Association of AURKA Polymorphisms with the Development and Progression of Hepatocellular Carcinoma in Egyptian Patients

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ABSTRACT

Hepatocellular carcinoma (HCC) is a highly fatal disease and one of the most prevalent cancers globally. Aurora kinase A (AURKA) also known as STK15, BTAK, and AIKI plays a crucial role in oncogenic processes by promoting mitotic progression and chromosomal stability in centrosomes and mitotic spindles. HCC group consists of 47 individuals, including 20 females and 27 males with a mean age of 60.62 ± 8.06 years. The distribution of HCC cases was based on age (ranging from 43 to 80 years) and hepatitis C virus (HCV) status (19 Positive and 28 Negative). Genotyping of AURKA T91A (Ile31Phe) rs2273535 T/A and G169A (rs1047972 G/A) Val57Ile polymorphisms was performed using the PCR-RFLP test. Our results revealed a significant increase in the median values of the traditional tumor biomarker (AFP) in the HCC group compared to the control group ($p < 0.001$). However, the analysis of allele frequencies and genotypes ARUKA T91A (Ile31Phe) rs2273535 T/A and G169A (rs1047972 G/A) Val57Ile between the HCC patients and control group. These findings suggest that while AURKA polymorphisms may not be directly associated with HCC susceptibility in Egyptian patients, further investigations are warranted to explore the potential role of AURKA in HCC development and progression, considering its involvement in mitotic processes and chromosomal stability.

Keywords: Hepatocellular carcinoma, AURKA, polymorphisms, alpha-fetoprotein, case-control study.

1. INTRODUCTION

Hepatocellular carcinoma is a prevalent and highly fatal cancer, ranking as the third most common disease worldwide (Samant *et al*., 2021). Hepatocellular carcinoma (HCC) is a common disorder worldwide and ranks 2nd and 6th most common cancer among men and women in Egypt (Omar *et al*., 2013). Several risk factors, including chronic hepatitis B and C virus infections, excessive alcohol consumption, cirrhosis, exposure to carcinogens such as aflatoxin B1, and genetic and epigenetic changes, have been associated with hepatocarcinogenesis. However, it is worth noting that a significant proportion of infected individuals with HBV or HCV do not develop HCC during their lifetime, indicating that inherited factors may play a crucial role in determining an individual's susceptibility to HCC.

Hepatocarcinogenesis is a complex, multistep process involving factors such as hepatitis C virus (HCV) or chronic hepatitis B virus (HBV) infection, cirrhosis, exposure to carcinogens, and a large number of single nucleotide polymorphisms (SNPs)(Weng *et al*., 2010). Single-nucleotide polymorphisms, or SNPs, are the most common kind of genetic variation occurs when a single nucleotide in a gene's shared nucleotide sequence differs among individuals or paired chromosomes of the same species. SNP

located in gene promoters or other regulatory can impact gene expression thereby influencing the onset and progression of specific diseases (Cheng *et al*., 2013).

The group of serine/threonine kinases identified as Aurora kinases, consists of Aurora A (AURKA), Aurora B (AURKB), and Aurora C (AURKC), playing a vital role in cell division by regulating mitosis, the process of chromosomal segregation. In addition to mitosis, Aurora kinases have been implicated in the regulation of meiosis (Tang *et al*., 2017).

Single nucleotide polymorphisms (SNPs) of AurkA and PNPLA3 have been linked in several earlier studies to ovarian carcinoma, gastric cancer, and hematological malignancies, among other cancers (Huang *et al*., 2012)

Functional investigations comparing the 31Ile variant with its 31Phe counterpart, have shown that the former exhibits increased amplification and aneuploidy in human colon cancers. Furthermore, it enhances both cell proliferation *in vitro* and tumorigenicity in nude mice to a greater extent. Although AURKA 31Phe is expressed at a level similar to AURKA 31Ile, the 31Phe variant exhibits a stronger affinity for the E2-ubiquitin-conjugating enzyme UBE2N in human cells and colocalizes more prominently with 31Ile in the centrosomes. The ability of AURKA to stimulate cell growth and transformation is inversely associated with this interaction.

