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Impact of Estrogen Receptor 2 Gene Polymorphisms and Expression, on Risk and Progression of Hepatocellular Carcinoma among Women with Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background*:* Given that risk for hepatocellular carcinoma (HCC) is linked to liver cirrhosis. There is accumulating evidence that estrogen influences liver function via estrogen receptors (ERs), which are dysregulated in many cancers all over the body. **Objective:** We aimed to investigate the ESR2 genes (rs1256049 and rs-4986938) polymorphism and mRNA expression in patients with nonalcoholic fatty liver (NAFLD), in correlation with the risk and progression of HCC among Egyptian women with NAFLD. **Patients and Methods***:* Case-control study enrolled fifty women with NAFLD and fifty healthy women. Serum E2 level was determined using ELISA. Genotyping of ESR2-SNPs (rs1256049 and rs4986938) was performed by PCR-RFLP. ERβ2 mRNA values were explored by RT- PCR.

Results: There were non-significant differences concerning ERβ2 mRNA expression levels among studied groups. Concerning genotype of ESR2 (rs1256049), in NAFLD group the frequencies of the AA, GA were higher than in controls (OR = 22.62,95% CI = 2.8- 182.6, P <0.001 and OR = 8.7,95% CI = 1.77- 42.69, P <0.001 respectively). Additionally, A allele OR = 13.5,95% CI = 4.58- 39.76, P <0.001). There were non-significant differences in frequencies of GG, GA, and A allele between case and control groups P >0.001. Regarding ESR2 (rs1256049), the only significant parameter was A allele OR = 4.71,95% CI = 1.96-11.33, P <0.001 in cirrhotic NAFLD compared to non-cirrhotic NAFLD. ESR2 (rs1256049) polymorphism was significantly higher in HCC. Thus, it could be a predictor of HCC among NAFLD patients. BMI, HOMA-IR, E2, and FIB-4 scores were highly correlated with HCC in both cirrhotic and non-cirrhotic NAFLD groups by applying a logistic regression test.

Conclusion: ESR2 (rs1256049) polymorphism was significantly higher in HCC. Thus, it could be a predictor of HCC among NAFLD patients.

Keywords: NAFLD, HCC, Cirrhotic, ESR2 genes, Genotype.

INTRODUCTION

Consistent evidence indicates that the prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing and is supposed to be the most indicator for liver transplantation [1]. Emerging evidence indicates that about 40% of the population had NAFLD [2]. Furthermore, steatohepatitis affects around 1.5%–6.5% of the general population worldwide. Nowadays, it is widely recognized that histological, lobular inflammation, and hepatocyte ballooning, which is the main histopathological criterion of steatohepatitis is associated with the rapid progression of liver fibrosis[3].

Several pieces of evidence have shown that cirrhosis is a precancerous disorder [4]. There is convincing evidence that the current pandemic of obesity and its associated NAFLD led to cirrhosis. Nowadays, it is widely recognized that the prevalence of HCC-related NAFLD is growing and about 15–20% of NAFLD patients have HCC in Western countries [5]. From a clinical perspective, the currently well-documented diagnosis of HCC depends on noninvasive diagnostic markers [6]. In recent years, considerable attention has focused on liver function regulations and the role of sex hormones, in particular estrogen, in liver growth and function regulations [7]. Additionally, estradiol prevents lipid accumulation and liver steatosis by decreasing lipogenesis and enhancing lipolysis [8].

Estrogen Receptors 2 (ERs) are expressed in many tissues and organs all over the body. Intriguingly, ER dysregulation over or under-expression could be led to many diseases and cancer [9]**.** Overall, there is modest evidence to support the association of ER (ESR1 and ESR2) with risk, clinicopathological features, and progression of cancers [10]**.**

Nonetheless, there is evidence that ESR2 influences the precancerous genes [11]. There is convincing evidence that under-expression of ESR2 is related to susceptibility and severity of gall bladder cancer [12-13].

Remarkably, HCC etiology varies according to the different epigenetic and genetic dysregulations. Even though the understanding of the pathogenesis and etiology of the disease has improved, still we needed further studies. Thus, we aimed to investigate whether polymorphism of ESR2 genes rs1256049 and rs-4986938 are associated with NAFLD and to determine a possible impact of this polymorphism on the risk and progression of HCC of Egyptian women with NAFLD.

MATERIALS AND METHODS

The current study was conducted on one hundred participants; 50 women with NAFLD and fifty healthy volunteers without liver disease, were included as the control group. The flowchart of the study is demonstrated in figure 1.

