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GENETIC POLYMORPHISM AND EXPRESSION OF GAS5 AND MIR-137 RELATED TO SUSCEPTIBILITY OF HEPATITIS B VIRUS INFECTION

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ABSTRACT:
Background: Long non coding RNA growth arrest specific 5 (GAS5) have been related to liver fibrosis through epigenetic mechanisms like promoter hyper methylation. On the other hand, microRNA 137 (miR-137) targets genes involved in viral replication and immune response, as well as modulates HBV gene expression. Genetic polymorphisms of these two microRNAs may influence their expression and thus HBV susceptibility.
Aim: This study aims to detect the implications of GAS5 rs2067079 and miR- 137 rs1625579 in susceptibility to HBV infection as well expression levels of these microRNAs.
Methods: This case control investigation encompassed 50 healthy individuals plus 50 HBV patients, GAS5 and miR-137 level detected via a twostep RT-qPCR approach, while TaqMan SNP genotyping assays were used to genotype both rs2067079 and rs1625579.
Results: The male/female ratio was 2.13 in patient group and regarding sex and age, a notable variation was noted between instances. Liver function tests showed significant differences between patients and controls. The recessive T allele of rs2067079 was found to be a risk factor for HBV infection (OR : 1.45; CI : 2.03-4.78), as well CT+TT genotypes in dominant model showed increased risk of HBV infection. While T allele of rs1625579 was a HBV infection risk factor (OR: 2.05; CI: 1.62-2.49), and GT+TT genotypes in dominant model showed increased risk of HBV infection. Regarding GAS5 and miR-137 expression, they considerably diminished within patients versus controls, where GAS5 level substantially lowered in TT comparing to CT and CC genotypes of rs2067079, while miR-137 was diminished in TT vs. GT and GG genotypes of rs1625579 in HBV instances.
Conclusion: Current investigation revealed that rs2067079 and rs1625579 contributed to the HBV pathogenesis and Gas5 and miR-137 expression respectively, therefore, they might use as possible genetic indicators of HBV vulnerability.

KEYWORDS: Gas5; miR-137; rs2067079; rs1625579; Hepatitis

INTRODUCTION:

Chronic hepatitis B virus (HBV) infections can result in serious clinical issues, such as cirrhosis, fibrosis, and hepatocellular carcinoma (HCC) [1]. Development of HBV infection can be influenced by a variety of factors. Host genetic and demographic characteristics are among the most significant determinants of HBV clinical course. For instance, the development of HBV infection in various populations can be influenced by single nucleotide polymorphisms (SNPs) in the genetic and genes regulatory elements that accountable for viral infection as well as alteration in antiviral cytokines expression [2].
Long non-coding RNAs (lncRNAs) are RNA molecules with lengths exceeding 200 nucleotides, that once known as non-proteins coding RNA, they are now recognized as crucial regulators of gene expression and cellular function such as epigenetic regulation of gene expression, post-transcriptional regulation, nuclear architecture & organization, cellular processes & signaling, as well as, subcellular organization [3].
LncRNA growth arrest specific 5 (GAS5) is requisite to growth stopping and cell cycle slow down [4]. Moreover, it regulates gene expression, apoptosis induction, prevents tumor growth, and inhibits T-cell proliferation [5, 6]. GAS5 had been found to play a role at hepatitis C virus (HCV) infection along with liver fibrosis [7]. Despite this, its role in HBV infection remains largely unknown.
MicroRNAs (miRNAs) have a vital function in pathophysiology and pathogenesis of various liver diseases [8, 9]. MiR-137 is appearing as a crucial regulator in hepatic diseases, notably in hepatocellular cancer, liver fibrosis, and non-alcoholic fatty disease [10]. Nonetheless, additional studies are required to entirely comprehend its molecular mechanisms in HBV infection. Several studies have hinted that non-coding RNAs polymorphisms may linked with HBV infection risk [11-13]. Though, the relationship betwixt GAS5 rs2067079 and miR-137 rs1625579 accompanying HBV infection has not yet been investigated in Iraqi population.

PATIENTS AND METHODS

The current case-control study included 50 healthy individuals and 50 HBV patients attending the Al Ramadi Teaching Hospital in Anbar, Iraq. HBV infection was diagnosed based on the hepatitis-B surface-antigen (HBsAg) detection [14]. Each participant provided written informed consent and detailed personal and medical history. Routine laboratory tests to assess liver function were also performed. The control group had normal liver function tests and negative anti-HBs, anti-HBc IgG and HBsAg assessments.