We hypothesized that polymorphisms in AurkA may play a crucial role in the development of HCC. While extensive research has been conducted on the role of AurkA in human cancer metastasis, prognosis investigation into the association of AurkA gene SNPs, environmental carcinogens, and susceptibility in HCC, as well as the clinical characteristics remain limited. Therefore, we conducted a case-control study to examine the association between AurkA SNPs and susceptibility to HCC, as well as the pathological progression of HCC, in Egyptian patients.

2. MATERIALS AND METHODS

2.1 Study subjects

Ninety-four Egyptian patients receiving treatment at Menoufia University's National Liver Institute Hospital and 47 healthy individuals served as the subjects of this case-control research. The Institutional Ethics Committee approved the study code of the ph.D proposal: SREC290124B10051 by the date 29-01-4024, and all participants provided informed consent. Each individual underwent a comprehensive medical history, clinical assessment, and laboratory tests including CBC, liver enzymes analysis, kidney function markers, alpha-fetoprotein (AFP) level measurement, and viral hepatitis indicators. Ll types of malignancy were excluded except for the subjects of HCC. Any remaining serum samples were stored at -80 ºC for future analysis.

2.2. Sample Collection:

Peripherally blood samples were withdrawn from all subjects for routine assessments, which included CBC, CRP, ALT, AST, prothrombin time assessment, and kidney function tests using commercially available assays.

2.3 AURKA genotyping:

The ABIOpureTM total DNA extraction kit (Bothell, WA 98021 USA) was used to extract DNA from peripheral whole blood in accordance with the kit methodology. Genotyping of ARUKA T91A (Ile31Phe) rs2273535 T/A and G169A (rs1047972 G/A) Val57Ile performed using a restriction fragment length polymorphism (PCR-RFLP) analysis. The following primers were used for the Phe31Ile SNP investigation:

Forward: 5′-
CTTTCATGAATGCCAGAAAGTT-3′

Reverse: 5′-
CTGGGAAGAATTTGAAGGACA-3′

For the Val57Ile SNP investigation, the following primers were used:

Forward: 5′-
CTTTCATGAATGCCAGAAAGTT-3′

Reverse: 5′-
CTGCTTCTGATTCTGAACCGGCTTG-3′

The resulting fragments were analyzed by electrophoresis on a 3% agarose gel.

2.4. Data analysis

The statistical analysis of the collected data was conducted using the IBM SPSS software package, version 20.0 (IBM Corp., Armonk, NY). Percentage and numerical data were used to convey quantitative information. The normal distribution of the data was evaluated through the Shapiro-Wilk test. Descriptive statistics, including the range (minimum and maximum values), mean, standard deviation, and median, were employed to characterize quantitative data. For the comparison of normally distributed quantitative data between the two study groups, the independent t-test was utilized. A significance level of $p < 0.05$ was established for all analyses.

3. RESULTS:

The comparison of the demographic data between the two groups is summarized in Table (1). The control group consisted of 47 participants with an age ranging from 43.0 to 80.0 years, and a mean age of 59.43 ± 9.99 years. The patient group included 47 patients, with an age range of 44.0 to 77.0 years, and a mean age of 60.62 ± 8.06 years.

Table (2) presents the comparison of serum creatinine between the HCC and control groups. The difference in serum creatinine between the two groups was not statistically significant ($p = 0.183$). However, serum AST levels were significantly high in the patient group compared to the control group ($p < 0.001$). Similarly, the serum ALT level was significantly elevated in the patient group compared to the control group ($U = 497.0$, $p < 0.001$). However, On the other hand, there was no significant difference in total bilirubin levels between the research groups ($p = 0.244$).

The overall findings indicates that the mean levels of direct bilirubin, AST (SGOT), and ALT (SGPT) were significantly higher in the HCC group compared to the other groups. In contrast, albumin levels in the HCC were were significantly lower. However, there was no discernible difference in the levels of total bilirubin or creatinine among the groups under study.

Significant differences in AFP levels between the two groups. The mean AFP level in the patient group was significantly higher compared to the control group. The patient group had a mean AFP level of 1105.77 ± 2554.81, while the the control group had a mean AFP level of 5.94 ± 1.0. These differences were statistically significant ($p < 0.001$), as shown in Table 5 and Figs. 7-11.