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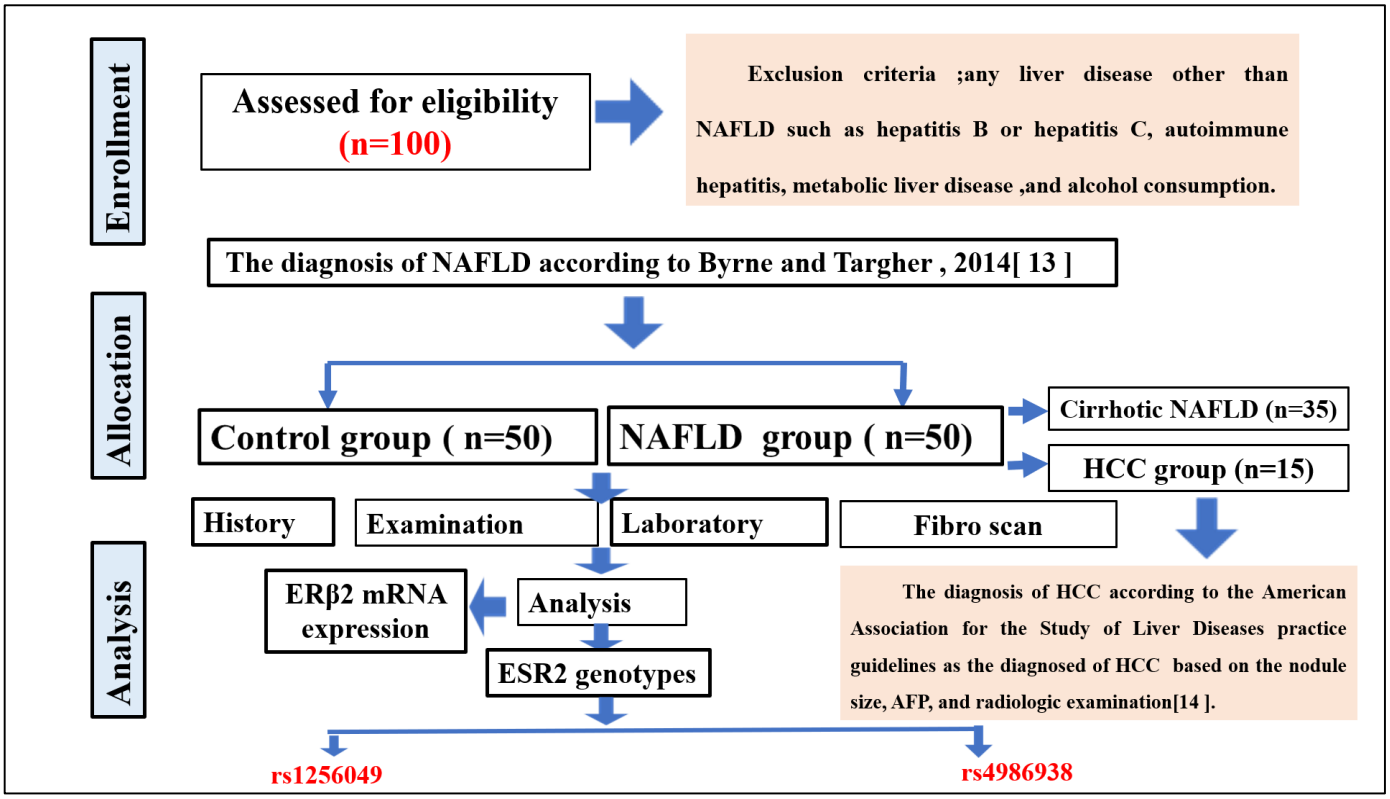

Figure (1): Flowchart of the study.

Ethical consent:

The study protocol was authorized by the Faculty of Medicine at Zagazig University's Ethical Committee and the reference number was IRB (Ethics number. 9944), and each participant signed a written informed consent document. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Blood sampling:

Blood samples were obtained from all the participants and laboratory tests were done according to operating procedures in Zagazig University Hospital Laboratories.

DNA extraction and genotyping of ESR2-SNPs:

We extracted DNA according to operating procedures in Zagazig University Hospital Laboratories. Genotyping of ESR2-SNPs (rs1256049 and rs4986938) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using a PTC-100 thermal cycler (MJ Research Inc, Watertown, Massachusetts, USA).

