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Evaluation of the diagnostic performance of Alpha-1-Antitrypsin in early detection of hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) constitutes one of the most frequent cancer types and accounting the vast majority of tumour-related fatalities worldwide. HCC is remain related to a bad prognosis in patients with an advanced disease stage. This study was conducted to evaluate the relationship between A1AT concentration, A1AT gene promoter methylation, and A1AT genotype variation, and HCC risk. In this case-control research, we investigated A1AT levels in plasma as a diagnostic biomarker for the earlier detection of HCC in 100 patient samples. We also checked DNA promoter methylation of the A1AT gene, and genotypes in all the studied groups. The levels of AFP and A1AT in plasma were determined using ELISA and nephelometric techniques, respectively. The genomic DNA extracted from blood samples has been examined for S and Z genotypes using the PCR-RFLP technique, as well as gene A1AT promoter methylation was assessed by methylation specific-PCR assay.The plasma data analysis showed that there was a significant difference between HCC and healthy control samples regarding the level of AFP and A1AT. The range of plasma A1AT concentration was 166.6±27.28g/L in patients and 129.8±15.87g/L in controls ($p < 0.001$). A1AT concentration was also associated with progressive tumour stages. Moreover, the roc curve stated that A1AT concentration is better in sensitivity than using AFP in early detection of HCC cancer patients as A1AT concentration at 135mg/L, had a sensitivity of 99% and a specificity of 79% for distinguishing cancer patients from healthy individuals. We concluded that the plasma A1AT concentration has higher sensitivity than AFP for early detection of HCC.

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Introduction

Hepatocellular carcinoma (HCC) is among the most frequently occurring type of primary liver tumour and an important cause of cancer death and morbidity across the world (1). Hepatitis C and B virus infection, misuse of alcohol, non-alcoholic fatty liver disease (NAFLD), and being obese are considered risk factors for the development of liver cancer (2). The development of liver cancer involves a variety of cellular mechanisms, including disorders of the apoptosis and cell cycle, cellular fibrogenesis processes, and inflammatory biological pathways. All of these are important biological targets for developing new pharmacological treatments (3).

Early detection is crucial for effective therapy and improved survival (4). Although the HCC detection accuracy has been significantly increased due to the advanced use of ultrasonography, magnetic resonance imaging (MRI), and other modes of imaging, their use has been embarrassed by weaknesses including, invasiveness, and inability to detect small tumours and the high cost (5). Biomarkers have many possible uses in cancer, such as early detection, screening, progression of the disease, surveillance, and prognosis. (6). Diagnostic biomarkers might improve disease identification such as various strategies for selecting features that are frequently employed to address diagnostic biomarker discovery to distinguish tumours from healthy samples (7). Many studies have been dedicated to discovering diagnostic biomarkers by searching for differentially expressed genes (DEGs), the mostly beneficial genes from huge unsuitable ones. Modern genome profiling investigation allows the detection of specific genetic mechanisms as well as the molecular patterns involved in HCC (8, 9). This technique can help in finding appropriate testing HCC biomarkers. Many studies utilizing this approach, identified various genes and their proteins that were considerably overexpressed in tumour samples and were identified as possible new HCC biomarkers (10, 11).

As a result, the identification of practical, affordable, non-invasive, and reproducible plasma biomarkers has been important for the diagnosis of HCC(12). A common biomarker for the detection of HCC is Alfa-fetoprotein (AFP), but because of its high prevalence of false-negative results for early-stage small tumours, its diagnostic efficacy is not optimal (13). Additionally, in some benign hepatic conditions such as cirrhosis or hepatitis without HCC, AFP may be high (14).

Alpha-1 antitrypsin (A1AT) is basically produced in the hepatocytes and acts as an anti-inflammatory molecule in the system of innate immunity by obstructing the damaging action of neutrophil proteases (15). Furthermore, besides its anti-protease activities, The anti-inflammatory

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properties of A1AT have been achieved by controlling cellular and molecular processes like cytokine expression and apoptosis (16, 17). In reaction to an inflammatory stimulus, it is discharged into the plasma, however, when the hepatocyte synthesis of an aberrant protein could not be completely discharged into the plasma as a result, the aberrant protein accumulates in the liver causing a chronic liver disease(18).

