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Z Mutation Allele Of Alpha1-Antitrypsin Deficiency In Hepatoma And Emphysema Pateints

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Abstract

Background: Alpha-1-antitrypsin deficiency (AATD) is one of the most common inherited metabolic disorders with the potential to cause severe injury to at least two organs system, namely the liver and lung. The gene for AAT is located on chromosome segment 14q 31-32 and is expressed in codominant fashion. More than 90 different allelic variants have been recognized to date, often differing by single point mutations. Wild type M allele (PiM) is the most common allele and is associated with a normally functioning AAT molecule. The most common type of severe deficiency variant is mutant Z allele (Pi*Z), which arises from single genetic point mutations causing a $Glu^{342} \rightarrow Lys$ substitution in the AAT molecule.

Many investigators used real time PCR to detect 2 mutations of AAT, this method was proved to be reliable and of high accuracy in comparison to other methods.

Patients and Methods : We used real time PCR in screening 3 groups of patients, group I {27 patients with hepatocelullar carcinoma (HCC)}, group II {20 patients with emphysema} and group III {15 healthy controls}.

Results: The results showed significant correlation between PiZ and hepatocelullar carcinoma in group I, while the results in group II were statistically non-significant, while all healthy controls were PiMM (wild type).

As all patients of group I (hepatocelullar carcinoma) were with past history of viral hepatitis (21 patients had HCV infection and 6 patients had HBV infection) we explained this high incidence of Z mutation of AAT in patients with history of chronic viral hepatitis by high prevalence of hepatitis C in Egypt which approximately more 10 times than that in USA.

The role of AAT in pathogenesis of hepatocelullar carcinoma is complex and needs further research.

Conclusion: We recommended screening these 2 risky groups of patients of possible AATD as there is a replacement therapy of AAT for patients with emphysema we also recommend evaluation of the antiviral therapy in patients with viral hepatitis and AATD.

Key words: α1- antitrypsin *(AAT)*,α1- antitrypsin deficiency*(AATD)*, hepatomcellular carcinoma*(HCC)*, protease inhibitor*(Pi)*.

Introduction

Protease inhibitor 1 (α_1-antitrypsin, AT) is the main serum inhibitor of proteolytic enzymes. In AAT-deficiency, enzymes such as neutrophil elastase can damage the lung tissues, leading to pulmonary emphysema. More than 90 different alleles have been identified so far for the protease inhibitor 1 (PI) gene. The three most important variants are type M (90% of population), type S (Pi*S) and type Z (Pi*Z). Homozygotes of type Z have a considerable reduction in the serum α_1-antitrypsin concentration and may develop pulmonary emphysema or hepatic cirrhosis. SZ-heterozygotes are less severely affected *(Crystal,et al.1989)*. Homozygous adult (PiZZ) are at risk developing cirrhosis and liver carcinoma *(Fabbretti,et al., 1992),* also heteroygotes of type Piz are associated with increased risk of primary liver carcinoma *(Zhou II,et al., 2000)*

The belief that AAT deficiency is disorder, which mostly affects white subjects, has been, in part, taken by the analysis of the world wide surveys performed by De serres,*2002*. He provided

evidence for significant prevalence of both PiZ and Pi*S in populations from the Middle East, North Africa, central and South-east Asia, suggesting that AAT deficiency has prevailed over racial and ethnic boundaries *(Pallardo, et al.,2000)*. Investigated the use of a real time PCR analysis to determine the pi status at high output level they concluded that the real time PCR Technique particularly attractive since it allow a rapid analysis of fresh blood sample as well the retrospective study of paraffin embedded archival tissues, their results suggested that the real time PCR Technique is highly accurate and reliable compared to the standard approaches used for genotyping of the PIZ locus. Within 60 minutes 30 samples can be analyzed and definite results were obtained immediately, obviating the need for further analysis such SSCP (Single–Strand Conformation Polymorphism) and DNA sequencing, as identical results were obtained when samples were tested by real time PCR in comparison to these two methods *(Pallardo, et al.,2000)*.

Pallardo and coworkers *(Pallardo, et al.,2000)* also concluded that, this method was the fastest and most convenient means of detecting the PiZ mutation at genetic level and of accurately determining a hetero-and homozygous carrier state.

