Coefficient	-0.034	0.5**	-0.168	-0.105	-0.032
		P-value	0.798	0.000	0.375	0.582	0.866
	BIL D	Correlation Coefficient	0.025	0.303*	-0.178	-0.115	-0.073
		P-value	0.848	0.019	0.348	0.544	0.701
	Albumin	Correlation Coefficient	0.007	-0.385**	0.045	0.065	0.030
		P-value	0.956	0.002	0.815	0.734	0.873

Spearman correlation was used to determine correlation—Spearman's rho coefficient. The bolded p values indicate statistical significance, which is less than 0.05. ALP, alkaline phosphatase; PLT, platelet count, ALT, alanine aminotransferase; AST, aspartate aminotransferase Hb: Hemoglobin ; TLC: total leukocyte count; BIL T: total bilirubin; BIL D: direct bilirubin

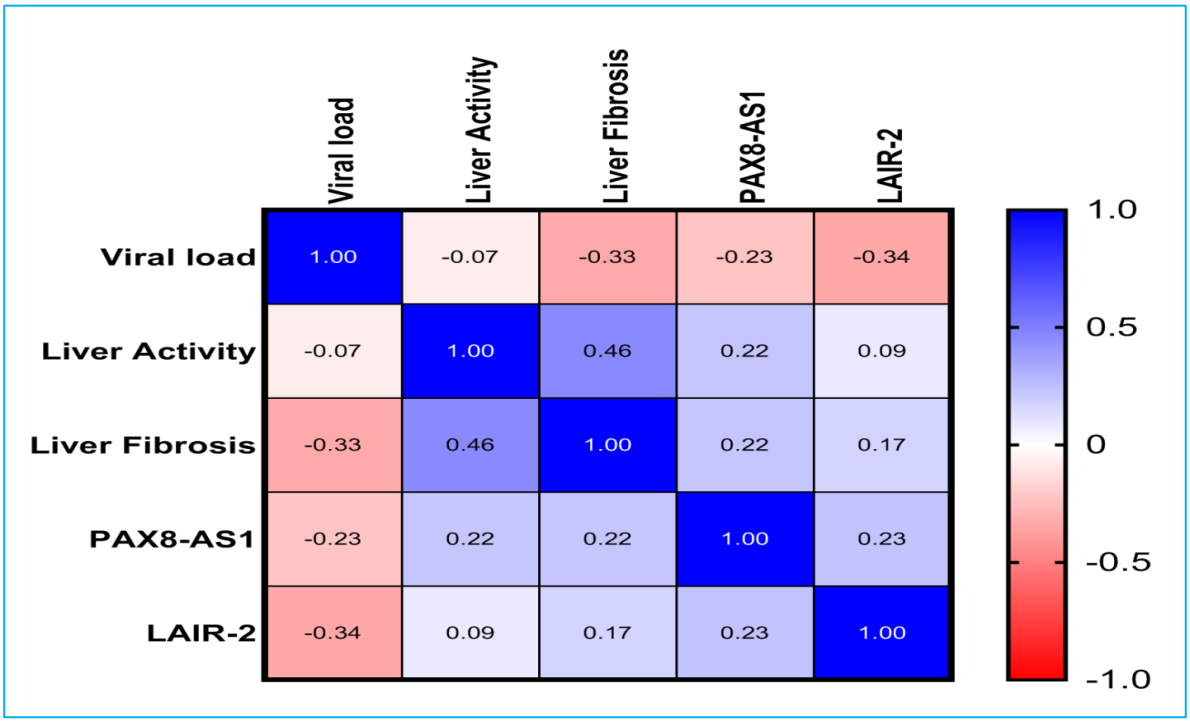

Figure 2. Correlations of laboratory and clinical data of HCV group.