Multiple clinical trials have detected elevated plasma A1AT values in a number of cancers, involving liver, breast, lung, pancreas prostate, esophageal, and bladder cancer (19). A1AT has also been reported as a possible clinical diagnostic marker for early identification of gastrointestinal injury (20). Various A1AT gene (SERPINA1) polymorphisms are linked to low plasma A1AT protein levels, and the most prevalent A1AT deficiency-associated polymorphisms are protease inhibitor S (PiS) and protease inhibitor Z (PiZ) variations (21). A1AT Z and S allele deficiencies have been linked to an elevated risk of developing malignancies such as hepatocellular carcinoma, bladder cancer, and lung cancer (22).

DNA methylation, an epigenetic process in which a methyl group is relocated to the C5 location on cytosine to form 5-methylcytosine, regulates the gene expression (23). Previous research has revealed that promoter DNA methylation may have a good role in the regulation of A1AT gene expression. The current case-control research was conducted on Iraqi patients to evaluate the relationship between A1AT concentration, A1AT gene promoter methylation, and A1AT genotype variation, and HCC risk. Plasma A1AT was also evaluated and compared to AFP, the most commonly utilized biomarker in HCC patients.

Materials and Methods

Study population and blood sample:

The present case-control study was carried out on 100 HCC patients divided into 91 males and 9 females (Mean 59.97 ± 7.85 years); all enrolled individuals were Iraqi being recruited at the National Liver Institute Hospital, Baghdad University, Iraq. Blood samples have been obtained as well from 100 healthy blood donors who were age and sex-matched and had no history of cancer or genetic illnesses and were free of any chronic diseases, living in the same geographical as control subjects divided into 78 males and 22 females with a 55.12 ± 11.36 years mean age. This study was done in accordance with international and national ethical guidelines (good clinical practice, Declaration of Helsinki) and the procedures were agreed according to the National Liver Institute Hospital Local Ethics Committee, NLI (001.09.2017/1). HCC patients had a localized lesion, according to computed tomography (CT) and ultrasonography scans. Blood samples were collected only from individuals who provided informed consent. A comprehensive medical history was supplied to all subjects. Patients who had any concurrent tumours other than HCC had been completely excluded from the study. Whole blood samples were withdrawn from all individuals for normal evaluation, which included liver functions, complete blood count (CBC), and kidney function tests. For genotyping, EDTA-peripheral blood was drawn and immediately frozen at -80^{o}C till analysis.

Measurement of Plasma A1AT and AFP Concentration

The level of A1AT in plasma was estimated using nephelometry and an Array™ Protein System auto-analyzer (Beckman Instruments, Brea, California, USA) using readily accessible antibodies (MININEPH, Birmingham, UK). COBAS CORE is utilised to assess AFP plasma levels. All biochemical parameters were determined using a COBAS 800 auto-analyzer (Roche, Switzerland).

DNA Extraction and Genotyping

ABIOpure™ DNA extraction kit (Bothell, WA 98021, USA) was used to extract genomic DNA from the peripheral leucocytes. Two serpinA1 gene single nucleotide polymorphisms (SNPs), Z variant and S variant (rs28929474, rs17580) respectively, which were related to A1AT deficits variants, have been genotyped by PCR followed by restriction fragment length polymorphism (RFLP) utilizing a set of primers used previously (24). Briefly, in a PCR thermal cycler (Techne, Russia), PCR amplification was performed with a 95 °C as initial denaturation for 5 minutes, followed by 95 °C denaturation, 56 °C annealing, and 72 °C extension, 45 seconds each step for 31 cycles, followed by 72 °C for 10 minutes as a final extension. 10 µl of the products of PCR had been digested with 2.5 U *Taq I* restriction enzyme (Thermo-scientific, USA) at 65 °C for 5-16 minutes for RFLP analysis, and the digested fragments were analyzed on a 3% agarose gel electrophoresis.