The following primers were used: for rs1256049, F: 5′-TTGCGCAGCTTAACTTCAAA-3′; R: 5′-ACCTGTCCAGAACAAGATCT-3′. for rs4986938, F: 5′-CAATGCATATCCTGCCTGTG-3′; R: 5′-GGTTTAGGGGTGGGGTAGACTG-3′ [15].

ERβ2 mRNA expression levels by real-time PCR:

The RNA was extracted from EDTA peripheral blood samples, according to the company's instructions. The mRNA expression of ERb was explored by Real-time PCR and the forward primer (-TGG TCC ATG GCC AGT TAT CA-), ERb reverse primer (-AGG TGT GTT CTA GCG ATC TTG CT-). GAPDH was used as a housekeeping gene. The expression level was determined using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Differences in sample characteristics were tested. One-way ANOVA test with post hoc test were performed for variables with normal distribution. Chi-square test was used to assess whether allele frequency distributions among controls and case groups were consistent with the Hardy–Weinberg equilibrium. Odds ratios (ORs) and confidence intervals (CIs) were calculated. The proper sample size was determined using PAWE-3D [16]. $P < 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS (Version 26).

RESULTS

Characteristics of the investigated patients and controls

As demonstrated in table 1 there were significant discrepancies between studied groups concerning metabolic risk parameters, liver function test, and E2 as well as alpha-fetoprotein in particular in the cirrhotic NAFLD group. As expected, age and BMI values were non-significant as we matched our groups to avoid the influence of obesity and age on our results. Regards ferritin it was expected to be non-significant and normal to exclude other causes of cirrhosis.

Table (1): Clinical, anthropometric and laboratory characteristics of studied groups

Variables	Control group (mean ± SD), (n=50)	Non-Cirrhotic NAFLD group (mean ± SD), (n=35)	Cirrhotic NAFLD group, (mean ± SD) (n=15)	P
Age (years)	34.9±9.37	34.2±10.1	35.8±12.9	0.883
SBP (mm Hg)	123.3± 3.07	132.6± 14.1*	129.3± 13.1	<0.001*
DBP (mm Hg)	86.3±4.8	88.5±7.30	92.3±7.74	<0.001*
Body mass index	33.6±3.940	33.3±3.126	32.3±3.126	0.768
FPG (mg/dL)	84.9±3.97	196.1± 8.1*	211.4±3.3$	<0.001*
FSI (lU/mL)	7.6±1.262	13.2±2.14*	16.2±3.74$#	<0.001*
HOMA-IR	1.61±0.1	5.4±1.21*	5.9±1.62$	<0.001*
INR	0.98±0.06	1.35±0.37*	1.66±0.43$#	<0.001*
Prothrombin time	10.9±0.28	11.9±1.9*	13.9±2.85$#	<0.001*
Albumin (g/dl)	4.1±0.21	3.9±0.38	2.75±0.57$#	<0.001*
AST (IU/L)	20.6±4.7	77.4±8.4*	82.5±8.98$	<0.001*
ALT(IU/L)	22.44±5.4	78.9±7.6*	80.6±18.3$	<0.001*
Total bilirubin (mg/dl)	0.9±0.13	1.13±0.2	2.16±0.54$	<0.001*
Direct bilirubin (mg/dl)	0.25±0.07	1.26±0.3*	1.26±0.3$	<0.001*
S. creatinine (mg/dl)	0.76±0.16	1.08±0.14*	2.87±0.4$	<0.001*
Alpha fetoprotein(ng/ml)	8.7±1.50	21.4±5.2*	185±25.98$#	<0.001*
Hepatic steatosis index	34.9±1.18	46.4±3.65*	54.9±1.18$	<0.001*
Ferritin (ng/mL)	20.12±3.2	21.22±3.1	25.11±4.6	0.654
E2 (pg/ml)	37.2±5.3	54.5±3.18*	65.9±7.44$	<0.001*
FIB-4 score	4.56±0.63	8.26±2.05*	12.2±0.22$	<0.001*
ERβ2 mRNA expression	0.87±0.13	0.85±0.15	0.75±0.11	0.127

SBP; systolic blood pressure, DBP; diastolic blood pressure, FPG; fasting plasma glucose, FSI; fasting serum insulin, HOMA-IR, homeostasis model assessments of insulin resistance, AST; aspartate aminotransferase, ALT; alanine aminotransferase, S; serum, E2;17β-estradiol, FIB-4 score; Fibrosis-4 score; *P < 0.05 when comparing non-cirrhotic NAFLD with control group, $P < 0.05 when comparing cirrhotic NAFLD with control group, #P < 0.05 when comparing cirrhotic NAFLD with non-cirrhotic NAFLD group

ERβ2 mRNA expression levels in studied groups

There was non-significant difference between studied groups regarding ERβ2 mRNA expression level, which was in the control group (0.83±0.20) and in case group (0.79±0.17) as shown in table 1 and figure 2.