Blue-red (cold-hot) correlation map. The correlations are close to 1 in blue and -1 in red. White has a near-correlation. Spearman correlation was used to determine correlation—Spearman's rho coefficient

Table3. The diagnostic accuracy of the biomarkers in the context of studied group

Parameters	Cutoff	AUC	P-value	Sensitivity	Specificity	PPV	NPV	95% CI
Pax8-Ast (HCV vs control)	**0.487**	**0.928**	**0.00**	**81.7%**	**96.7%**	**98%**	**72.5%**	**0.88-0.99**
LAIR2 (HCV vs control)	**1.71**	**0.943**	**0.00**	**91.7%**	**96.7%**	**98.2%**	**85.3%**	**0.86-0.99**

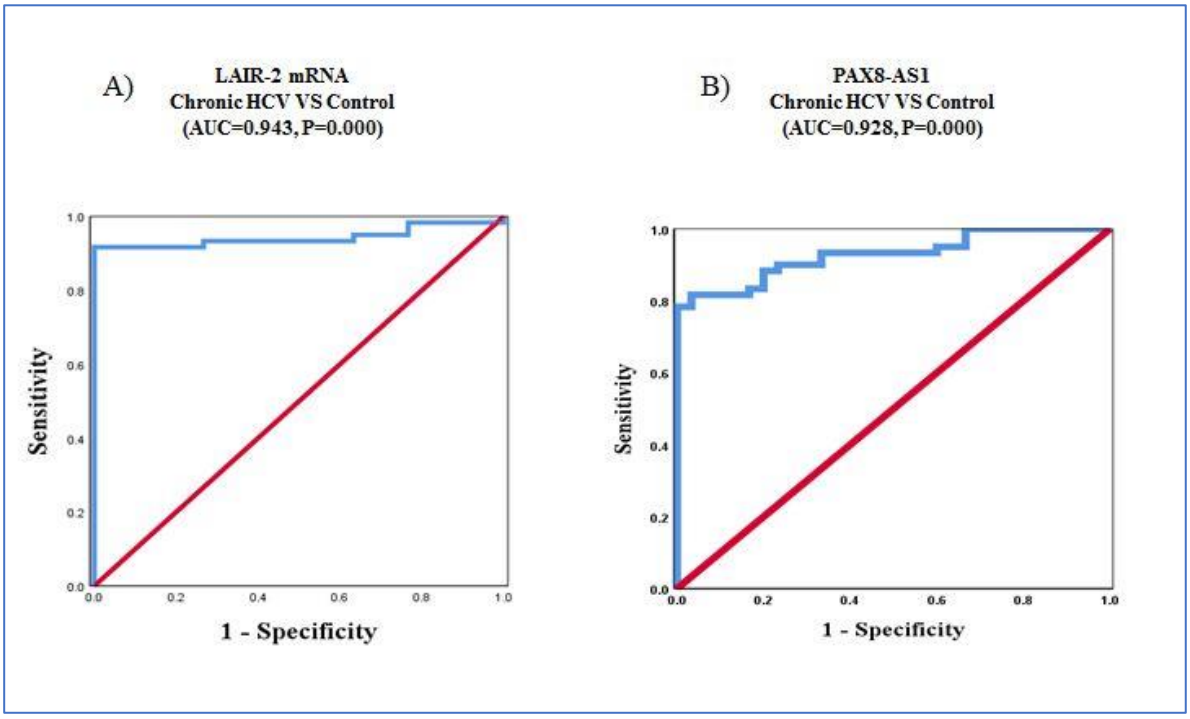

Figure 3. The diagnostic performance of the parameters under investigation.
The ROC curve analysis focuses on LAIR-2 mRNA in (A) and PAX8-AS1 in (B). There were 60 HCV cases and 30 healthy control

There was a significant difference in sex between studied groups among men (71.7%) compared to females (28.3%). Previous results agree with the present result. 55% (n = 80 443) of all registered cases of hepatitis C among persons over 18 years of age were male, while 45.0% (n = 65 713) were female (p = 0.03) [31]. Additionally, patients with minimal or no fibrosis exhibited an enrichment of the variant compared to patients with more advanced stages (12.6% vs. 6.6%; P = 0.005). Male patients were entirely responsible for the variance [32]. Viral eradication was more prevalent among females than males (44.6% vs. 33.7%, respectively; p = 0.001 [33]. Males are more predisposed than females to engage in intravenous drug use and circumcision [34].