Methylation-Specific PCR (MS-PCR)

MS-PCR was used to evaluate the methylation state of the SERPINA1 gene promoter in all participants. Bisulfite treatment was conducted on each DNA sample according to the previously defined (25) and the resulting treated DNA was amplified utilizing a set of primers intended for selective PCR-amplification of methylation and unmethylated promoters region of the SERPINA1 gene (24). Amplification got started with a 5-minute denaturation at 94 °C, followed by 35 cycles of 45-second denaturation at 94 °C, 30-second primer annealing at 60 °C, 30-second extension at 72 °C, and 10-minute final extension at 72 °C. MS-PCR amplification fragments were separated using 3% agarose gel electrophoresis.

Statistical analysis

IBM SPSS software programme version 20.0. (IBM Corporation, Armonk, New York) was used to feed and analyze the data into the computer. The Kolmogorov-Smirnov test was employed to confirm the normality of the variable distribution. The Chi-square test was used to compare categorical variables between groups. For distributed quantitative variables, student t-test and Mann-Whitney were used (for regular and non-normal distribution respectively). The receiver operating characteristic curve (ROC) and the area under the ROC curve represents the test's diagnostic performance. When examining more than two groups, ANOVA was utilized. The acquired findings were declared significant at ≤ 0.05.

Results

Hematological, biochemical, demographic, and biomarkers data between the studied groups

Table 1 outlines the hematological, biochemical, demographic, and biomarker data of the study groups. The

mean ages of healthy controls and HCC patients were 59.97 ± 7.85, and 55.12 ± 11.36, respectively and the studied groups didn't vary significantly with regard to sex. Patients with HCC showed significantly higher direct bilirubin, ALT, AST, and INR levels than the healthy group investigated, while albumin, TLC, and platelet count were considerably lower. The levels of creatinine, haemoglobin, or total bilirubin did not change in a statistically significant variations in creatinine, haemoglobin. among all participants. The cancer tissues were divided as stage I in 59 (59.0%), stage II in 37 (37.0%), and stage III in 4 (4.0%), of patients. The number of tumour foci is 1 lesion in 61 (61.0%) and 2 lesions in 24 (24.0%), 3 lesions in 9 (9.0%), and 6 (6.0%) patients had more than 3 lesions. Plasma data analysis indicated significant differences in AFP and A1AT concentrations between patients and controls (Table 2). A1AT level had a substantially higher area under the ROC curve (AUC) than AFP level ($P < 0.001$) (Figure 1A) as the AUC for A1AT and AFP levels were 0.906 (95% CI: 0.853 – 0.958), 0.930 (95% CI: 0.898 – 0.963) res-

Table 1. Selected clinical and demographic characteristics of patients and controls.

Variables	HCC (n =100) Mean ± SD	Control (n = 100) Mean ± SD	Test of sig.	p
Demographic data				
Age, years	59.97 ± 7.85	55.12 ± 11.36	t= 3.512*	0.354
Sex, n (%)	M, 91 (91.0%)-F, 9 (9.0%)	M, 78 (78.0%)-F, 22 (22.0%)	χ^2= 6.614*	<0.07
HCV carriers, n (%)	59 (59%)	0 (0%)	-----	
Hematological profile				
HB	12.54 ± 1.6	12.78 ± 1.45	t=1.094	0.275
RBCs.	4.35 ± 0.60	4.55 ± 0.51	t=2.486*	0.014*
TLC	5.33 ± 1.70	7.15 ± 1.96	U=2424.0*	<0.001*
PLT	124.1 ± 57.7	269.3 ± 72.6	U=641.50*	<0.001*
Pro. Conc.	24.16 ± 37.77	95.99 ± 4.65	U=490.0*	<0.001*
INR	1.25 ± 0.21	1.03 ± 0.04	t=10.583*	<0.001*
Biochemical parameters				
Creatinine	0.91 ± 0.23	0.94 ± 0.20	t=0.936	0.350
AST	62.76 ± 79.81	29.0 ± 8.22	U=1338.50*	<0.001*
ALT	46.02 ± 30.25	24.57 ± 8.55	U=2075.50*	<0.001*
Total bilirubin	1.28 ± 0.67	1.07 ± 0.15	U=4879.0	0.766
Direct bilirubin	0.53 ± 0.43	0.19 ± 0.08	U=1776.0*	<0.001*
Albumin	3.38 ± 0.62	4.29 ± 0.61	t=10.454*	<0.001*
Child pugh score				
A	59 (59.0%)			
B	37 (37.0%)			
C	4 (4.0%)			
Focal lesion				
Size (cm)	4.80 ± 2.91			
Number				
1	61 (61.0%)			
2	24 (24.0%)			
3	9 (9.0%)			
>3	6 (6.0%)			