Von Ashen and coworkers *(Von Ashen,et al., 2000)* used a multiplex method and used 2 sets of primers to amplify the gene regions covering the PiS or PiZ mutation sites. Mutation detection was performed on the real time PCR by melting curve analysis of detection probes labelled with two different fluorescent dyes, Lc-Red 640 and Lc-Red 705, unequivocal genotyping results were obtained for all investigated samples in assay time of 30 min, they concluded that real time PCR was a rapid, convenient and economic alternative to other methods which were described for the detection of α1-antitrypsin deficiency alleles(*Von Ashen,et al., 2000*).

The aim of this work: The aim of present work was to study the Z mutation patterns of α-1-antitrypsin in healthy controls, patients with hepatoma and patients with emphysema (as comparison risky group) this was done in an attempt to detect whether there is a relation between Z mutation of alpha-1-antitrypsin and these two diseases.

Patients and Methods

This study included 27 patients with hepatoma, 20 patients with chronic obstructive pulmonary disease (COPD) as well as 15 healthy control. The patients are selected from oncology unit and Department of Chest of El Hussein Hospital (Al Azhar University).

Statistical analysis was done using statistical paeleage for social science(SPSS 12.0) software program.

Patients were classified into three groups

a) ***Group I :*** Included 27 patients (21 males and 6 females with primary hepatoma, all patients with past history of chronic viral hepatitis. Their main age was 53.74± 4.74 years.

The diagnosis was based on full history taking complete clinical examination, routine liver function tests, abdominal ultrasonography and liver biopsy.

They had a past history of chronic hepatic disease (chronic viral hepatitis), 21 patients were positive for HCV antibody and 6 patients were positive for HBsAg.

b) ***Group II:*** Included 20 patients (17 males and 3 females) with chronic obstructive pulmonary disease (mainly emphysema). The main age of this group was 46.73 ± 4.86 years. This group was taken as other risky group for alpha-1-antitryopsin deficiency and all the patients were smokers.

The diagnosis was based on full history taking complete clinical examination, pulmonary function tests, routine liver function tests and abdominal ultrasonography.

c) ***Control Group (Group III):*** Included 15 healthy subjects (12 males and 3 females), their main age was 40.2 ± 2.98 years.

Each individual included in the study was subjected to:

1- Full medical history.

2- Complete clinical examination

3- Routine liver function tests including total bilirubin, direct bilirubin, total proteins, albumin, alanine aminotran-

sferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase. (GGT).

4- HCV antibody and HBs Ag by ELISA (DiaSorin) was done for group I and III .

5- Pulmonary function tests (for group II).

6-Assay of Z mutation of *AATD by real time PCR,* using Light Cycler system (Roche Diagnostic).DNA extraction from whole Blood by MAGNA Pure Compact nucleic acid Kit I in combination with the MAGNA Pure Compact instrument [MAGNA Pure Compact nucleic acid Kit I (Cat. No. 03730-972001) Roche Diagnostic Corporation]

Technical principle: The samples are lysed by incubation with proteinase K and special lysis buffer.

Magnetic Glass particles (MGPs) are added and the nucleic acids are bound to their surfaces. Unbound substances are removed by several washing steps, followed by elution of the purified nucleic acid.

7- High speed detection of α1-antitrypsin deficiency allele PiZ on the Light Cycler. (by testing DNA which were extracted from whole blood)

Needed material

I- DNA extractions of the samples (as explained before)

II- Real Time PCR instrument 2.0 or 1.5 (Roche Diagnostics, Mannheim, Germany)

III-Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.

IV-LighCycler capillaries (Roche Diagnostics):

Note: The LightCycler system provides adapters that allow LightCycler Capillaries to be centrifuged in a standard microcentrifuge rotor.

V- Amplification Primers (TIB MOLBIOL, Berlin) which include :

a) Forward Primers AAT Pi*Z with 25 bases in the following sequence 5`-TCCACgTgAgCCTTgCTCgAggCCTg`

b) Reverse Primes: AAT Pi*Z R with 23 bases in the following sequence 5`-TTgggTgggATTCACCACTTTTC.

Use PCR primers at a final concentration of 0.2-1μM,. the recommended starting concentration is 0.5μM each.

VI- Hybridization Probes (TIB MOLBIOL, Berlin, Germany) which include :

a) Donor Probes with 26 bases in the following sequence: 5`-CTTCAgTCC CTTTCTCgTCgATggTC - - FL.

b) Acceptor probes with 24 bases in the following sequence: 5`-LC Red 640-CACAgCCTTATgCACggCCTggAg - - PH.