3.1 AURKA genotyping

ARUKA T91A (Ile31Phe) rs2273535 T/A and G169A (rs1047972 G/A) Val57Ile genotyping was carried out using a polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) analysis.

The ARUKA T91A genotyping in patients and controls in accordance with Hardy Weinberg Equilibrium ($p > 0.05$). In the HCC group, the frequency of the TT, TA, and AA genotypes was 6.4%, 47.4%, and 36.2%, respectively, while in the control group the frequency was 14.9%, 63.8%, and 21.3%, respectively ($p = 0.1688$). No statistically significant association was found between the ARUKA T91A allele or genotype with the risk of HCC (Table 3 and Fig. 2).

In contrast, the genotype frequencies of ARUKA G169A in the HCC group were in accordance with the equilibrium criterion. contrast to 53.2%, 40.4%, and 6.4% The control group consisted of 19 (40.4%) GG, 26 (55.3%) GA, and 2 (4.3%) AA (observed) genotypes. There was a statistically significant positive association between the risk of HCC and the AA genotype and the A allele in the total sample (Table 2 and Fig. 3).

4. DISCUSSION

Hepatocellular carcinoma (HCC) is a significant global health concern (Jemal et al., 2017), with high incidence rates particularly in regions with high rates of hepatitis B and hepatitis C virus infections (El-Serag, 2012). The use of serum alpha-fetoprotein (AFP) in combination with hepatic ultrasonography is

recommended for HCC diagnosis and surveillance (Singal *et al*., 2009). While ultrasound is the main surveillance method, computed tomography (CT) and magnetic resonance imaging (MRI) are also employed in clinical practice due to their higher detection rates (Kobayashi *et al*., 1985).

AURKA, a cell cycle-regulated kinase plays a crucial role in various cellular processes, included mitotic entry, centrosome maturation, bipolar spindle formation, and chromosomal separation (Goldenson and Crispino, 2015). Increased expression of AURKA has been observed in several types of cancer, and it exhibits oncogenic activity by regulating tumor-suppressive and oncogenic proteins (Nikonova *et al*., 2013; Katayama and Sen, 2010), Two common non-synonymous SNPs F31I and V57I in the AURKA gene have been extensively studied. On the other hand, F31I SNP has been associated with a modest protective effect against cancer development in several meta-analyses. Gene polymorphism is a key factor that may enhance a person's risk of developing cancer (Sherwood, 2015; Cong *et al*., 2013).

This study investigated the association between AURKA polymorphisms and the development of hepatocellular carcinoma in an Egyptian population. The results did not support a significant correlation between the AURKA rs1047972 variation and HCC susceptibility in the Egyptian population. The frequency of different genotypes did not show significant differences between the control and patient groups. These findings contradict a previous study by Gu *et al*. (2007) that reported an association between the AURKA rs1047972 mutation and cancer risk.

Similarly, the AURKA rs2273535 polymorphism was not associated with an increased risk of HCC in this study. This result is in contrast to studies that have found an increased risk of breast cancer and oral cancer in individuals with the AURKA rs2273535 polymorphism (Dai *et al*., 2014; Lee et al, 2015. Our results, however, the findings of this study align with those of Wand *et al*. (2018), who reported an association between the AURKA rs2273535 polymorphism and HCC susceptibility (Bin Wang1 *et al*., 2018).

5. CONCLUSION

This study did not find a significant association between the AURKA rs1047972 and rs2273535 polymorphisms and the risk of developing HCC in the Egyptian population. These findings contribute to the understanding of the genetic factors involved in HCC development and suggest that the AURKA polymorphisms may have different effects depending on the specific cancer type and population studied. Further research with larger sample sizes and diverse populations is warranted to validate these findings and explore the potential role of AURKA in HCC.

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7. AUTHOR CONTRIBUTIONS:

The conception and design of this study were collaboratively developed by all authors. H.A. was responsible for the collection of samples, while material preparation and data analysis were conducted by H.A. and M.S. The manuscript drafting and revisions were equally shared among F.A., M.E., M.S., and G.B. All authors provided valuable input and feedback during the manuscript's evolution, and they collectively approved the final version of the manuscript.