The biochemical parameters of these studied groups showed a significant increase in ALT, AST, ALP, and direct bilirubin and a significant decrease in creatinine, urea, and Platelet Count (PC) in the HCV group compared to a control group. These results agreed with [35,] who reported a significant statistic in PC between patients with CHC and healthy controls (PC 226.03 ±68.36 vs. 188.9±46.49, P-value = 0.02). These results agreed with the present result. Both groups have a considerable difference in liver function tests [36]. Other studies support our study; age, PC, AST, ALT, and alkaline phosphatase levels significantly differed between the patients [37]. A case-control comparison indicated significantly increased AST: ALT levels, and bilirubin was

sensitive and specific in different hepatitis and controls [38]. A statistically significant increase ($P < 0.01$) in urea and creatinine levels was observed in patients with renal failure, both without HCV and HCV, as compared to the control group [39].

The current study found no statistically significant difference in albumin, INR, and total bilirubin, hemoglobin and TLC in HCV groups compared to the control group. results agreed with [40], who found no statistical significance between HCV and control patients regarding haemoglobin and total bilirubin [41]. While albumin indicates synthetic liver function and nutritional condition, AST and ALT levels indicate ongoing liver inflammation and tissue destruction with a chronic HCV infection [42].

It has been reported that the lncRNA PAX8-AS1 was downexpressed in patients with ovarian cancer and it was associated with the disease progression, [43]. As well, patients with thyroid cancer (TC) showed downregulation of PAX8-AS1, and those with lower levels showed a more favorable prognosis. These findings identified the PAX8-AS1 as associated with the prognosis of TC[44]. PAX8-AS1 expression is lower in Papillary Thyroid Cancer (PTC) PTC samples, and as indicated by the previous study's findings, the upregulation of PAX8-AS1 suppressed PTC cell growth and enhanced apoptosis [45]. Pax8-AS1 demonstrated the highest diagnostic value for HCV. Previous reports have implicated the lncRNA Pax8-AS1 in papillary thyroid carcinoma, cervical cancer, and pediatric acute lymphoblastic leukaemia [46, 47].

ROC curve study of blood leukocyte lncRNAs showed Pax8-AS1's diagnostic value for HCV, with 96.7 and 81.7% sensitivity and specificity. The current investigation contributed to developing new HCV diagnostic biomarkers. Based on earlier studies on PAX8-AS1 and cancer risk, it was hypothesized that decreasing PAX8-AS1 regulation might impact HCC risk by disrupting PAX8-AS1-PAX8 interaction and PAX8 expression. PAX8 inhibits cell differentiation and death, contributing to cancer etiology [48].

This present study identified that serum LAIR-2 upregulation could be implicated in HCV. Lebbink et al. reported that SF from individuals with RA has markedly increased levels of LAIR-2 [49]. Another study examined LAIR-2 secretion in vivo in healthy control participants and RA patients to determine its efficacy as a biomarker for RA and found elevated levels in pregnant women. Some researchers have found lower LAIR-2 mRNA expression in chorionic villous samples of preeclampsia-prone pregnancies 50. Therefore, investigators have associated the concentrations of sLAIR-1 and LAIR-2 in plasma and urine specimens from rheumatoid arthritis individuals with inflammatory conditions [51]. Achieng et al. investigated the molecular basis of elevated sLAIR2, which may be a receptor inhibitor for LAIR-1[52].

In the present research, there was a negatively correlated between LAIR-2 and hematology laboratory tests in HCV patients. Similarly, the concentrations of sLAIR2 were significantly increased in children with severe malaria anemia (SMA) compared to non-SMA. The findings present a supplementary explanation for the reduced LAIR1 transcripts noted in children with SMA, matching with prior studies in other inflammatory disorders that showed an association between increased sLAIR2 levels and decreased LAIR1 receptor expression [53]. These present findings agree with previous research in individuals with RA, which demonstrated that the amount of LAIR2 in blood was linked to an increase in the shedding of membrane-bound LAIR1 [54].

"In this study, we observed that LAIR-2 had a positive correlation with AST, direct and total bilirubin, and ALP, indicating its potential association with liver dysfunction. On the other hand, LAIR-2 showed an inverse correlation with albumin, which is commonly used as a marker of liver synthetic function.