t: Student t-test. - **U:** Mann Whitney test- ***:** Statistically significant at $p \leq 0.05$

Table 2. Comparison between the two studied groups according to biochemical parameters and A1AT concentration.

Biochemical parameters	HCC (n = 100)	Control (n = 100)	Test of Sig.	p
AFP (ng/ml)				
<200	62 (62.0%)	100 (100.0%)	χ^2= 46.914*	<0.001*
>200	38 (38.0%)	0 (0.0%)		
Mean ± SD.	1192.2 ± 3180.6	6 ± 1	U= 943.50*	<0.001*
A1AT concentration mg/dl				
Mean ± SD.	166.6 ± 27.28	129.8 ± 15.87	t= 11.669*	<0.001*

t: Student t-test χ^2: Chi-square test U: Mann Whitney test *: Statistically significant at $p \leq 0.05$

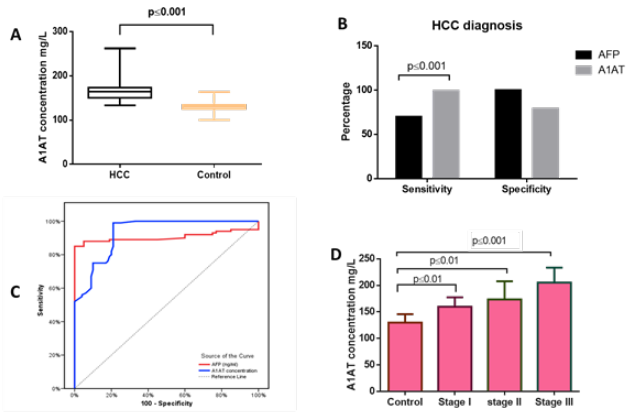

Figure 1. ROC curve for AFP (ng/ml) and A1AT concentration for diagnosing HCC.

pectively. The optimum sensitivity and specificity for differentiating HCC patients from healthy controls at an AFP cut-off point of 18 ng/ml were 70.0% and 100%, respectively). While, the observed cut-off value for the A1AT level from the ROC curve was 135 mg/L, with 99% sensitivity and 79% specificity in distinguishing HCC patients from healthy controls (Table 3, Figure 1A, B). The range of plasma level of A1AT was 166.6 ± 27.28 mg/L (median 164 mg/L) in patients and 129.8 ± 15.87 mg/L (median 130 mg/L) in controls (p <0.001) (Figure 2C). While the level of AFP concentration in the HCC patients is 1192.2 ± 3180.6 mg/L and the range of the healthy control group is 6 ± 1 g/L (p<0.001).

The association between the tested biomarkers and Clinicopathological Features of HCC Patients

Table 3 shows the link between the median mean values of A1AT concentrations detected in patients' serum and the clinicopathological characteristics of the HCC cases. There were no statistically significant differences in the concentrations of plasma A1AT in HCC cases stratified by age (<60 and ≥ 60), however, the mean values of A1AT concentration in male is higher significantly than in females. In this study, 64 patients (64%) had < 5 cm size tumours, while 36 patients (36%) had tumours more than 5 cm in size. According to the statistical results, the mean concentration value of A1AT in plasma was considerably higher in patients with big tumours (<5 cm) than in smaller (≥5 cm) (P < 0.049) (Table 3). The range of A1AT levels measured in HCC patients with small and large tumour sizes were 133 – 261 g/L and 136 – 262 g/L, respectively. The mean levels of plasma A1AT concentration in all HCC

Table 3. Relation between A1AT concentration and clinical data in the cases group.