VII- LightCycler FastStart DNA MasterPlus Hybprobe Kit (from Roche Diagnostics).

The principle

HybProbe probes consist of two different short oligonucleotides that bind to an internal sequence of the amplified fragment during the annealing phase of the amplification cycle.

One probe is labeled at the 5`-end with a Light Cycler Red fluorophore (LightCycler Red 610, 640, 670, or 705); it is also 3`- phosphorylated, so it cannot be extended. The other probe is labeled at the 3`- end with fluorescein. When hybridized to the template DNA, the two probes are close enough to allow fluorescence resonance energy transfer (FRET) between the two fluorophores.

During FRET, fluorescein (the donor fluorophore) is excited by the light source of the Light Cycler Instrument. Fluorescein transfers part of this excitation energy to the Light-Cycler Red dye (the acceptor fluorophore). Then, the Light Cycler Red dye emits fluorescence, which is measured by the Light Cycler instrument.

Experimental protocol :

Program the Light Cycler Experimental Protocol before preparing the reaction mixes.

Normally, a Light Cycler protocol that uses Light Cycler FastStart DNA Master^{PLUS} HybProbe contains the following parts.

- Pre-incubation (activation of FastStart DNA polymerase and denaturation of the DNA).
- Amplification of the target DNA
- Melting curve for amplicon analysis (optional; only for mutation detection).

• Cooling the rotor and thermal chamber.

A total of 18 µl of master mix and 2 µl of genomic DNA (40-100 ng) were added to each glass capillary placed in adapters. 2µl H_2O as negative control was added to one capillary tube. Sealed capillaries were centrifuged briefly (5 seconds) with the adapters in a micro centrifuge and were put in the Light Cycler rotor.

The following PCR protocol was used for amplification and melting curve analysis :

• Denaturation at 94°C for 2 min

Amplification

Cooling was for 30s at 40°C (Temperature transition rate 20°C/s)

• On-line PCR and PiZ Mutation Detection with Hybridization probes Fluorescence monitoring.

With the hybridization probes specific for the PiZ allele, the different genotypes result in different fluorescence signal intensities. The detection probe has a T_m 69.5°C with the wild type sequence and is stable at the PCR annealing temperature (55.0°C), whereas base pairing with PCR products from a homozygous mutant individual is impaired due to relatively low T_m (64°C). This apparent variability is signal intensity does not affect the quality of the melting curves monitoring of the melting behavior starts at 40°C.

Determination of PiZ Genotypes by melting Curve analysis:

Individuals with two PiZ alleles exhibit a single peak at 64°C, in heterozygous two peaks are detected, and individuals with the wild type sequence on both alleles result in a single peak at 69.5°C

Table (1) a variable elution volume is possible for "Blood sample" (Choice of 100 or 200 µL elution volume).

Sample	Elution volume	DNA concentration	DNA yield
500 µL of whole blood (on EDTA)	100 µL	129 ng/ul	12.9 µg
500 µL of whole blood (on EDTA)	200 µL	79 ng/ul	15.8 µg

Table(2) Preparation of PiZ Hybridization probe Master Mix for 20µl reaction

	Volume (µl)	Final
Master Hybridization probes	2	1x
$MgCl_2$ (25 mM)	0.8	2.0
Primers (5 µM each)	1 + 1	mM
Probes (4 µM each)	1 + 1	0.25
H_2O (PCR grade)	11.2	µM
Total volume	18	0.2 µM

Table(3) Procedure on Light Cycler

Parameter	Value		
Cycle	40		
Type	Quantification		
	Segment 1	Segment 2	Segment 3
Target temperature (°C)	95	55	72
Incubation time (s)	0	10	15
Temperature transition rate (°C/s)	20	20	20
Acquisition mode	None	Single	Note

Table (4) Melting curve Analysis

Parameter			
Cycle	1		
Type	Melting curves		
	Segment 1	Segment 2	Segment 3
Target temperature (°C)	94	40	70
Incubation time (s)	0	5	0
Temperature transition rate (°C/s)	20	20	0.2
Acquisition mode	None	None	Step

Table(5) Melting temperatures of PiZ alleles

Locus	Allele	Pairing	T_m (observed)
PiZ	WT PiZ	G-C match G-T mismatch	69.5°C 64.0°C
WT, wild type sequence			

Results

Table (6) : Frequency of different genotypes of alpha-1-antitrypsin among different studied groups

	No.	MM No. (%)	MZ No. (%)	ZZ No. (%)
Group I Patients with HCC)	27	14(51.9)	11(40.7)	2(7.4)
Group II (patients with Emphysema)	20	17(85)	2(10)	1(5)
(Healthy control)	15	15(100)	0(0)	0(0)

Table (7): Homozygous ZZ mutations in group I (patients with HCC) versus group III (healthy control).