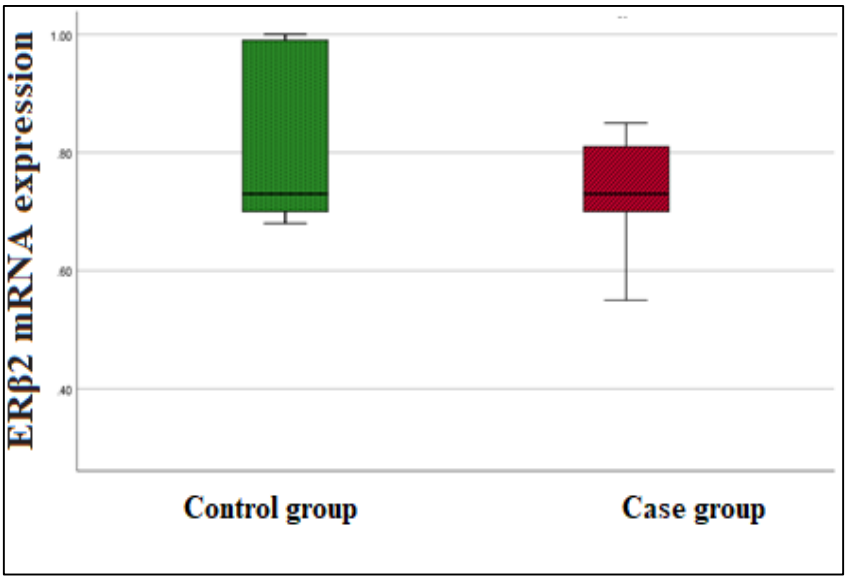

Figure (2): ERβ2 mRNA expression levels among studied groups

The clinicopathological features of HCC patients are demonstrated in table 2.

Table (2): Clinicopathological features of HCC patients

	HCC patients (n =16) n (%)	P value
Stage		0.480
Stage I/II	6 (37.5%)	
Stage III/IV	10 (62.5%)	
Tumor size		0.157
<5 cm	12 (75%)	
>5 cm	4 (25%)	
Lymph node metastasis		<0.05*
-Absent	14 (87.5%)	
-Present	2 (12.5%)	
Distant metastasis		<0.05*
-Absent	14 (87.5%)	
-Present	2 (12.5%)	
Child–Pugh grade		0.882
-A	4 (25%)	
-B	6(37.5%)	
-C	6(37.5%)	
Portal vein thrombosis		0.157
-Negative	12 (75%)	
-Positive	4 (25%)	
Number of tumor lesions		0.889
-Single	8 (50%)	
-Multiple	8 (50%)	
Site of lesions		0.882
-Right lobe	6(37.5%)	
-Left lobe	4 (25%)	
-Both	6(37.5%)	

* Significant *P* value (*P*<0.05)

Distribution of ESR2 genotypes and allele frequencies in healthy controls and patients with NAFLD. Among studied subjects (n=100). Genotype and allele frequencies of the ESR2 (rs1256049), and (rs4986938) of the case and control groups are presented in table 3. Regards ESR2 (rs1256049), the AA genotype, GA genotype, and A allele were significantly increased in those with NAFLD than control group as shown in table 3.

Regarding ESR2 (rs4986938)

As we proved in table 3, there were non-significant differences in frequencies of GG, GA, and A allele between case and control groups.

Table (3): Distribution of ESR2 genotypes and allele frequencies in healthy controls and patients with NAFLD

Gene	Genotype	Healthy control		NAFLD patients		OR (95%)	p
		N=50		N=50			
		n	(%)	n	(%)		
rs1256049	GG	47	(94%)	27	(54%)		
	GA	2	(4%)	10	(20%)	8.7(1.77-42.69)	<0.001*
	AA	1	(2%)	13	(26%)	22.62(2.8- 182.6)	<0.001*
	G allele	96	(96%)	64	(64%)		
	A allele	4	(4%)	36	(36%)	13.5(4.58- 39.76)	<0.001*
rs4986938	GG	45	(90%)	44	(88%)		
	GA	4	(8%)	5	(10%)	1.27(0.32- 5.07)	P=0.727
	AA	1	(2%)	1	(2%)	1.022(0.06- 16.86)	P=0.987
	G allele	94	(94%)	93	(93%)	1.17(0.38-3.64)	
	A allele	6	(6%)	7	(7%)		P=0.774

