Interplay of Sphingosine1 phosphate, Adiponectin and Sex Hormones with Gender disparity among patients with Hepatocellular Carcinoma

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Research

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Abstract

Background

circulating sphingosine 1-phosphate (S1P) showed oncogenic roles in various cancers. It showed conflict data in Hepatitis B virus- hepatocellular carcinoma (HCC). Adiponectin and sex hormones were contributing factors for gender disparity in HCC. Up to date, no clinical study among HCC patients addresses interplay of S1P, adiponectin and sex hormones that was described in experimental studies. The current study aimed to evaluate circulating SIP, adiponectin and sex hormones among sex stratified HCC subgroups and to assess gender disparity of theses parameters regarding HCC development and diagnosis, their interplay and association with clinic -morphological and staging of HCC.

Method

We measures serum S1P, adiponectin, testosterone (T), estradiol (E) and sex hormone binding globulin (SHBG), calculated free and bioavailable T and E/T ratio among HCV – HCC group in comparing with comparable HCV- cirrhotic and healthy groups with their sex stratified male and female subgroups

Results

Among all, male and female HCC patients, S1P was significant higher than corresponding cirrhotic and healthy subjects with cut off value ≥ 113ng/l as screening test (sensitivity 95%, specificity 56%) for HCC diagnosis. Compared to sex respective cirrhotic subgroups, male-HCC had significant higher SHBG and lower adiponectin and estradiol while opposite profile was observed among female-HCC. Female-HCC had significant higher SIP and adiponectin than male-HCC. Although S1P was positively correlated with adiponectin, testosterone and negatively with E/T ratio among male and female HCC, adiponectin was correlated negatively with testosterone and SHBG and positively with estradiol and E/T ratio among entire HCC group but reverse associations were observed in female (not- male) subgroup. Associations of large tumor size and higher T-class TNM staging with higher adiponectin and testosterone and lower E/T ratio and link of multiplicity with higher estradiol in females while association of higher testosterone among higher T-class TNM staging male were observed. Portal vein thrombosis was related to lower SHBG and higher testosterone in male and female respectively.

Conclusion

We suggested S1P as screening test for diagnosis of HCC among HCV-cirrhotic and healthy subjects. There was gender disparity as regard association and interaction of S1P, adiponectin and sex hormone with respect of HCC development and its clinic-morphological features and staging.

Introduction

Hepatocellular carcinoma (HCC) is more frequently observed and aggressive in men than in women (1). This gender disparity is multifactorial: hepatitis C viral infection (HCV), sex hormones and adiponectin play a contributing role (2, 3). Adiponectin role in HCC is still unclear with diverse actions, both protective and oncogenic actions were previously reported (4, 5). Testosterone may decrease adiponectin level based on cell lines, animal models and epidemiological studies while estrogen may stimulate its release (3, 6). Although many studies supported protective role of estradiol, others referred to its oncogenic role in HCC. Enhanced aromatization with conversion of androgen to estrogen was described in HCC tissue and can be assessed by calculated estradiol to testosterone (E/T) ratio (7, 8). Total testosterone (T) circulates in three forms mainly ~ 60% bound to sex hormone-binding globulin (SHBG) which is synthetized by liver. Both fraction bound to albumin (40–50%) and free testosterone (1%) represent active testosterone forms are estimated by bioavailable testosterone (BAT) and free androgen index (FAI) respectively(9). Among HCV-cirrhotic male, FAI but not T was related to fibrosis and steatosis(10).In spite of extensive molecular studies, little conflict clinical studies described role of testosterone (usually total and seldom free forms), estradiol, E/T and SHBG in male HCC of various etiologies and rarely stratified by sex or HCV etiology(11–16)

In addition, adiponectin receptors have intrinsic ceramidase activity that is increased 20 fold by adiponectin binding particularly adiponctin R2 with increased sphingosine 1-phosphate (S1P) production (17). S1P is produced by de-acylation of ceramide to sphingosine by ceramidase, followed by phosphorylation by sphingosine kinase (SphK). S1P as a pleotropic molecule enhances cell proliferation and mobility, immune cell recruitment, angiogenesis, and metastasis and it is diagnostic and prognostic biomarker in various cancers (18).