	N	A1AT concentration mg/dl			Test of Sig.	p
		Min. – Max.	Mean ± SD.	Median		
Age						
< 60	43	133 – 261	166.93 ± 265	164	t= 0.092	0.927
> 60	57	136 – 262	166.42 ± 28.40	164		
Sex						
Male	91	136 – 262	168.29 ± 27.77	165	t= 1.945*	0.005*
Female	9	133 – 176	150 ± 13.90	143		
Stages						
I	59	133 – 215	159.64 ± 17.99	160		
II	37	136 – 262	173.62 ± 34.10	166	F= 8.200*	0.001*
III	4	180 – 243	205.25 ± 28.18	199		
Size						
< 5 cm	64	133 – 261	162.63 ± 26.53	157.5	t= 1.992*	0.049*
> 5 cm	36	136 – 262	173.78 ± 27.50	166		
Number						
1	61	133 – 262	167.21 ± 29.22	163		
2	24	138 – 243	166.58 ± 27.87	165	F= 0.250	0.861
3	9	138 – 214	169 ± 20.49	165		
>3	6	140 – 174	157.50 ± 11.24	156		
AFP						
< 200	62	133 – 261	162.68 ± 22.35	161.5	t= 1.715	0.092
> 200	38	137 – 262	173.11 ± 33.14	166		
ALT						
<40	**53**	133 – 261	166.47 ± 29.75	163	t=0.065	0.948
>40	**47**	136 – 262	166.83 ± 24.51	165		
AST						
<40	**32**	137 – 215	164.25 ± 246	161.5	t=0.599	0.550
>40	**68**	133 – 262	167.76 ± 28.77	164		

F for ANOVA test t: Student t-test *: Statistically significant at p ≤ 0.05

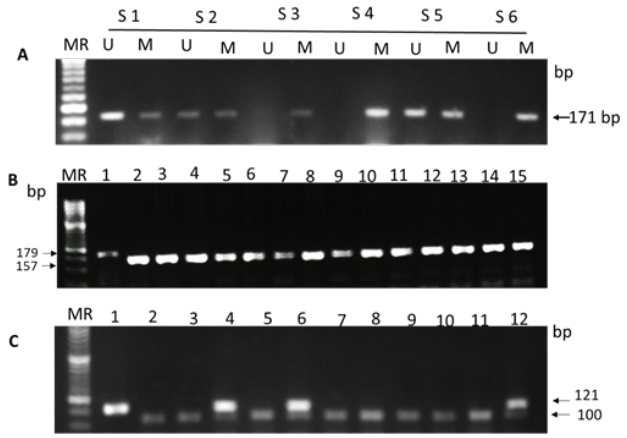

Figure 2. A: Representative examples of MS-PCR reactions for promoter methylation analysis of Serpin A1 gene in HCC tumours samples and healthy control. U: Unmethylated gene; M: Methylated gene; S1: sample 1; MR: 50 bp DNA size marker. **B:** SerpinA1 gene (Z allele) genotyping in HCC patients using The PCR-RFLP technique. *Taq I* enzyme digestion of the 179-bp PCR amplified M allele resulted in two fragments of 157 and 22 bp (lanes 2-15), whereas the Z allele provides an undigested fragment. Lane 1 (control) is a 179-bp PCR product that has not been digested. MR; 50 bp DNA marker. **C:** PCR-RFLP was used to determine the genotype of the SerpinA1 gene (S allele) in HCC patients. *Taq I* digest of the 121-bp PCR product produces three fragments of 121, 100, and 21 bp for the Pi MS genotype (lanes 4, 6, and 12), whereas the S allele yields an undigested fragment. Pi MM normal genotype provides two 100 and 21 bp fragments. Lane 1 (control) is a 121-bp PCR product that has not been digested. MR, is a 50-bp DNA marker.