	ZZ	
	No.	%
Group I (n=27)	2	7.41
Group III (n=15)	0	0.00
Fisher's exact test (P-value)	0.408 N	

Table (8): Homozygous ZZ mutations in group II (patients with emphysema) versus group III (healthy control).

	ZZ	
	No.	%
Group II (n=20)	1	5.00
Group III (n=15)	0	0.00
Fisher's exact test (P-value)	0.571 N	

Table (9): PiZ (both hetero and homozygous) in group II (patients with emphysema versus group III (healthy control)

	ZZ + MZ	
	No.	%
Group II (n=20)	3	15.00
Group III (n=15)	0	0.00
Fisher's exact test (P-value)	0.174 N	

Table (10): Heterozygous MZ mutations of AAT in group I (patients with HCC) versus group III (healthy control).

	MZ	
	No.	%
Group I (n=27)	11	40.74
Group III (n=15)	0	0.00
Fisher's exact test(P-value)	0.003*	

Table (11): Heterozygous MZ mutations of AAT in group II (patients with emphysema) versus group III (healthy control).

	MZ	
	No.	%
Group II (n=20)	2	10.00
Group III (n=15)	0	0.00
Fisher's exact test (P-value)	0.319 N	

Table (12) : Homozygous ZZ mutations of AAT in group I (patients with HCC) versus group II (patients with emphysema)

	ZZ	
	No.	%
Group I (n=27)	2	7.41
Group II (n=20)	1	5.00
Fisher's exact test (P-value)	0.613 N	

Table (13): Heterozygous MZ mutations of AAT in group I (patients with HCC) versus group II (patients with emphysema).

	MZ	
	No.	%
Group I (n=27)	11	40.74
Group II (n=20)	2	10.00
Fisher's exact test (P-value)	0.02*	

Table (14): Incidence of hepatitis C virus and hepatitis B virus in patients of group I(patients with HCC)

		Hepatitis	
		No.	%
B+		6	22.22
C+		21	77.77
Total		27	100.00
Chi-square	X^2	10.704	
	P-value	0.001*	

Note:
N insignificant where P-value >0.05
* Significant where P-value <0.05

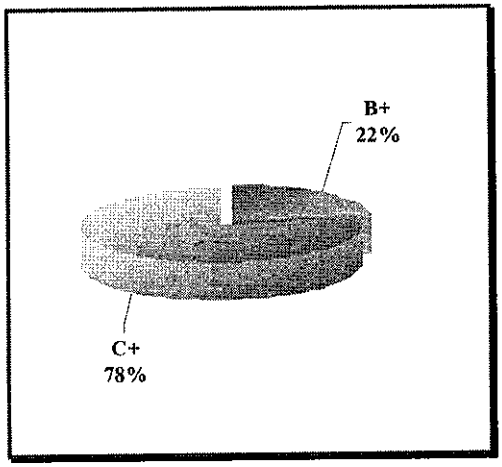

Fig. (1) Incidence of hepatitis C virus and hepatitis B virus in group I(patients with HCC).