All participants were excluded if they had evidence of other liver illnesses, antiviral therapies or immunosuppressive medications, positive anti-HIV or anti-HCV antibodies, or smoking. Ethics Committee of University of Anbar approved this research (179 in 20/8/2024).

Blood samples handling: 5 ml of venous blood was withdrawn from each subject. 3 ml was placed into tube with serum separator gel, samples allowed to coagulate for 15 minutes before being centrifuged for 10 minutes at 4000×g to serum obtaining, which kept in -20°C.

Enzyme-linked immunosorbent assay (ELISA) was used for assess HBeAg (Sunlong- Biotech, China). Total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were quantitatively measured for all participants according to manufacturer's instructions of ready-to-use kits (Linear Chemicals, Spain), TSB has been calculated in µmol/L units, whereas APL, ALT and AST have been represented as U/L.

The left 2 ml. of blood were placed in anticoagulant tube and used for DNA purification and genotyping, while 0.25 ml. was transferred from anticoagulant tube and mixed with 0.75 ml. TRIzol for RNA purification and genes expression.

Detection of GAS5 and miR-137 expression: Total RNAs were purified from blood samples via TRIzol extraction method. While GAS5 and miR-137 expression were performed via employing the specific primers obtained from Macrogen (Korea) as detailed in Table 1 via twostep RT-qPCR approach (Mic qPCR Cycle, BioMolecular System/ Australia) [15].

Table 1: Primers of gene expression

Primers	Sequences 5′- 3′	References
miR-137	**F: TTATTGCTTAAGAATACGCGTAG** **R: GTGCAGGGTCCGAGGTATTC**	**[16]**
U6	**F: CTCGCTTCGGCAGCACA** **R: AACGCTTCACGAATTTGCGT**	**[16]**
GAS5	**F: CAACTTGCCTGGACCAGCTT** **R: TCAAGCCGACTCTCCATACC**	**[17]**
GAPDH	**F: CTTCTGAGTGGCAGTGATGG** **R: TTTGGAGTCCCTGTTCGTAC**	**[16]**

Genotyping of rs2067079 and rs1625579: DNA purified from whole blood as directed by DNA purification kit manufacturer (Geneaid/ Taiwan),. Concentration and purity of nucleic acids (DNA and RNA) were determined via Nanodrop (Bioneer/Korea) [18]. TaqMan SNP genotyping assays were employed for rs2067079 and rs1625579 genotyping based on manufacturer's instructions and as described via Mahmoud *et al*. [19].

Statistical analysis: The results presented as mean ± S.E., and T-test were employed to determine the significance of the comparison between two samples. Chi-square ($\chi 2$) (IBM SPSS– version 28) was used to determine the significance of different percentages. Whenever a P-value < 0.05 was considered statistically significant.

RESULTS:

This case–control study included 50 patients with chronic HBV infection and 50 control. Among the patients, the male/female ratio was 2.13 in patient group and 2.52 in control. Significant difference was observed between cases regarding sex and age (p= 0.012 and 0.003, respectively) (Table 2). Liver function tests showed significant differences ($p < 0.001$) between patients and controls as shown in Table 3.

Table 2: The age and sex distribution of the HBV patients

Sex No. (%)						
Male	**Female**			**Total**	**χ2**	**P-value**
34 (68)	**16 (32)**			**50 (100)**	**12.96**	**0.012****
Age group (years) No. (%)						
20-29	**30-39**	**40-49**	**≥50**			
17 (34)	**19 (38)**	**11 (22)**	**3 (6)**	**50 (100)**	**16.533**	**0.003****
**** (P≤0.01).**						

Table 3: Distribution of LFT levels in HBV patients and control

LFT	Patients (Mean ± SE)	Control(Mean ± SE)	T test	P- value
ALT (U/L)	26.31 ± 4.62	7.19 ± 1.04	3.99	0.004**
AST (U/L)	24.06 ± 2.98	8.29 ± 1.14	4.17	0.0002**
ALP (U/L)	114.86 ± 8.67	63.57 ± 6.92	5.27	0.0003**
TSB (mg/dl)	1.9 ± 0.67	0.81 ± 0.2	3.11	0.007**
** (P≤0.01)				