stages were considerably higher than in controls (Figure. 1D). The mean concentrations of plasma A1AT in cancer patients with stage I was substantially lower than in the other progressive stages ($P < 0.05$). The A1AT levels range identified in cancer patients stage I, II, and III, were 133 – 215 (median 160), 136 – 262 (median 166), and 180 – 243 (median 199) respectively (Figure. 1D). In HCC patients, the range of A1AT concentration does not differing significantly in those patients who had AFP more than 200g/ L and patients who had AFP less than 200 g/L. Moreover, the mean values of A1AT concentration were not changed significantly with number of the focal lesions in the HCC patients. According to the level of ALT and AST the A1AT concentration has no significant difference when comparing >40 and<40 g/L HCC patients. A1AT concentration was shown to be favourably correlated with AFP levels in HCC patients ($r = 0.199$, $p = 0.047$), However, there was a lack of association between A1AT concentration and the other clinicopathological data (data not shown).

Promoter DNA Methylation and SERPINA1 Genotype

Using the PCR-RFLP technique, all subject's DNA samples were analyzed for the existence of SERPINA1 gene Z or S alleles. *Taq I* digests the amplification products of the PCR using either S or Z primers resulting in 2 fragments for the wild MM genotype. The existence of mutant S or Z alleles in the amplified fragments eliminates the restriction site of the *Taq I* enzyme. In the all patients and control samples examined, there was not a noticeable Z mutation on PCR-RFLP electrophoresis results (Tables 4 and 5; Figure 2). But the results indicated that presence of 8 (8%) samples carry heterozygous of the MS genotype in HCC cases and 9 (9%) samples carry the same genotypes in the healthy control samples without a significant difference in the distribution of the S allele between the cases and control samples. The results showed the pres-

Table 4. Comparison between the two studied groups according to genotype and allele frequency of SerpinA1 S allele.

Genotype and allele frequency of SerpinA1 S allele	HCC (n = 100)	Control (n = 100)	OR (95%CI)	p
Genotype				
AA®	92 (92.0%)	91 (91.0%)		
AT	8 (8.0%)	9 (9.0%)	0.879 (0.325 – 2.379)	0.800
TT	0 (0.0%)	0 (0.0%)	–	–
$^{HW}p_1$	**0.677**	**0.637**		
Allele				
A®	192 (96.0%)	191 (95.5%)		
T	8 (4.0%)	9 (4.5%)	0.884 (0.334 – 2.340)	0.804

OR: Odds ratio CI: Confidence interval ®: reference group *: Statistically significant at $p \leq 0.05$

Table 5. Comparison between the two studied groups according to genotype and allele frequency of A1AT methylation.

Genotype and allele frequency of A1AT methylation	HCC (n = 100)	Control (n = 100)	OR (95%CI)	p
Genotype				
MM®	8 (8.0%)	51 (51.0%)		
U/M	92 (92.0%)	49 (49.0%)	11.969(5.261 – 27.230)	<0.001*
UU	0 (0.0%)	0 (0.0%)	–	–
Allele				
M®	108(54.0%)	151(75.5%)		
U	92(46.0%)	49(24.5%)	2.625(1.715 – 4.018)	<0.001*

OR: Odds ratio CI: Confidence interval ®: reference group *: Statistically significant at $p \leq 0.05$

ence only of the normal homozygous MM genotype and the absence of the Z genotype of *SERPINA1* in this group of HCC patients. While only 8% of the tested samples are heterozygous S variant (MS) genotypes in HCC patients.