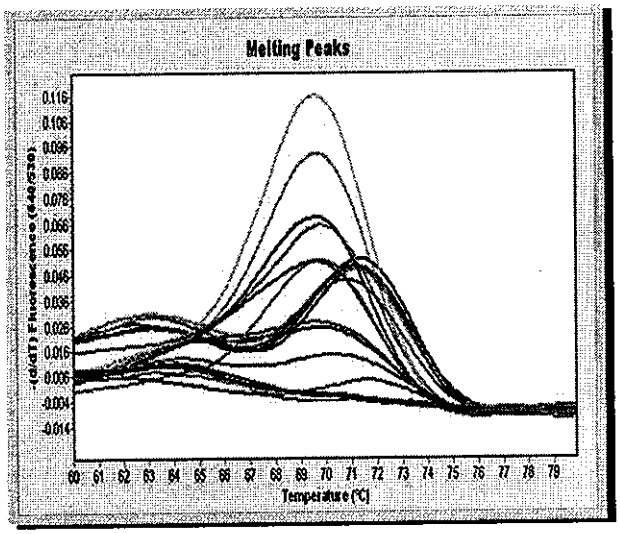

Fig.(2) Melting curves peaks

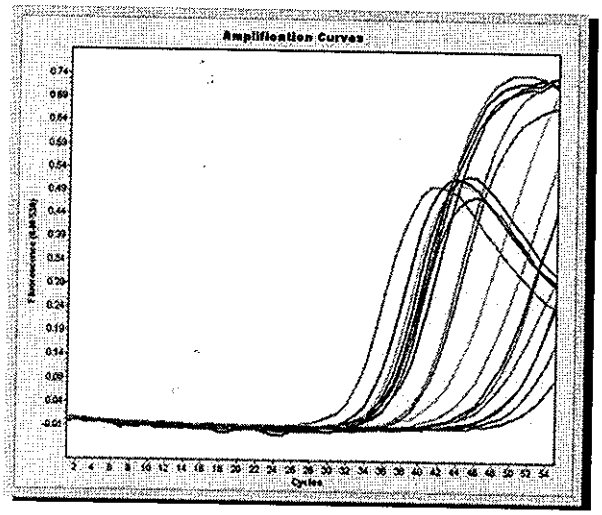

Fig.(3)amplification curves of target area

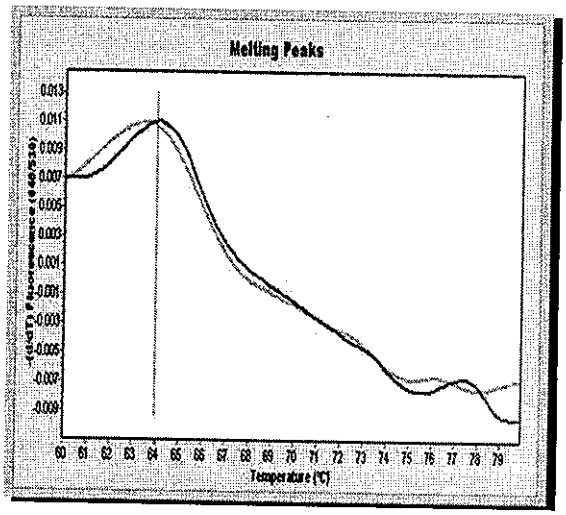

Fig.(4)the maximaum melting peaks

Discussion

Our current study, which for our knowledge is the first genetic study, applied on Egyptian patient for Z-alleles of α1antitrypsin.

In this study we screened 27 patients with primary hepatocellular carcinoma (HCC) who was giving past history of chronic viral hepatitis, 20 patients with history of early onset emphysema and 15 healthy control.

We used real time PCR-Light-Cycler, with hybridization probes. Generally, hybridization was performed with two different, short oligonucleotides that hybridize to two adjacent internal sequences of the amplified PCR fragment during the annealing phase of the PCR cycles, one probe is labeled at the 5` end with a light-Cycler red fluorophore and is phosphorylated at the 3` end. The other probe is labelled with fluoresein. Only after hybridization the probes are in close proximity, resulting in fluorescence resonance energy transfer between the two fluorophores. During the latter, fluorescein, the donor fluorophore, is stimulated by the light source of the Light-Cycler instrument, and part of the resulting energy is transferred to Light-Cycler red, the acceptor fluorophore. The emitted fluorescence of Light-Cycler red fluorophore is measured.