These findings suggest that LAIR-2 could be involved in liver-related diseases and may serve as a useful biomarker for liver injury.The critical functions of the LAIR-2 as prognostic markers have been previously revealed through extensive preclinical studies. Nonetheless, there was no association between LncRNA-PAX8-AS1 and LAIR-2 mRNA and liver activity or liver fibrosis. In the present research, LAIR-2 co-expressed with PAX8-AS1 efficiently distinguished HCV disease from normal controls with high specificity and sensitivity. Furthermore, they were significantly assessed among patients and differentiated conditions in ROC analysis, indicating diagnostic biomarkers for early detection.

Study limitations should not be declined. The sample size might restrict the study's interpretation. Patient selection bias may be present as we obtained samples from one institution. Applying the research findings to other populations requires care. This study's conclusions must be replicated on a larger scale or in

a varied racial population. Despite the small sample size, the findings improve our understanding of HCV.

5.CONCLUSIONS

This is the initial research to examine lncRNA-PAX8-AS1 and mRNA LAIR-2 in HCV. Additionally, molecular alterations in lncRNAs and mRNAs may shed light on HCV and its complications. LAIR-2 may regulate from interaction between collagen and LAIR-1. The present study demonstrated that up-regulation of LAIR-2 on the cell surface dramatically inhibited LAIR-1 binding to collagen, making LAIR2 biomarkers for HCV immune infiltration assessment. LAIR2 may indicate exhausted T cell populations, improving patient survival.

Acknowledgements: None.

Conflict of interest: There are no conflicts of interest.

Funding: None.

Author Contribution: Aya M. Ibrahim.; investigation, methodology, resources, designing the initial draft formal analysis; data curation; software; visualization.; Olfat Gamil Shaker.; investigation methodology, resources, supervision of the practical part and Project administration, Abeer I. Abd El-Fattah.; investigation, review of the manuscript and editing it. Ahmed A. Youssef.; Reviewed the manuscript and helped in editing it. All authors revised and approved the final paper**.**
The authors declare that there are no conflicts of interest. All the authors, Aya Mohamed Ahmed Ibrahim, Abeer I. Abd El-Fattah, Olfat Gamil Shaker and Ahmed A. Youssef agree that it should be submitted to this journal.

List of Abbreviations: Hepatitis C virus (HCV), Long noncoding RNAs (lncRNAs), Cluster differentiation (CD8+), Leukocyte-associated immunoglobulin-like receptor (LAIR) Paired-box gene 8-antisense RNA 1(PAX8-AS1), Regulatory T cells (Tregs), Cholangiocarcinoma (CCA), microRNAs (miRNAs), complete blood count(CBC).

REFERENCES

1. Kishta S, Tabll A, Omanovic Kolaric T, Smolic R, Smolic M. Risk factors contributing to the occurrence and recurrence of hepatocellular carcinoma in hepatitis C virus patients treated with direct-acting antivirals. Biomedicines. 2020;8(6):175.

2. Papatheodoridi A, Papatheodoridis G. Hepatocellular carcinoma: the virus or the liver? Liver International. 2023; 43:22-30.

3. Henriot P, Castry M, Luong Nguyen LB, Shimakawa Y, Jean K, Temime L. Meta-analysis: Risk of hepatitis C virus infection associated with hospital-based invasive procedures. Wiley Online Library; 2022.

4. Garvey CM. An Exploration of the Influence of Stigma and Trauma in the Illness Representations of those Veterans who Decided to Initiate Treatment for Hepatitis C Virus: Northeastern University; 2020.

5. Rockey DC, Friedman SL. Fibrosis regression after eradication of hepatitis C virus: from bench to bedside. Gastroenterology. 2021;160(5):1502-20. e1.

6. Hofmann M, Tauber C, Hensel N, Thimme R. CD8+ T cell responses during HCV infection and HCC. Journal of Clinical Medicine. 2021;10(5):991.

7. Klöhn M, Schrader JA, Brüggemann Y, Todt D, Steinmann E. Beyond the usual suspects: hepatitis e virus and its implications in hepatocellular carcinoma. Cancers. 2021;13(22):5867.

8. Zhou Y, Zhang Y, Moorman JP, Yao ZQ, Jia ZS. Viral (hepatitis C virus, hepatitis B virus, HIV) persistence and immune homeostasis. Immunology. 2014;143(3):319-30.