We also looked at the methylation condition of the SERPINA1 gene promoter in all individuals under investigation in this study. Figure 2A shows a number of examples of MS-PCR findings from the promoter methylation study. Table 5, summarizes the SerpinA1 methylation. In HCC samples, only 8 (8%) samples were fully methylated of SERPINA1 promoter and hemimethylated (partially methylated) in 91 (91%) samples. The percentage of fully-methylated SERPINA1 promoters was substantially greater in control than tumour samples (51% versus 8%, respectively) ($p < 0.001$).

The relation between *SERPINA1* gene methylation of the promoter region and clinicopathological features of HCC patients was investigated. In tumour stage, I, none of the *SERPINA1* gene promoters was fully methylated while 4 patients were fully methylated in either stage II, or III. On the other hand, the hemimethylated patients were higher in stage I than in stages II and III where the difference was significant $p < 0.001$. Moreover, the level of AFP and A1AT concentration was higher in hemimethylated patients in comparison to fully methylated patients ($p < 0.001$). No correlation was found between the promotor methylation of the *SERPINA1* gene with other clinicopathological characteristics of HCC patients (data not shown).

Discussion

HCC is the second-prominent cause of cancer death and is a common aggressive tumour of the gastrointestinal tract characterized by rapid development and dissemination (4). A large number of patients with HCC are detected at a late stage since their early symptoms are not always visible (26). In the current study, we investigated A1AT concentration, *SERPINA1* gene variants and the methylation condition of the *SERPINA1* gene promoter in blood samples from HCC patients and healthy control individuals in comparison with AFP as an HCC plasma biomarker. The mean plasma concentrations of A1AT in HCC patients were considerably higher compared with healthy control individuals ($p = 0.001$, Table 2). A comprehensive examination found that the A1AT plasma level increased in correlation with the severity of the HCC. Furthermore, the mean A1AT level in all grades and stages of HCC patients was higher compared to in controls ($p < 0.01$). Furthermore, patients with high HCC stage III had substantially higher plasma AIAT concentrations than normal controls and HCC patients with stage I, II (Figure 1D). In accordance with a prior report (27), we stated that, the higher A1AT concentrations in the plasma of individuals with advanced tumours stage (III) than in those with early stages (I and II). High-risk people must be routinely screened for HCC in order to detect it early. AFP remains the most often utilized biomarker in HCC patients, although its limited sensitivity and specificity, particularly for early-stage disease (28). The capacity of AFP to diagnose HCC was demonstrated in the current investigation to be somewhat subpar. These inconsistencies confirm the insufficiency of AFP as a biomarker, as revealed by earlier research (29). Therefore, to enhance HCC diagnosis, novel and trustworthy biomarkers are required (30). According to the ROC curve study, plasma A1AT has higher sensitivity but not specificity than AFP in distinguishing HCC patients from healthy persons. While plasma AFP levels demonstrated a sensitivity of 70% and a specificity of 100% for discriminating controls from HCC patients at the recommended cut-off value (Fig. 2). With an AUC of 0.93, the anticipated probability based on plasma A1AT concentration showed 99% sensitivity and 79% specificity (Figure 1A). These results imply that plasma A1AT concentration is a superior diagnostic for the early identification of HCC than plasma AFP biomarkers.