8. COMPLIANCE WITH ETHICAL STANDARDS

8.1. Funding:

Not applicable

8.2. Conflict of interest:

The authors declare that there are no conflicts of interest**.**

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Table 1. Demographic data between the studied groups

	Patients (n = 47)		**Control (n = 47)**		**Test of sig.**	**P**
	No.	**%**	**No.**	**%**		
Gender						
Male	27	78.7	21	44.7	χ^2= 11.525*	0.345
Female	20	21-3	26	55.3		
Age (years)						
Min. – Max.	44.0 – 77.0		43.0 – 80.0		t= 0.637	0.526
Mean ± SD.	8.06±60.62		9.99±59.43			

χ^2: **Chi-square test** **t: Student t-test** p: p-value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$

Table (2): Comparison between the two studied groups according to biochemical pareameters.

Biochemical pareameters	Patients (n = 47)	Control (n = 47)	Test of sig.	p
Creatinine				
Min. – Max.	1.0 – 2.10	0.70 – 1.20	U= 931.0*	0.181
Mean ± SD.	0.20±1.28	0.16±0.98		
Median (IQR)	1.3(1.2– 1.4)	1.0(0.9 – 1.10)		
AST				
Min. – Max.	13.0 – 210.0	20.0 – 40.0	U= 352.50*	<0.001*
Mean ± SD.	36.34±58.28	5.79±32.64		
Median (IQR)	50.0(40.0 – 68.0)	34.0(30.0 – 37.0)		
ALT				
Min. – Max.	11.0 – 239.0	11.0 – 33.0	U= 497.0*	<0.001*
Mean ± SD.	38.48±48.11	6.71±26.13		
Median (IQR)	40.0(30.0 – 53.0)	28.0(21.0 – 32.0)		
Total bilirubin				
Min. – Max.	0.52 – 3.50	0.70 – 1.20	U= 951.50	0.244
Mean ± SD.	0.68±1.38	0.12±1.08		
Median (IQR)	1.20(0.9 – 1.85)	1.10(1.0 – 1.2)		
Direct bilirubin				
Min. – Max.	0.11 – 2.10	0.06 – 0.30	U= 337.00	<0.001*
Mean ± SD.	0.44±0.57	0.08±0.19		
Median (IQR)	0.40(0.24 – 0.75)	0.20(0.10 – 0.24)		
Albumin				
Min. – Max.	2.10 – 4.40	3.50 – 5.50	t= 8.438*	<0.001*
Mean ± SD.	0.61±3.24	0.61±4.31		
Median (IQR)	3.20(2.80 – 3.70)	4.40(3.7 – 4.9)		
AFP				
Min. – Max.	2.40 – 9902.0	4.30 – 8.0	U= 277.0	<0.001*
Mean ± SD.	2554.81±1105.77	1.0±5.94		
Median (IQR)	104.30(15.1 – 633)	6.0(5.25 – 6.60)		

Table 3: ARUKA gene comparison between the two research groups.

Genetics	Patients (n = 47)		Control (n = 47)		χ^2	p
	No.	%	No.	%		
AURKA rs2273535						
TT	3	6.4	7	14.9	3.573	0.168
TA	27	57.4	30	63.8		
AA	17	36.2	10	21.3		
HWP1	p_1= 0.074		p_1= 0.053			
Allele						
A	33	35.1	44	46.8	2.662	0.103
T	61	64.9	50	53.2		
AURKA rs1047972						
GG	19	40.4	25	53.2	2.154	McP =0.344
GA	26	55.3	19	40.4		
AA	2	4.3	3	6.4		
HWP	p_1= 0.061		p_1= 0.808			
Allele						
G	64	68.1	69	73.4	0.643	0.423*
A	30	31.9	25	26.6		