Animal and molecular studies suggested role of SphK and S1P signaling in HCC including cell proliferation, epithelial mesenchymal transition, progression, angiogenesis, invasion and metastasis (19). Sparse inconsistent data exist about S1P tissue over-expression in HCC compared to normal adjacent tissues (20, 21) or alterations of its serum level that was measured by high performance tandem mass liquid chromatography spectrometry in heterogeneous HCC patients mainly hepatitis B virus (HBV) etiology focusing in Chinese population as compared to healthy subjects or non - comparable cirrhotic patients(22–24). However, sphingosine is a biomarker of chronicity and fibrosis in HCV but not HBV (25, 26). Effect of sex and menopause on S1P level and its regulation by sex hormones in experimental studies are uncertain (27, 28).

Purpose of the current study was to evaluate the serum S1P, adiponectin, SHBG and sex hormones levels that included total testosterone, calculated BAT and FAI, estradiol and E/T ratio among male and female subgroups with HCV related HCC patients in comparison to sex respective matched

corresponding cirrhotic and healthy subjects, verify possible interplay of studied biomarkers and their association with clinical and morphological data stratified by sex in HCC; an aspect not studied before

Subjects And Methods

In the current cross sectional case-control study, 80 HCV- cirrhotic patients HCC (males/female [m/f](40 /40) who formed HCC group, 60 HCV- cirrhotic patient (m/f : 30/30) who formed cirrhotic group and 50 healthy control (m/f: 25/25) were included in our analysis. The three groups were age and sex matched and each group was further subdivided into male and female subgroups. The study was done in harmony with the Declaration of Helsinki and was permitted by the local ethics committee along the period from February 2019 to November 2020 at the Department of Internal Medicine, Minia University Hospital, Egypt. All participates had signed a written informed consent before inclusion in the study.

Diagnosis of chronic HCV infection was based on presence of anti-HCV antibodies for ≥ 6 months and detection HCV RNA. Diagnosis of cirrhosis was based on abdominal ultrasound and laboratory data. Severity of liver dysfunction was assessed by Child Pough and model end stage liver disease (MELD) scores (29). The diagnosis of HCC was based on European Association for the Study of the Liver (EASL) guideline 2012 by dynamic imaging CT or MRI (30). Staging of HCC was based on The Barcelona Clinic Liver Cancer (BCLC) and Tumor Node Metastasis (TNM) system according to The American Joint Committee on Cancer (AJCC) (31, 32)

All participates had conventional assessments including thorough history taking, physical examination, and abdominal ultrasonography. They underwent routine laboratory tests. All women were menopausal.

Exclusion criteria were a history of malignancy elsewhere, diabetes mellitus or endocrine disorders, any organ failure, recent infection, fertile women, alcohol intake or any local or systemic treatment for HCC, other causes of cirrhosis or hormonal treatment.

Biochemical assays:

Fasting venous blood samples were collected at 9 AM. EDTA containing tube was used for complete blood count. Prepared serum was used for assessment liver function test (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin), viral infection status, renal function and fasting blood sugar. Citrated blood sample used to separate plasma for prothrombin time and calculation of international normalized ratio. CHILD and MELD scores were calculated. Serum aliquots were stored at 80□C for measurements of S1P, adiponectin and testosterone, estradiol and SHBG that were quantitatively determined by ELISA kites supplied by bioassay Technology laboratory Biotech Co, Shanghai, Korain) according to the manufacturer's instructions. BAT, FAI and E/T were calculated (8, 9).