Therefore, in the case of an M-allele, the expected melting temperature will be higher than the expected melting temperature in the case of Z-allele where a mismatch of one base pair is included with melting temperature 69.5 C^0 for M-allele and 64 C^0 for Z-allele. The isolated alleles in the three groups, group I (patients with HCC), 11 patients were Pi MZ (40.74%), p value 0.003) and 2 patients were Pi ZZ (7.4%), other 14 patients were Pi MM (51.85%). In group II (patients with early emphysema), we isolated two patients with Pi MZ (10%) and one patient was Pi ZZ (5%), other 17 patients were Pi MM (85%) while all healthy controls (15) were Pi MM

Our results showed increased α1antitrypsin Z mutation variants in patients with HCC (40.74%) MZ, (7.4%) ZZ, this agree with *(Helal et al., 1999)* and also *(Abdel-Sattar et al., 1995)* who used isoelectric focusing for screening Egyptian patients with hepatocelluler carcinoma and other chronic hepatic disorders for AAT phenotyping, their study showed (25%) MZ phenotypes, (8.3%) ZZ phenotypes in patients with HCC, also our results coinside with many investigators in non-Egyptian patients *(Reintoft and Hagerstrand .1986), (Abdul-Nasser and Eriksson.1996),* who observed that heterozygous type Pi MZ of

ATT were associated with increase of incidence of HCC. This positive correlation between Z alleles of α1antitrypsin and hepatocelluler carcinoma explained by direct cellular injury resulting from abnormal high quantities of oxygen free radicals originating from an increase in proteases in the liver which have not been neutralized by the low circulating levels of α1antitrypsin *(Schmidt and Perlmutter ,2005)*

Further more,in the patients carrying the Z-allele, liver involvement might be also explained by accumulating hypothesis, which suggested that intracellular globules of altered unsecretable α1antitrypsin material might cause liver damage *(Schmidt and Perlmutter ,2005).*

In our results there were positive correlation between HCC and viral infection. (77%) of patients of group I were giving past history of HCV and (22.22%) were giving past history positive HBV. This results agree with other studies on Egyptian patients with HCC for HCV and HBV markers, HCV Ab (88%), HBsAg (13%) *(Serri, et al,1995.)*, HCV Ab (69.84%), HBc Ab, (47.62%) *(Abdou Sabri, et al.,1995)* HBsAg (11.8%) (HBc Ab (68%), HCV Ab, (89%) *(Raouf, et al.,1996)*, HCV Ab, (70%), HBc Ab (48%), *(Qabil et al.,2002).* HCV Ab (90%) *(Authman and Ashraf,2000)*, HCV Ab, (76.67%) HBc Ab, (28.67%) *(Ashour et al., 2004).*

Similar conclusion in non Egyptian patients is obtained by, *(Propst, et al ,1997).* Pathogenesis of HCC due to viral infection, in HBV explained by that HBV is DNA virus that integrates into the host genome, and this interaction is believed, in part, to be carcinogenic. Besides, the virus encodes a 17 Kda protein, HBx, which is know to be a causative agent in the formation of HCC. On the contrary, HCV is a RNA virus that does not integrate into host genome but likely induces HCC through host protein interactions or via the inflammatory response to the virus. Products encoded in the HCV genome interfere with and disturb intracellular signal transduction. Some HCV proteins, such as the core protein, NS3 and NS5A, have seen to have a regulatory effect on cellular promoters, to interact with a number of cellular proteins; and to be involved in programmed-cell death modulation in certain conditions.

So how we can explain this significant correlation between α1antitrypsin heterozygous Z-allele and HCC in patients with viral infection (P value 0.003, while in some of non Egyptian studies especially in USA, there are no association between α1antitrypsin heterozygous Z-allele and viral infection in cases of HCC *(Rabinovitz et al.1992), (Bowlus et al., 2005).*

This was explained by the high prevalence of HCV infection in Egyptian population (20%) *(Darwish et al., 1997)*, (22.54%) *(Fakeeh and Zaki,1999)* While prevalence of HCV in USA is 1.6% *(Armstrong,et al., 2006).* So the prevalence of Hepatitis virus C infection in Egyptian populations is at least 10 times more than that in USA

Previous studies have been conflicting as to whether patients with heterozygous α-1 antitrypsin deficiency, particularly Pi types MZ and SZ are at increased risk of developing liver injury and progression to end-stage liver disease *(Hodges et al., 1981), (Vennarecci et al., 1996).* An alternative explanation may be that individuals carrying single PiZ are more susceptible to other hepatic insults such as viral infection, toxins or the coexistence of the other liver diseases *(Propst et a.l,1997).* While the association between homozygous Z mutation and HCC is well established *(Sharp,1982), (Eriksson et al., 1986), (Eriksson, 1987)* this coinside with our results (7.4% of hepatoma patients had PiZZ mutations). There is no uniformly accepted mechanism explaining the pathphysiology of liver injury although there is general agreement that it is linked to intracellular accumulation of mutant α1AT *(Teckman et al., 2002).* Amino acid substitution in the α1AT molecule lead to abnormal folding and accumulating of α1AT within cellular endoplasmic reticulum, a process that may be further aggravated by defective degradation of the aggregated mutant molecules in some individuals. *(Carrel and Travis,1985), (Wu Y. Whitman et al., 1994).* The accumulation theory" is further supported by the rare " null" variants (PI*Qo), who are incapable of synthesizing α 1AT and