Distribution of ESR2 genotype (rs1256049) and allele frequencies in patients with NAFLD

In the current study, patients with NAFLD which stratified into non-cirrhotic NAFLD and cirrhotic NAFLD, concerning ESR2 (rs1256049), the only significant parameters were A allele. Patients with cirrhotic NAFLD had significantly higher frequencies compared to the other group of NAFLD, as shown in table 4.

Table (4): Distribution of ESR2 genotype (rs1256049) and allele frequencies in patients with NAFLD, which were stratified to non-cirrhotic NAFLD and cirrhotic NAFLD

Gene	Genotype	Non-cirrhotic NAFLD		Cirrhotic NAFLD		OR (95%)	p
		N=35		N=15			
		n	(%)	n	(%)		
rs1256049	GG	20	(57.1%)	7	(46.7%)		
	GA	8	(22.9%)	2	(13.3%)	0.71(0.121- 4.20)	P = 0.7098
	AA	7	(20%)	6	(40%)	2.44(0.610-9.82)	P = 0.2064
	G allele	48	(68.6%)	16	(53.3%)		
	A allele	22	(31.4%)	14	(46.7%)	4.71(1.96-11.33)	<0.001*

Distribution of ESR2 genotype (rs1256049) and allele frequencies in patients with non-HCC patients with NAFLD and HCC patients with NAFLD.

Among studied NAFLD patients we further evaluated ESR2 (rs1256049) as significant in the NAFLD group, the AA, GA genotypes, and A allele were significantly increased in those with HCC than non-HCC group as shown in table 5. Interestingly these results revealed that ESR2 (rs1256049) polymorphism was significantly higher in HCC. Thus, ESR2 (rs1256049) could be a predictor of HCC among NAFLD patients.

Table (5): Distribution of ESR2 genotype (rs1256049) and allele frequencies in patients with non-HCC patients with NAFLD and HCC patients with NAFLD

Gene	Genotype	Non-HCC (NAFLD)		HCC		OR (95%)	p
		N=34		N=16			
		n	(%)	n	(%)		
rs1256049	GG	28	(82.3%)	3	(18.8%)		
	GA	2	(5.9%)	5	(31.2%)	23.3(0.07-117.04)	<0.001*
	AA	4	(11.8%)	8	(50%)	18.6 (3.442-101.23)	<0.001*
	G allele	58	(85.3%)	11	(68.8%)		
	A allele	10	(14.7%)	21	(31.2%)	11.071(4.1-29.8)	<0.001*

Influence of ESR2 rs1256049 polymorphism on ERβ2 mRNA expression levels in HCC patients

According to the results of the current study, there were non-significant differences between ESR2 rs1256049 genotype GG (0.79±0.12), GA (0.76±0.134), and AA (0.808±0.162) regarding ERβ2 mRNA expression levels in HCC patients; P =0.442 (Figure 3).

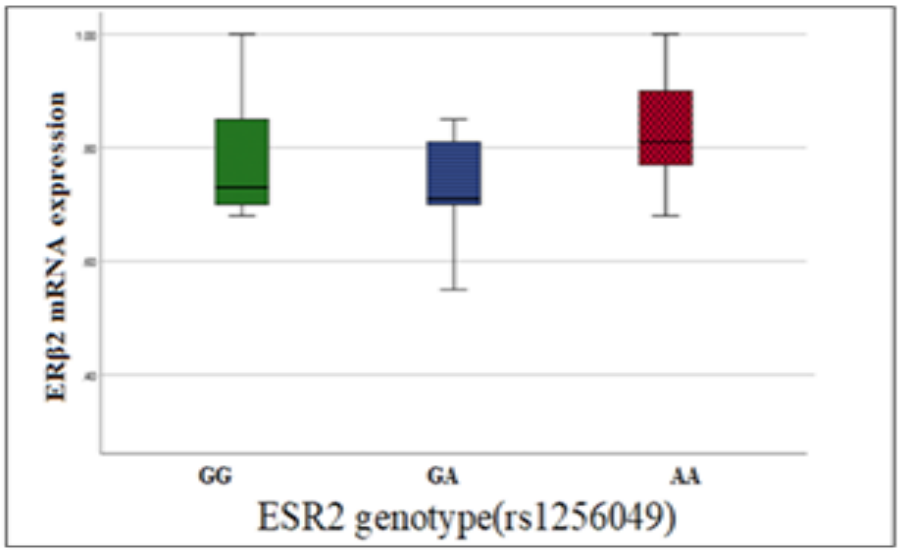

Figure (3): Influence of ESR2 rs1256049 polymorphism on ERβ2 mRNA expression levels in HCC patients