STATISTICAL ANALYSIS:

SPSS version 20 was used for data analysis. Normally distributed quantitative data were expressed as mean ± SD and compared by unpaired t-test. Non -normally distributed quantitative data was expressed as median and interquartile (25% and 75%IQ) and compared using Mann-Whitney test. Qualitative variables were presented as percentage and compared by Chi-square test. Correlation analysis was performed using Spearman rank test. Receiver operating characteristic (ROC) curve was used to determine the diagnostic performance of SIP for HCC. Specificity, sensitivity, and cutoff values were detected via areas under the ROC curve (AUC). $P < 0.05$ was considered to be statistically significant.

Table (1): Clinical and laboratory Data of the Studied Groups.

Variables	HCC Group (n = 80)	Cirrhotic Group (n = 60)	Healthy Group (n = 50)
Age (years) [a]	61.4 ± 10	60 ± 7.6	60.7 ± 6.6
Male gender (%)	40(50%)	30(50%)	25(50%)
Hemoglobin% (gm/dl) [a]	11.5± 2.3***	11.9 ± 2.7**	13.2 ± 1.2
White blood cells count(x103) [b]	6.1(4.5–8.3) ‡‡	5.5(4.4–6.3) **	6.3(5.7–7.7)
Platelet count (x103) [b]	153(131–225) **	163(143–212) **	219(179–271)
Alanine aminotransferase (U/l) [b]	53(33–65) ‡‡‡***	34(21–49)	30(23–33)
Aspartate aminotransferase(U/l) [b]	49 (33–78)‡‡‡***	36(26-53.2) ***	25(23–33)
Albumin (gm/dl) [a]	3.3 ± 0.67***	3.3 ± 0.8***	4.05 ± 0.43
Bilirubin (mg/dl) [b]	1.3(0.9–1.9) ***	1.2(0.8–1.4) ***	0.56(0.4–0.8)
International normalized ratio [a]	1.3 ± 0.25***	1.4 ± 0.32***	1.1 ± 0.2
Random blood sugar (mg/dl) [a]	130 ± 17	128 ± 18	124 ± 15
Creatinine (mg/dl) [a]	0.98 ± 0.3	0.95 ± 0.3	.93 ± 0.3
Child calss A/B/C n (%)	24/28/28 30/35/35	22/18 /20 36.7/ 30/ 33.3	-----
Child score [a]	8.6 ± 2.6	8 ± 2.5	-----
MELD score [a]	12.2 ± 3.9	12 ± 3.4	-----
Sphingosine-1 phosphate (ng/l) a	146 ± 31‡‡***	129 ± 22.4***	34.1.8 ± 13.9
Adiponectin (mg/l) [b]	3.4(2.6–6.5) ‡ ***	3(2.4–4.1) ***	12.1(8.5–15.8)
Total testosterone (ng/ml) [b]	1.5(0.51–3.8)	1.2(0.66–3.4)	3.9(0.35–0.89)
Sex hormone binding globulin(mmol/l) [b]	15.2(10.2–29.6)	14.3(10.6–19.8) *	19.7(14.3–24.1)
Bioavailable testosterone (ng/ml) [b]	0.63(0.29–1.8)	0.73(0.36–1.7)	2.5(0.2–5.8)
Free androgen index (ng/ml) [b]	0.04(0.02–0.09)	0.04(0.02–0.09)	0.12(0.01–0.26)
Estradiol (pg/ml) [b]	29.4(16.8–22.7) ***	27.8(20.5–35) ***	57(49.5–66.7)
Estradiol to testosterone ratio x10-3 [b]	17.3(5–10)	25.9(6.1–45.6)	31.9(5.6–47)

HCC = Hepatocellular carcinoma; MELD: Model of End stage Liver Disease;

a = normally distributed quantitative data are expressed as mean ± standard deviation and compared using independent sample t- test between each two groups

Gender and child class are expressed as number (percentage) and compared by Chi square test

b = not- normally distributed quantitative data are expressed as median and interquartile (25%-75%) and compared using Mann Whitney U test between each two groups. $P < 0.05$ is considered to be statistically significant.