It has been proposed that an elevated level of A1AT can distinguish between cancer and chronically benign conditions as well as between clinical remission and recurrence (31). A1AT and 50 more proteins were shown to differ noticeably between HCC tissues and precancerous lesions in a proteomics analysis, indicating that changes in the expression of proteins were frequent during the course of hepatocarcinogenesis (32). Individuals with HCC had plasma A1AT levels that were considerably higher than liver cirrhosis or chronic hepatitis individuals (33). The precise mechanism and effect of high A1AT plasma levels in HCC are unknown. The findings of the Sawaya et al. study confirmed the hypothesis that, A1AT synthesis by tumour cells corresponds with local inflammatory and enzymatic properties, both of which are likely involved in tumour cell protection (34). In cases of widespread inflammation and infection, A1AT plasma levels can rise by three to five times (35). Strong anti-inflammatory capabilities are another benefit of A1AT (36-38). Compared to the plasma concentration of AFP, we observed an increase in the level of ATA1 in the current investigation. The phases of the development of liver cancer were directly correlated with this rise. A1AT is essential for innate immune defense, and Interleukin (IL)-6 and IL-17, two pro-inflammatory cytokines, are produced during the acute-phase protein response and can cause an increase in plasma concentration of A1AT (39). Increased production of A1AT both locally and systemically as a result of the acute-phase protein response leads to significantly higher tissue A1AT concentrations, where its ability to bind cell surface domains and interact with proteins, proteases, peptides, and cytokines may have significant effects on the control of inflammation (40). Additionally, the present investigation revealed that the concentration of A1AT increased in men than in women as shown in Table 3. HCC has been linked to several sociodemographic factors such as significant male preponderance, which is probably due to a concentration of risk factors in males as well as variations in sex hormones (41). Recent findings show that specific SERPINA1 gene mutations induce A1AT proteins to have reduced anti-protease function (42, 43). We also examined the subjects for the presence of S and Z-deficient A1AT alleles. We found no Z variants in any of the 100 HCC samples examined. However, we found 9 heterozygous S deficient alleles in HCC samples. These findings revealed that 91% of HCC patients had a normal MM genotype and only 9% had PiMS deficient allele but we could not find any significance in the percentage of S deficient allele between the HCC and healthy control samples. Eigenbrodt et al. identified a relationship between end-stage liver disorders and A1AT Z heterozygosity, including alcoholic liver disease, HCV infection, cryptogenic cirrhosis, and primary hepatic

malignancy (44). Bowlus et al. on the other hand, demonstrated that obesity and male gender, but not viral hepatitis or alcohol, predispose those with A1ATD to severe hepatic disease (45). A new study found that, A1AT mutations S and Z are related with sinusoidal iron buildup and hyperferritinemia in non-alcoholic fatty liver disease, but not advanced liver disorders (46). It has been discovered that, A1AT promoter DNA methylation regulates its production in cells (47).

As a result, we investigated the status of SERPINA1 gene promoter methylation in 100 HCC and 100 healthy control samples. In tumour samples, the promoter of the SerpinA1 gene was either hemimethylated or fully methylated (Table 5). The percentage of fully methylated SERPINA1 gene in healthy samples was considerably greater than tumour samples (51% versus 8%, $p < 0.001$). There was a relationship between the gene promoter methylation stage of the HCC tumour, AFP concentration and A1AT concentration while no association with other clinicopathological features. The A1AT gene, which is transcriptionally active, is hypomethylated, while the hypermethylated gene is inactive (47), It's likely that in comparison to normal tissues and tumours with fully methylated promoters, hemimethylated promotors produce more A1AT protein. Our results revealed that the A1AT concentration is high in cancer tissue than normal due to the over-production of A1AT by tumour cells themselves due to hemimethylated gene promotor and eventually, increases the plasma A1AT levels in HCC patients. To validate our findings, a larger sample size investigation must be carried out.

Conclusion

The present study suggested that blood A1AT concentration has a superior sensitivity than AFP measurement in detection of HCC and might be used as a promising biomarker for the early diagnosis. Also, our findings further point to the possibility that the higher expression of the A1AT gene in tumour tissues as a result of promoter hemi-methylation may be a key factor in the rise of plasma A1AT concentrations in HCC patients.

Ethics approval and consent to participate

This study was done in accordance with international and national ethical guidelines (good clinical practice, Declaration of Helsinki) and the procedures were agreed upon according to the National Liver Institute Hospital Local Ethics Committee, NLI (001.09.2017/1). All enrolled individuals were Iraqi being recruited at the National Liver Institute Hospital, Baghdad University, Iraq.

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Authors' Contributions

MS, MC, and AA designed the study. MC performed sample collection, and experiment. MS, MC, and AA performed analysis and interpretation of results. MS, MC, and AA prepared the draft and revised form of the manuscript.

Data Availability Statement

Please contact the authors for data requests.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Competing interests

All of the authors declare no conflicts of interest.

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No conflicts of interest were declared by all authors.

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