9. Rios DA, Valva P, Casciato PC, Frias S, Soledad Caldirola M, Gaillard MI, et al. Chronic hepatitis C liver microenvironment: role of the Th17/Treg interplay related to fibrogenesis. Scientific Reports. 2017;7(1):13283.

10. Jenne CN, Kubes P. Immune surveillance by the liver. Nature immunology. 2013;14(10):996-1006.

11. Hakim MS, Rahmadika N, Jariah RO. Expressions of inhibitory checkpoint molecules in acute and chronic HBV and HCV infections: Implications for therapeutic monitoring and

personalized therapy. Reviews in Medical Virology. 2020;30(2):e2094.

12. Beauchamp T. Investigating the role of inhibitory and immunoregulatory pathways in the development of memory CD8 T cells following acute infection. 2020.

13. Marchiano M, Iervasi E, Pesce G, Rumbullaku M, Foti C, Fumarulo R, et al. Increased concentration of soluble leukocyte-associated immunoglobulin-like receptor (LAIR) in sera from patients with blistering diseases: possible pathophysiological implications? Minerva Medica. 2022.

14. Singh A, Mommers-Elshof E, Vijver SV, Jansen JHM, Gonder S, Lebbink RJ, et al. Leukocyte-associated immunoglobulin-like receptor-1 blockade in combination with programmed death-ligand 1 targeting therapy mediates increased tumour control in mice. Cancer immunology, immunotherapy : CII. 2024;73(1):16.

15. Simone R, Pesce G, Antola P, Merlo DF, Bagnasco M, Saverino D. Serum LAIR-2 is increased in autoimmune thyroid diseases. PLoS One. 2013;8(5):e63282.

16. Chen Z, Yu M, Yan J, Guo L, Zhang B, Liu S, et al. PNOC expressed by B cells in cholangiocarcinoma was survival related and LAIR2 could be a T cell exhaustion biomarker in tumor microenvironment: characterization of immune microenvironment combining single-cell and bulk sequencing technology. Frontiers in Immunology. 2021;12:647209.

17. Vishnubalaji R, Shaath H, Alajez NM. Protein coding and long noncoding RNA (lncRNA) transcriptional landscape in SARS-CoV-2 infected bronchial epithelial cells highlight a role for interferon and inflammatory response. Genes. 2020;11(7):760.

18. Carnero E, Barriocanal M, Prior C, Pablo Unfried J, Segura V, Guruceaga E, et al. Long noncoding RNA EGOT negatively affects the antiviral response and favors HCV replication. EMBO reports. 2016;17(7):1013-28.

19. Huang Jf, Guo Yj, Zhao Cx, Yuan Sx, Wang Y, Tang Gn, et al. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. Hepatology. 2013;57(5):1882-92.

20. Shen Y, Tong Z, Zhou Y, Sun Y, Xie Y, Li R, et al. Inhibition of lncRNA-PAX8-AS1-N directly associated with VEGF/TGF-β1/8-OhdG enhances podocyte apoptosis in diabetic nephropathy. Eur Rev Med Pharmacol Sci. 2020;24(12):6864-72.

21. Yu X, Cao Y, Tang L, Yang Y, Chen F, Xia J. Baicalein inhibits breast cancer growth via activating a novel isoform of the long noncoding RNA PAX8-AS1-N. Journal of cellular biochemistry. 2018;119(8):6842-56.

22. Jiang Q, Sun L, Lu Y, Han S, Hou L, Lou K, et al. Identification of related long non-coding RNAs and mRNAs in subclinical hypothyroidism complicated with type 2 diabetes by transcriptome analysis—a preliminary study. Endokrynologia Polska. 2020;71(3):213-26.

23. Vračarić V, Dejanović B, Janjić N, Zirojević M, Savić Ž, Latinović-Bosnjak O, et al. Non-invasive Assessment of Liver Damage Degree in Patients with Viral Hepatitis: Is it Feasible and Acceptable? Hepatitis Monthly. 2024;24(1).

24. Souza GHDPE, Silva LD, Vieira DA, Rocha GA, Lima AS, Vidigal PVT. High-density lipoprotein cholesterol and systemic arterial hypertension are associated with hepatic necroinflammatory activity in patients with chronic hepatitis C. Arquivos de Gastroenterologia. 2023;60(3):287-99.