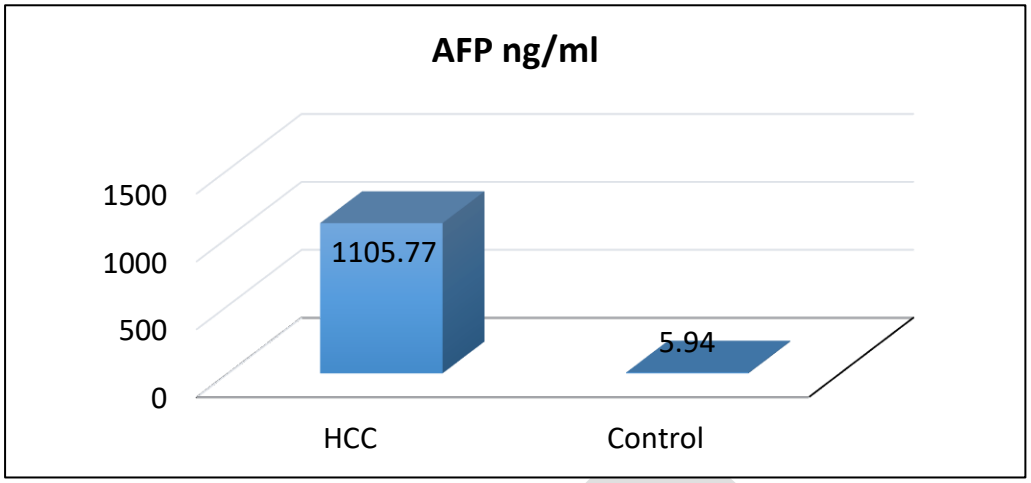

Fig. (1): Comparison between the two studied groups according to AFP.

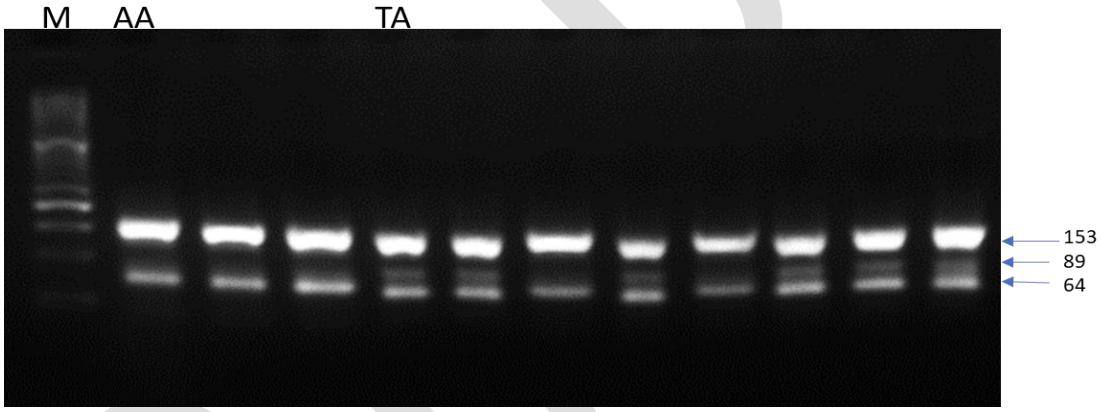

Fig. (2): PCR-RFLP analysis of the ARUKA T91A (Ile31Phe) rs2273535 T/A polymorphism. ApoI restriction enzyme cannot cleave the AA genotype and generates just one band (153 bp). While the TT allele is cleaved by an enzyme and yields two fragments (89 bp and 64 bp). The heterozygote TA genotype generates three bands (153 bp, 89 bp, 64 bp). Lane M = 50 bp DNA ladder.