Logistic regression analysis for prediction of HCC among case group

To find the predictors of HCC, the present study provides evidence that BMI, HOMA-IR, E2, and FIB-4 scores were independently associated with HCC in both cirrhotic and non-cirrhotic NAFLD groups by applying a logistic regression test (Table 6).

Table (6): Logistic regression analyses for detection of HCC among women with NAFLD

Variable	B	S.E.	Wald	P value	Exp(B)	95% C.I. for EXP(B)	
						Lower	Upper
BMI	3.617	1.813	3.980	0.046	37.241	1.065	1301.716
HOMA-IR	1.337	0.435	9.441	0.002	3.808	1.623	8.934
E2	0.142	.041	12.031	0.001	1.152	1.064	1.248
FIB-4	0.739	0.269	7.561	0.006	2.093	1.236	3.543
Constant	-23.428	11.586	4.089	0.043	0.000		

DISCUSSION

There is convincing evidence that HCC is more common in males than females [17]. Nowadays, it is widely recognized that the liver is a hormone-sensitive organ, and it is influenced by gonadal hormones, such as estrogen [18]. Considering the important role of estrogen, estrogen may regulate growth, differentiation, and metabolism in addition to liver function [19].

Emphasis has been given to the underlying molecular mechanisms of NAFLD. Regarding gender roles in the pathogenesis of HCC, several studies have reported that the prevalence of cirrhosis was higher in women compared to men [20]. To gain further insights, we performed our study on Egyptian women. We enrolled fifty women as control and fifty women with NAFLD. Among NAFLD, patients were classified as non-cirrhotic (n=35) and cirrhotic NAFLD (n=15). According to our results, 11 patients had HCC in the non-cirrhotic group (31.4%) and 5 patients had HCC in the cirrhotic group (33.3%). From this consideration, we hypothesized that there are non-significant differences regarding the percentage of HCC in both groups.

Several pieces of evidence have shown that liver cirrhosis is precancerous disorder. However, ongoing research revealed that among NAFLD patients, HCC can grow in patients without cirrhosis. According to **Piscaglia *et al.*** [21] study, cirrhosis was present in only about 50% of NAFLD-HCC patients. A similar finding observed by **Ertle *et al.*** [22], who observed that NAFLD/NASH could be a strong risk factor for HCC, even in the absence of cirrhosis.

Leung *et al.* [23] conducted their study on NAFLD-associated HCC and cirrhotic patients to evaluate the characteristics of HCC in cirrhotic and non-cirrhotic NAFLD. They detected that HCC could develop in NAFLD without cirrhosis. Interestingly, at diagnosis non-cirrhotic NAFLD-associated HCCs are larger than those in cirrhotic, conferring a poorer prognosis. These results are in agreement with the results of the **Mohamad *et al.*** study [24].

It is noteworthy to mention that estrogen protects liver function and structures as well as prevents liver diseases including HCC, and this has been attributed to the anti-proliferative and anti-inflammatory activities brought about by E2 through binding to and activation of estrogen receptor beta (ERβ) [25]. **Brady** observed that estrogens endorse a variety of protective mechanisms, including the maintenance of mitochondrial structure and function, and the enhancement of innate immunity [26].

In light of the fact that epigenetic and genetic regulations of estrogen are associated with HCC. Though some reports are controversial [27]. Hence, we decided to investigate whether polymorphism of ESR2 genes rs1256049 and rs-4986938 are associated with NAFLD and to determine a possible impact of this polymorphism on risk and progression of HCC among Egyptian women with NAFLD.

Up to now, no Egyptian studies have specifically explored the association of ESR2 polymorphism and ERβ2 mRNA expression with NAFLD or its associated cirrhosis and HCC. We have performed this study to explore this relationship. To increase the reliability of the present study we investigated ERβ2 mRNA expression in peripheral blood in all studied groups and we did not find any significant differences between the studied groups. This is consistent with previous studies, for example **Iyer *et al.*** [28], who conducted their study on patients with chronic hepatitis C and hepatocellular carcinoma to evaluate ERβ2 mRNA expression, and according to this study's results, there were no statistically significant changes in the expression of ERb under different clinical features.