25. MA Ibrahim A, Ghazy AN, Youssef AA. Hepatocellular Carcinoma Incidence and Risk Factors: Insights into the Significance of Genetic Heterogeneity. ERU Research Journal. 2023;2(3):483-501.

26. Zhang Y, Lv K, Zhang C, Jin B, Zhuang R, Ding Y. The role of LAIR-1 (CD305) in T cells and monocytes/macrophages in patients with rheumatoid arthritis. Cellular Immunology. 2014;287(1):46-52.

27. Dai L, Lue SW, Hansenne-Cervantes I, Karas C, Iyke NE, Parish A, et al. Active Peptides and

Motifs within Collagen and Other ECM Proteins. American Journal of Molecular Biology. 2023;13(4):241-60.

28. Greeviroj P, Lertussavavivat T, Thongsricome T, Takkavatakarn K, Phannajit J, Avihingsanon Y, et al. The world prevalence, associated risk factors and mortality of hepatitis C virus infection in hemodialysis patients: a meta-analysis. Journal of Nephrology. 2022;35(9):2269-82.

29. Aghaei AM, Gholami J, Sangchooli A, Rostam-Abadi Y, Olamazadeh S, Ardeshir M, et al. Prevalence of injecting drug use and HIV, hepatitis B, and hepatitis C in people who inject drugs in the Eastern Mediterranean region: a systematic review and meta-analysis. The Lancet Global Health. 2023;11(8):e1225-e37.

30. Niu J, Lin Y, Guo Z, Niu M, Su C. The Epidemiological investigation on the risk factors of hepatocellular carcinoma: a case–control study in Southeast China. Medicine. 2016;95(6).

31. Jumabayeva A, Nersesov A, Kulzhanov M, Nefedova M, Nuraliyeva G, Rakhimbekova G, et al. Prevalence of viral hepatitis B, C, and D in Kazakhstan. The Scientific World Journal. 2022;2022(1):9102565.

32. Schott E, Witt H, Neumann K, Taube S, Oh D-Y, Schreier E, et al. A Toll-like receptor 7 single nucleotide polymorphism protects from advanced inflammation and fibrosis in male patients with chronic HCV-infection. Journal of hepatology. 2007;47(2):203-11.

33. Bakr I, Rekacewicz C, El Hosseiny M, Ismail S, El Daly M, El-Kafrawy S, et al. Higher clearance of hepatitis C virus infection in females compared with males. Gut. 2006;55(8):1183-7.

34. Qureshi H, Arif A, Riaz K, Alam SE, Ahmed W, Mujeeb SA. Determination of risk factors for hepatitis B and C in male patients suffering from chronic hepatitis. BMC Research Notes. 2009;2:1-6.

35. El-Deeb GS, EL-Edel RH, Shehab El-Deen SAE-M, Laban MS. Mean Platelet Volume as a Fibrosis Marker in Patients with Chronic Hepatitis C. Afro-Egyptian Journal of Infectious and Endemic Diseases. 2014;4(4):172-83.

36. Fatima S, Naz L. Differences in clinical and biochemical profiles between acute and chronic hepatitis b and c patients. int j biol res. 2015;3(1):57-61.

37. Cheong JY, Kim DJ, Hwang SG, Yang JM, Kim YB, Park YN, et al. Serum markers for necroinflammatory activity in patients with chronic viral hepatitis and normal or mildly elevated aminotransferase levels. Liver International. 2011;31(9):1352-8.

38. Jain P, Batta AK, Singh P. Comparative study of serum levels of gamma-glutamyl transferase, Aspartate aminotransferase (AST), Alanine Transaminase (ALT), AST: ALT, and bilirubin in patients with chronic hepatitis. Indian J Med Biochem. 2022;26(3):74.

39. Dheyauldeen SO, Nadem AS, Obaid SR, Abdalkareem JS, Majid MT. Study of some physiological parameters in renal failure patients with hepatitis C virus infection. Research Journal of Biotechnology Vol. 2024;19:11.

40. Ali DM, AlMoslemany MA, Mohamed O, Raafat K. Albumin-Bilirubin Score as a Non-Invasive Serum Biomarker for Advanced Liver Fibrosis and Cirrhosis in Egyptian Patients with Chronic Hepatitis C Infection: A Case-Control Study. African Journal of Gastroenterology and Hepatology. 2022;5(2):32-47.