therefore do not have intracellular accumulation. *(Fabbretti et al.,1992), (Norman et al.,1997).* Histological evidence of accumulated α1AT in liver biopsy specimens is typically seen as intracytoplasmic deposition of periodic acid-Schiff (PAS)-positive, diastase-resistant globules in hepatocytes, primarily localized to periportal areas. Intense immunohist-ochemical staining with antibodies against α 1AT is also characteristic. Despite these observations, little information is available about the mechanism by which retained α1AT molecules eventually induce hepatocellular injury *(Graziadei et al., 1996).*

Studies by Lomas and Mahadeva shown that this substitution reduces the stability of the monomeric form of the protein and increases the likelihood that it will undergo polymerization in vitro by a novel "Loop-Sheet" insertion mechanism *(Lomas and Mahadeva,2002).*

Our results in group II (20 patients with emphysema) one patient was PiZZ (5%) and 2 patients were heterozygous PiMZ (10%), these findings agree with other Egyptian investigators *(Safwat Maiy,1990), (Radhwan et al., 1992), (Gouda et al., 1998).* Pathogenesis of Alpha 1 antitrypsin deficiency in lung due to associated insufficient amounts of Alpha 1 antitrypsin in lung to protect the tissues from damage by the enzymes predominantly neutrophil elastase normally controlled by this inhibitor as explained by the protease and antiprotease theory of emphysema which occur in association with homozygous ZZ alleles of alpha-1 Antitrypsin. This relation is well established *(Janoff, 1985).* Also, polymeric alpha-1-antitirypsin co-localize with neutrophils in emphysematous alveolli and are chemotactic this explain accelerate tissue destruction in Z-mutant alpha-1antitrypsin *(Mahadeva et al 2002).* As we detected 2 patients with PiMZ alleles who were (10%) of group II (patient with emphysema), this is relatively high but not statistically significant as P value (0.319), this can be explained because of our patients were smokers and were emphysematous while many studies concluded that MZ mutations of alpha-1-antitrypsin can lead to COPD in patients with MZ mutation of alpha-1-atitrypsin if they are smokers *(Dahl,et al.,2001),* also *(Janoff, 1985)* observed that low incidence of PiMZ of alpha-1-antitrypsin in bronchiectasis. So the diagnosis of mutant Z allele of alpha-1-antitrypsin is important as α-1-protienase inhibitor replacement therapy is available *(Brantly,et al.,1988).* Antiprotease therapy with alpha-1-protease inhibitor reduces the incidence of lung infections and slowing deterioration of the lung function and cause reduction of mortality *(Lieberman, et al., 1986)*

In this study it is concloded significant correlation of mutant Z allele of AAT with HCC, also showed that patients with PiMZ are of high risk to develop HCC when they get viral hepatitis infection and so mutant Z allele can be considered as a risk factor of HCC in Egyptian patients with viral hepatitis.

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الدراسة الجزيئية لنقص (ألفا-1-انتيتربسين) في الأورام الكبدية ومرض التمدد الرئوى

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يعتبر نقص (ألفا-1-انتيتربسين) من الأمراض التي تنتقل وراثياً بسبب طفرات جينية مصاحبة ويعتبر الحاملون لهذه الطفرات الجينية أكثر عرضة لمرض تمدد الرئة المبكر في البالغين وأمراض الكبد في البالغين والأطفال ومعظم الأشخاص الذين يعانون من نقص (ألفا-1-انتيتربسين) لا يتم تشخيصهم ومن الناحية الإكلينيكية يكون وجود هذه الطفرات أكثر احتمالاً في المرضى الذين يعانون من تمدد رئوي مبكر تحت سن الستين حتى لو كان لديهم تاريخاً في التدخين كذلك في حالات أمراض الكبد خاصة في الأطفال حديثي الولادة والبالغين في حالات التهاب الكبد المزمن وحالات الأورام الكبدية وبالرغم من عدم وجود علاج قاطع لهذا المرض فإن التشخيص المبكر له وبدء إجراءات التحكم في العوامل المساعدة على تطور المرض من الممكن أن يكون لها أهمية في تقليل درجة المرض على سبيل المثال معظم هؤلاء المرضي الذي يعانون من نقص (ألفا-1-انتيتربسين) يمكن الأ يعانوا من مرض التمدد الرئوي إذا تجنبوا التدخين وتناولوا (ألفا-1-انتيتربسين) كعلاج عن طريق الاستنشاق أو الحقن.