In this context, a study by **Ohnishi *et al.*** [29] showed that the expression of estrogen receptors may be rather suppressed in primary HCC indicating the pathogenic role of estrogens in HCC development.

As a matter of fact, ERs are expressed in hepatic cells and tissues and their epigenetic and genetic dysregulation of them are related to many liver disorders and contribute to HCC. In line with the results of previous studies, the **Baldissera *et al.*** [8] study demonstrated that estrogen receptors polymorphisms are related to HCC.

In considering the fact that ER is associated with HCC, previous studies have evaluated the influence of anti-estrogen in the treatment of HCC [30, 31]. Surprisingly they detected that antiestrogen had no benefit on clinicopathological features of HCC.

These differences could be that the etiology of HCC is complex not only related to estrogen. Regarding ESR2 (rs1256049) polymorphism, the findings of the current result were that the GA and AA genotypes as well as the A allele in patients with NAFLD were higher than in the control group. Regarding rs4986938 SNP, the GG, GA, and AA numbers and frequencies were not meaningful.

Among NAFLD groups, regarding ESR2 (rs1256049) polymorphism. There was a significantly high frequency of the mutant A allele in non-cirrhotic NAFLD compared to cirrhotic NAFLD. While the GG, GA, and AA genotypes frequencies were not significantly different between both groups.

In short, we revealed for the first time that ESR2 (rs1256049) polymorphism GA and AA genotypes and mutant A allele were significantly higher in NAFLD, in particular the HCC subgroup. From this consideration, we hypothesized that ESR2 (rs1256049) polymorphism could be a predictor of HCC among NAFLD patients.

To attain the independence assumption, we performed an additional test. The present study aimed to explore the potential clinical significance of metabolic dysfunction parameters and liver function tests concerning HCC. The present study provides evidence that BMI, HOMA-IR, E2, and FIB-4 scores were independently associated with HCC in both cirrhotic and non-cirrhotic NAFLD groups by applying a logistic regression test.

Similar results were observed by **Schlesinger *et al.* [32],** who found that the high value of BMI is an independent risk factor of HCC. Moreover, another study conducted by **Ohki *et al.* [33]** demonstrated that obesity is a risk factor for HCC in patients with NASH. Regarding diabetes mellitus (DM), **Hossain *et al.* [34]** detected that DM is a risk factor for fibrosis progression among NAFLD.

In conclusion, there were non-significant differences concerning ERβ2 mRNA expression levels among the studied groups. Additionally, the current study revealed that the AA, GA genotype as well as A allele of ESR2 (rs1256049) were significantly increased in patients with NAFLD in particular HCC than the control group. A allele of ESR2 (rs1256049) was significantly higher in cirrhotic NAFLD compared to non-cirrhotic. Thus, it could be a predictor of HCC among NAFLD patients.