41. Zuckerman E, Zuckerman T, Douer D, Qian D, Levine AM. Liver dysfunction in patients infected with hepatitis C virus undergoing chemotherapy for hematologic malignancies. Cancer: Interdisciplinary International Journal of the American Cancer Society. 1998;83(6):1224-30.

42. Kostadinova L, Shive CL, Zebrowski E, Fuller B, Rife K, Hirsch A, et al. Soluble Markers of Immune Activation Differentially Normalize and Selectively Associate with Improvement in AST, ALT, Albumin, and Transient Elastography During IFN-Free HCV Therapy. Pathogens & immunity. 2018;3(1):149-63.

43. Liu G, Tian J. PAX8-AS1/microRNA-25–3p/LATS2 regulates malignant progression of ovarian cancer via Hippo signaling. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis. 2024;829:111858.

44. Liang J, Deng Y, Zhang Y, Wu B, Zhou J. Identification and clinical value of a new ceRNA axis (TIMP3/hsa-miR-181b-5p/PAX8-AS1) in thyroid cancer. Health science reports. 2024;7(2):e1859.

45. Zhou P, Xu T, Hu H, Hua F. Overexpression of PAX8-AS1 inhibits malignant phenotypes of papillary thyroid carcinoma cells via miR-96-5p/PKN2 Axis. International Journal of Endocrinology. 2021;2021(1):5499963.

46. Bahari G, Hashemi M, Naderi M, Sadeghi-Bojd S, Taheri M. Long non-coding RNA PAX8-AS1 polymorphisms increase the risk of childhood acute lymphoblastic leukemia. Biomedical Reports. 2018;8(2):184-90.

47. Zhang Y, Li F, Chen J. MYC promotes the development of papillary thyroid carcinoma by inhibiting the expression of lncRNA PAX8-AS1: 28. Oncology Reports. 2019;41(4):2511-7.

48. Chaves-Moreira D, Morin PJ, Drapkin R. Unraveling the mysteries of PAX8 in reproductive tract cancers. Cancer research. 2021;81(4):806-10.

49. Lebbink RJ, van den Berg MC, de Ruiter T, Raynal N, van Roon JA, Lenting PJ, et al. The soluble leukocyte-associated Ig-like receptor (LAIR)-2 antagonizes the collagen/LAIR-1 inhibitory immune interaction. The Journal of Immunology. 2008;180(3):1662-9.

50. Founds SA, Conley YP, Lyons-Weiler JF, Jeyabalan A, Hogge WA, Conrad KP. Altered global gene expression in first trimester placentas of women destined to develop preeclampsia. Placenta. 2009;30(1):15-24.

51. Olde Nordkamp MJ, van Roon JA, Douwes M, de Ruiter T, Urbanus RT, Meyaard L. Enhanced secretion of leukocyte-associated immunoglobulin-like receptor 2 (LAIR-2) and soluble LAIR-1 in rheumatoid arthritis: LAIR-2 is a more efficient antagonist of the LAIR-1-collagen inhibitory interaction than is soluble LAIR-1. Arthritis and rheumatism. 2011;63(12):3749-57.

52. Guo N, Zhang K, Gao X, Lv M, Luan J, Hu Z, et al. Role and mechanism of LAIR-1 in the development of autoimmune diseases, tumors, and malaria: A review. Current research in translational medicine. 2020;68(3):119-24.

53. Perbellini O, Falisi E, Giaretta I, Boscaro E, Novella E, Facco M, et al. Clinical significance of LAIR1 (CD305) as assessed by flow cytometry in a prospective series of patients with chronic lymphocytic leukemia. haematologica. 2014;99(5):881.

54. Nordkamp MJO, van Roon JA, Douwes M, de Ruiter T, Urbanus RT, Meyaard L. Enhanced secretion of leukocyte-associated immunoglobulin-like receptor 2 (LAIR-2) and soluble LAIR-1 in rheumatoid arthritis: LAIR-2 is a more efficient antagonist of the LAIR-1–collagen inhibitory interaction than is soluble LAIR-1. Arthritis & Rheumatism. 2011;63(12):3749-57.