In HBV instances, GAS5 and miR-137 expression were particularly diminished comparing to controls (Table 4). Table 5 revealed the rs2067079 genotype and allele repetitions for all participants, where T allele prevailed in cases (58% vs 35% in control) and C allele dominated in control (65% vs 42% in cases), additionally C allele was protective agent against HBV (OR: 0.32; CI : 0.17- 0.51), while T allele was risk factor (OR: 1.45; CI: 2.03-4.78). In dominant model (CC vs. CT + TT), rs2067079 genotypes repetitions was significantly different (p = 0.002) between HBV patients and control subjects, and CT+TT genotypes showed increased risk of HBV infection (76%) (Table 5).

Concerning rs1625579, T allele dominated in cases (84% vs 30% in control) and G allele dominated in control (70% vs 46% in cases), moreover G allele was protective factor against HBV infection (OR: 0.90; CI: 0.75-1.02), while T allele was risk factor (OR: 2.05; CI: 1.62-2.49). In dominant model (GG vs GT + TT), rs2067079 genotypes distribution differed substantially (p < 0.001) comparing HBV patients and control, and GT+TT genotypes indicated greater HBV infection risk (84%) (Table 5).

Through examining rs2067079 impact in GAS5 expression, we identified low GAS5 level in TT genotype comparing to CT or CC genotypes (Table 6). Regarding rs1625579 impact in miR-137 expression, TT genotype carriers revealed diminished miR-137 level comparing to GT and GG genotypes amongst HBV instances (Table 7).

Table 4: Comparison of the expression of GAS-5 and miR-137 in HBV patients and control.

Genes	Patients (Mean ± SE)	Control (Mean ± SE)	T test	P- value
GAS-5	0.48± 0.11	1.46 ± 0.82	2.19	0.049*
miR-137	0.69± 0.14	1.25 ± 0.74	1.81	0.031*
* (P≤0.05)				

Table 5: Association of rs2067079 and rs1625579 with the risk of HBV infection.

	Genotypes	Patients No.(%)	Control No.(%)	OR(CI)	χ2	P value
GAS5 rs2067079						
Codominant model	CC	12 (24)	24 (48)		8.201	0.005**
	CT	18 (36)	17 (34)			
	TT	20 (40)	9 (18)			
Dominant model (CC vs. CT + TT)	CC	12 (24)	24 (48)		6.250	0.002**
	CT + TT	38 (76)	26 (52)			
Recessive model (TT vs. CT + CC)	TT	20 (40)	9 (18)		5.877	0.002**
	CT + CC	30 (60)	41 (82)			
Allele	C	42 (0.42)	65 (0.65)	0.32 (0.17-0.51)	10.632	0.0004**
	T	58 (0.58)	35 (0.35)	1.45 (2.03-4.78)		
miR-137 rs1625579						
Codominant model	GG	8 (16)	26 (52)		23.166	0.0008**
	GT	15 (30)	18 (36)			
	TT	27 (54)	6 (12)			
Dominant model (GG vs GT + TT)	GG	8 (16)	26 (52)		14.439	0.0006**
	GT + TT	42 (84)	24 (48)			
Recessive model (TT vs. GT + GG)	TT	27 (54)	6 (12)		19.946	0.0007**
	GT + GG	23 (46)	44 (88)			
Allele	G	46 (0.46)	70 (0.70)	0.90 (0.75-1.02)	27.092	0.0009**
	T	84 (0.84)	30 (0.30)	2.05 (1.62-2.49)		
** (P≤0.01)						

Table 6: Relationship between rs2067079 genotypes and of *GAS5* gene expression

Genotype	Mean ± SE of GAS5 gene expression (Folding)		t-Value	P value
	Patients	Control		
CC	0.29± 0.1 b	1.92± 0.6 a	3.91	0.0003**
CT	0.74± 0.4 b	1.43± 0.7 a	3.21	0.0002**
TT	0.35± 0.2 b	1.65± 0.8 a	3.88	0.0001**
Different letter in same column indicates a substantial difference, **(P≤0.01).				

Table 7: rs1625579 genotypes and miR-137 gene expression relationship

Genotype	Mean ± SE of miR-137 gene expression (Folding)		t-Value	P value
	Patients	Control		
GG	0.71± 0.3 b	1.50± 0.4 a	2.88	0.01**
GT	0.68± 0.4 b	1.75± 0.7 a	3.19	0.0002**
TT	0.39± 0.1 b	0.98± 0.5 a	2.61	0.037 *
Different letter in same column indicates a substantial difference, *(P≤0.05), **(P≤0.01).				