إن تأثير الكبد يحدث في وجود الجين (زد) في صورة كاملة أو جزئية (جين واحد أو اثنان) وعادة توجد هذه الجينات على الذراع الطويل للكروموزوم رقم (14).

ويعتبر هذا المرض من أكثر الأمراض الجينية التي ترتبط بأمراض الكبد في الأطفال. وهناك حوالي 40٪ من المصابين بهذه الطفرات الجينية والذي لا يصابون بأمراض كبدية في فترة الطفولة يعانون من أمراض تليف الكبد في فترة البلوغ كما أن 15٪ من هؤلاء يعانون من أورام الكبد وليس من المعلوم تماماً كيفية حدوث المرض لكن تغير أحد الأحماض الأمينية في الجين الوراثي الناتج عن الطفرة الوراثية من النوع (زد) يسبب تغيراً كيميائياً في تركيب (ألفا-1-انتيتربسين) مما يؤدي الى ترسبه في الخلايا الكبدية محدثاً تأثيرات شديدة الضرر عليه وبالنظر الى أن نسبة قليلة من مرضى نقص (ألفا-1-انتيتربسين) هي التي تتعرض للأمراض الكبدية فإن العوامل المحفزة يمكن أن يكون لها دور في اظهار المرض مثل التهاب الكبد الفيروسي وادمان الكحوليات.

الهدف من هذه الدراسة

إجراء مسح للمرضى الذين يعانون من الأورام الكبدية (مرضى لهم تاريخ بالإصابة بفيروس كبدى مزمن) وذلك لدراسة الطفرة الجينية (من النوع زد) فى هؤلاء المرضى ومقارنتهم بمجموعتين أحدهما من الأشخاص الأصحاء والأخرى من مرضى التمدد الرئوى باستخدام تقنية البلمرة التسلسلية (بى– سى– أر).

المرضى والنتائج :

وقد تم إجراء هذه الدراسة على عدد 27 مريض من مرضى الأورام الكبدية (21 من الذكور و 6 إناث) وعدد 20 مريض من مرضى التمدد الرئوى (17 من الذكور وثلاثة من الإناث) ومجموعة من الأصحاء وعددهم 15 شخصا (12 من الذكور وثلاثة من الإناث) وقد أظهرت النتائج فى مجموعة الأورام الكبدية عدد 11 نمط جيني من النوع زد (المخلط) (معه جين طبيعى من النوع أم) وحالتين من النمط الجينى (زد) (الخالص) (كلا الجينين من النوع زد) وباقي المرضى في هذه المجموعة وعددهم 14 مريض كانوا من النوع (إم إم) (النوع الطبيعي)، وفى المجموعة الثانية (مرض التمدد الرئوى) تم تشخيص حالتين من النوع (زد) المخلط وحالة من النمط الجينى (زد) (الخالص) وباقى المرضى 17 من النوع الطبيعى (إم) وجاءت المجموعة الثالثة كلها من النوع (إم) الطبيعى.

وهذه النتائج تظهر وجود (النمط الجينى زد) بنسبة ذات دلالة إحصائية فى مرض الأورام الكبدية وبنسبة 15٪ فى مرضى التمدد الرئوى ولذلك فقد يعتبر هذا النمط الجينى (زد) فى حالة وجوده عامل خطورة إضافى لحدوث الورم الكبدى فى المرضى المصريين المصابين بالفيروسات الكبدية وهو أيضاً موجود بنسبة تماثل النسب العالمية تقريباً في مرضى التمدد الرئوي ومن المهم تشخيصه في هؤلاء المرضى لوجود علاج بديل عن طريق الاستنشاق يؤدي الى تحسن كبير في أعراض المرض.