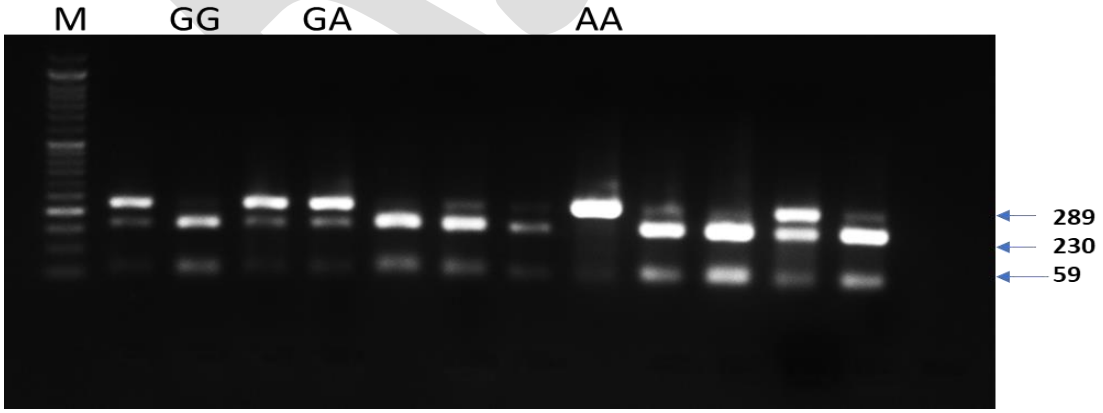

Fig. (3): PCR-RFLP analysis of the ARUKA G169A (rs1047972 G/A) Val57Ile mutation. The AA genotype cannot be cleaved by the BstU I restriction enzyme with a single band that is 289 bp long. *BstUI*, however, cleaves the G allele, producing two pieces (230 bp and 59 bp). Three bands are produced by the heterozygote (289 bp, 230 bp, and 59 bp). Lane M = 50 bp ladder DNA marker

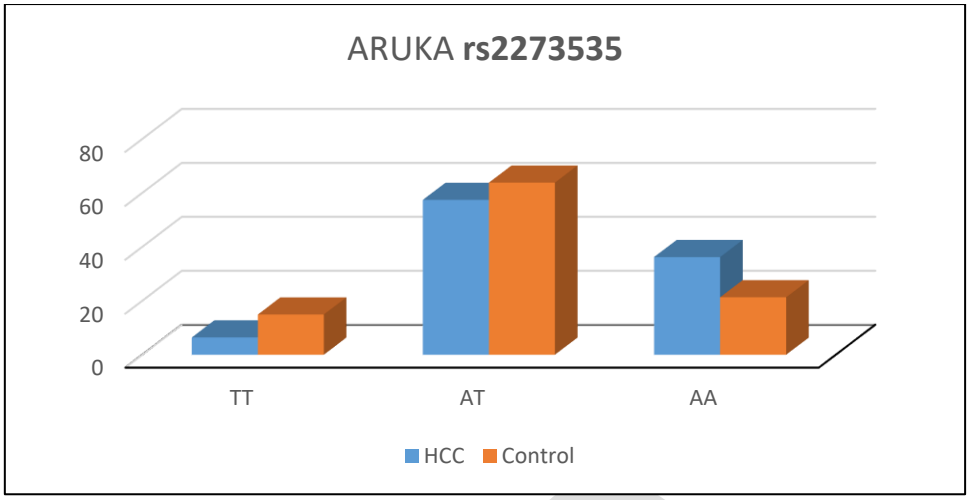

Fig. (4): A comparison between the two studied groups according to ARUKA rs2273535 polymorphism.

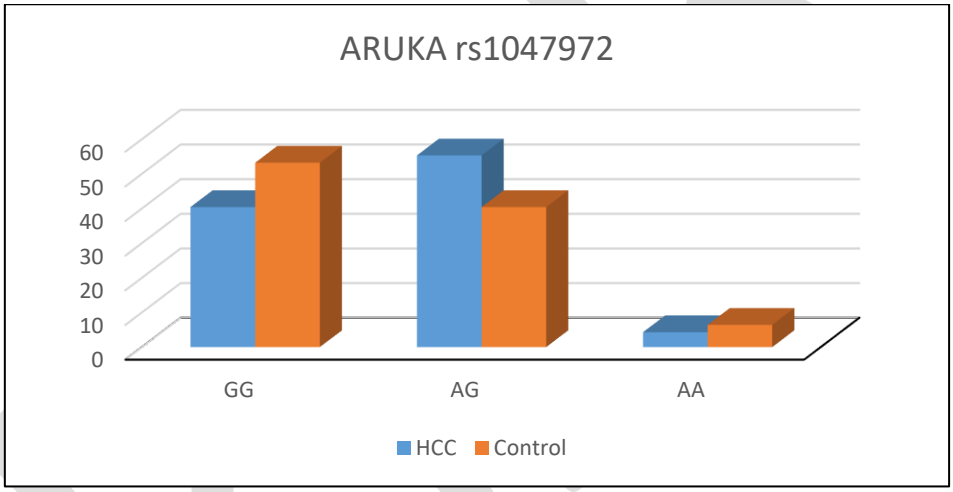

Fig. (5): A comparison between the two studied groups according to ARUKA rs1047972.