DISCUSSION:

Non-coding RNAs possess vital assignments at HBV related diseases, they regulate gene expression and influence many aspects of the infection process, such as viral replication, immune response, fibrosis and liver cancer development. This study appeared that males were more infected with HBV than females with male to female ratio of approximately 2:1, and the 30–39 years age group was registered the higher percentage (38%) among other age groups (Table 2), these results agreed with other local and international studied [20- 22]. Enhanced liver enzymes may be due to increased production and secretion, or catabolism reduction, in HBV patients, noteworthy altitudes in LFT identified comparing with control (Table 3), these results are in line to previous investigations [23, 24]. These results are expected as the liver is an organ of HBV reproduction, which leads to hepatocyte death and an increase in these molecules' releasing [25].

GAS5 expression was considerably diminished in HBV cases than control, our findings aligned with earlier studies [19, 27]. GAS5 dysregulation indicated in various cancers, cardiovascular conditions, inflammation-associated diseases, and viral infections [28]. Momentously, GAS5 is universally recognized as a tumor suppressor. It had revealed to suppressing liver fibrosis as well inhibiting HCC cells translocation and invasion via sequestrating miR- 21 which effect cell maintaining [27]. GAS5 overexpression inhibits innate immune responses via lowering of IFN- α and IFN- λ amount after viral infection [29].

With regard to miR-137 expression, our investigation indicates diminished miR- 137 level in HBV instances contrasting to control. HBV X protein (HBx) has been shown to alter many genes and epigenetic molecules expression and function such as lncRNAs and miRNAs, leading to multiple pathways malfunction. HBx knockdown reduced the miR-137 methylation and reconditioned its expression. [15]. Further, table 4 indicated a potential positive relationship between GAS5 and miR- 137 levels, likewise Wozniak, M., & Czyz, M. reported positive relation betwixt GAS5 and miR- 137 [30].

Regarding rs2067079, CT and TT genotypes linked with elevated HBV risk whilst CC genotype was protective agent against HBV, this finding is consistent with a previous study conducted in Egyptian cohort. [19]. The GAS5 polymorphism found to affected hepatocellular carcinoma, colorectal cancer, and oral cancer. [31]. Furthermore, our findings revealed that the risky rs2067079 TT genotype had significantly lower serum GAS5 expression than CT or CC genotypes in HBV instances. The guiding ChIP - seq facts about various cells kinds provided that rs2067079 locus identified as effective promoter. Due to possibility that genetic variations in regulatory components could regulate expression levels, studied SNP may impact GAS5 transcriptional behavior. Moreover, rs2067079 had wealthy influence on GAS5 secondary structure that is vital to their efficiency [32].

Mutated T allele and TT genotype of rs1625579 appeared primarily detected in HBV instances in contrast to control. Further, we identified a worthy association betwixt miR-137 level diminish and TT genotype in contrast to GT and GG genotypes in HBV instances, these results were consistent with earlier research [19], proposing a possible role of studied SNP in HBV development. The rs1625579 SNP located in miR-137 intron, related to schizophrenia development in European and Asian cohorts [33]. Genetic variations in miR-137 can interrupt its normal function via impacting its transcription or its bind capacity to target genes, which can lead to unusual expression and possibly engagement to various disorders. It was denoted that miR-137 non-coding elements variants result in faulty mRNA structure, splicing, and stability [34].

Considering our observations in HBV infections, further with bigger cohort and larger independent population investigations are required to validate current results. Furthermore investigations are needed to assess rs1625579 and rs2067079 precisely roles in the tissue grade.

CONCLUSION:

To sum up, GAS5 and miR-137 expression were diminished in HBV instances. Furthermore, rs2067079 and rs1625579 participated HBV infections and may serving as prospective markers to HBV susceptibility. CC genotype of rs2067079 was a protective agent whilst CT and TT genotypes exhibited a raised HBV risk. On the other hand, GG genotype of rs1625579 signified as protective agent whilst TT genotype noted as HBV risk factor. These conceivable markers may offer a preferable perception of HBV infections and could facilitate the development of potentially curative strategies.

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