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REFERENCES

1. **Charlton M, Burns J, Pedersen R *et al.* (2011):** Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology, 141: 1249-1253.
2. **Younossi Z, Koenig A, Abdelatif D *et al.* (2015):** Global epidemiology of non-alcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence and outcomes. Hepatology, 64: 73-84.
3. **Kanwal F, Kramer J, Mapakshi S *et al.* (2018):** Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. Gastroenterology, 155: 1828–1837.
4. **Forner A, Llovet J, Bruix J (2012):** Hepatocellular carcinoma. Lancet, 379: 1245-1255.
5. **Estes C, Razavi H, Loomba R *et al.* (2018):** Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology, 67: 123–133.
6. **Marrero J, Kulik L, Sirlin C *et al.* (2018):** Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology, 68: 723–750.
7. **Shen M, Shi H (2015):** Sex hormones and their receptors regulate liver energy homeostasis. Int J Endocrinol., 15:1–12. doi: 10.1155/2015/294278
8. **Baldissera V, Alves A, Almeida S *et al.* (2016):** Hepatocellular carcinoma and estrogen receptors: polymorphisms and isoforms relations and implications. Med Hypotheses, 86: 67-70.
9. **De Maria N, Manno M, Villa E (2002):** Sex hormones and liver cancer. Mol Cell Endocrinol., 193: 59-63.
10. **Kalra M, Mayes J, Assefa S *et al.* (2008):** Role of sex steroid receptors in pathobiology of hepatocellular carcinoma. World J Gastroenterol., 14:5945–61.
11. **Zhou Z, Qiao J, Shetty A *et al.* (2014):** Regulation of estrogen receptor signaling in breast carcinogenesis and breast cancer therapy. Cell Mol Life Sci., 71: 1549. doi: 10.1007/s00018-013-1376-3
12. **Castiglione F, Taddei A, Rossi Degl'Innocenti D *et al.* (2008):** Expression of estrogen receptor beta in colon cancer progression. Diagn Mol Pathol., 17: 231-236.
13. **Byrne C, Targher G (2014):** NAFLD: A multisystem disease. J Hepatol., 62:47–64.
14. **Liovet J, Br U, Bruix J (1999):** Prognosis of hepatocellular carcinoma: The BCLC staging classification. Semin Liver Dis., 19: 329-38.
15. **Mahdavipour M, Zarei S, Fatemi R *et al.* (2017):** Polymorphisms in the estrogen receptor beta gene and the risk of unexplained recurrent spontaneous abortion. Avicenna Journal of Medical Biotechnology, 9:150–154.
16. **Gordon D, Haynes C, Blumenfeld J *et al.* (2005):** PAWE-3D: visualizing power for association with error in case/control genetic studies of complex traits. Bioinformatics, 21:3935–37.
17. **Bray F, Ferlay J, Soerjomataram I *et al.* (2018):** Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin., 68(6):394–424.
18. **Wang A, Lee K, Kim S *et al.* (2006):** The expression of estrogen receptors in hepatocellular carcinoma in Korean patients. Yonsei Med J., 47 (2006), pp. 811-816
19. **Nagasue N, Ito A, Yukaya H *et al.* (1986):** Estrogen receptors in hepatocellular carcinoma. Cancer, 57(1): 87-91.
20. **Phipps M, Livanos A, Guo A *et al.* (2020):** Gender matters: characteristics of hepatocellular carcinoma in women from a large, multicenter study in the United States. Am J Gastroenterol., 115(9):1486–1495.
21. **Piscaglia F, Svegliati-Baroni G, Barchetti A *et al.* (2016):** Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. Hepatology, 63(3):827–838.
22. **Ertle J, Dechêne A, Sowa J *et al.* (2011):** Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer, 128(10):2436–2443.

23. **Leung C, Yeoh S, Patrick D *et al. et al.* (2015):** Characteristics of hepatocellular carcinoma in cirrhotic and non-cirrhotic non-alcoholic fatty liver disease. World J Gastroenterol., 21(4):1189–1196.
24. **Mohamad B, Shah V, Onyshchenko M *et al.* (2016):** Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. Hepatol Int., 10(4):632–639.
25. **Brady C (2015):** Liver disease in menopause. World J Gastroenterol., 21:7613–7620.
26. **Iavarone M, Lampertico P, Seletti C *et al.* (2003):** The clinical and pathogenetic significance of estrogen receptor beta expression in chronic liver diseases and liver carcinoma. Cancer, 98:529–534.
27. **Palmisano B, Zhu L, Stafford J (2017):** Role of estrogens in the regulation of liver lipid metabolism. Adv Exp Med Biol., 1043: 227–256.
28. **Iyer J, Kalra M, Kaul A *et al.* (2017):** Estrogen receptor expression in chronic hepatitis C and hepatocellular carcinoma pathogenesis. World J Gastroenterol., 23(37): 6802-6816.
29. **Ohnishi S, Murakami T, Moriyama T *et al.* (1986):** Androgen and estrogen receptors in hepatocellular carcinoma and in the surrounding noncancerous liver tissue. Hepatology, 6: 440–443.
30. **Engstrom P, Levin B, Moertel C *et al.* (1990):** A phase II trial of tamoxifen in hepatocellular carcinoma. Cancer, 65:2641–2643.
31. **Di Maio M, Daniele B, Pignata S *et al.* (2008):** Is human hepatocellular carcinoma a hormone-responsive tumor? World J Gastroenterol., 14:1682–1689.
32. **Schlesinger S, Aleksandrova K, Pischon T *et al.* (2013):** Abdominal obesity, weight gain during adulthood and risk of liver and biliary tract cancer in a European cohort. Int J Cancer, 132(3):645–657.
33. **Ohki T, Tateishi R, Shiina S *et al.* (2009):** Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. Gut, 58(6):839–844.
34. **Hossain N, Afendy A, Stepanova M *et al.* (2009):** Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol., 7(11):1224–1229.