Significant difference when HCC patients compared to cirrhotic patients $p < 0.05$= ‡, $p < 0.01$=‡‡, $p < 0.001$=‡‡‡

Significant difference when compared to healthy subjects $p < 0.05$= *, $p < 0.01$=**, $p < 0.001$=***

Results

Clinical and laboratory Characteristics of studied groups and subgroups:

Although HCC group was matched to cirrhotic group as regard severity of liver disease (child or MELD score), the former had significant higher liver enzymes, leucocyte count, S1P and adiponectin. The two groups had significant higher S1P and significant lower adiponectin and estradiol than healthy subjects. The detailed laboratory characteristics were shown in Table 1.

In the next step, we apportioned studied HCC, cirrhotic and healthy subject groups in corresponding male and female subgroups whom laboratory data were shown in table (2). Male and female subgroups were similar in age, liver markers and severity either within HCC group or within cirrhotic patient except haemglobin % was significant lower in female patients subgroups while ALT was significant higher among female HCC and significant lower in cirrhotic female subgroup compared to disease respective male subgroups. Among male subgroups, HCC patients had significant higher S1P, SHBG and significant lower adiponectin, estradiol and E/T ratio (p = 0.03, < 0.001, 0.03, 0.002, 0.02 respectively) and comparable testosterone forms (total, BAT and FAI) compared to cirrhotic patients. Meanwhile, female- HCC subgroup had significant higher S1P, adiponectin, estradiol and E/T ratio and significant lower total testosterone and SHBG (p = 0.007, < 0.001, < 0.001, < 0.001, 0.04, 0.001 respectively) and comparable level of BAT and FAI compared to cirrhotic female subgroups.

In comparison to sex respective matched healthy subjects, both HCC and cirrhotic subgroups had significant higher SIP and significant lower adiponectin (p < 0.001 for all). In addition, HCC and cirrhotic male subgroup had significant lower testosterone forms (total, BAT, FAI) and estradiol (p < 0.001 for all) than healthy male subjects. Among female subgroups, significant higher FAI and lower estradiol among both HCC (p = 0.01, 0.03) and cirrhotic patients (p = 0.001, < 0.001) meanwhile lower SHBG among HCC and lower E/T ratio among cirrhotic (p = 0.01, < 0.001) respectively compared to healthy subjects.

In comparison male versus female of corresponding groups (disease respective group): serum SIP and adiponectin were lower among male HCC subgroups (0.001, < 0.001); testosterone, FAI, BAT, SHBG were higher while estradiol and E/T ratio were lower among both cirrhotic and HCC male subgroups (p < 0.001 for all).

Morphological features and staging of HCC

We demonstrated tumors size > 5cm in diameter among 62.5% (50/80, m/f: 24/26), multiple tumor lesion in 53% (42/80, m/f: 20/22), portal vein thrombosis (PVT) among 27.5% (22/80, m/f: 12/10) among HCC patients. Neither lymph node metastasis nor distant metastasis was detected. Number of cases and m/f according to T-class (T1,T2 and T3) and TNM staging(I/II / IIIa) were similar 25: 13/12, 32: 16 /16, 23: 11/12 respectively; according to BCLC stages A, B, C, D, were 16 :8/8; 20:8/12, 16: 10/6, 28: 14/14 cases respectively. Male and female subgroups had comparable morphological features and staging.

Interplay of sphingosine 1 phosphate, adiponectin and sex hormones among HCC group

Firstly, we studied interplay of S1P, adiponectin and studied sex hormones among entire HCC group and sex stratified subgroups. S1P was correlated positively with adiponectin and estradiol in HCC group (r = 0.56, p < 0.001; r = 0.25, p = 0.02(Fig. 1,2). Among male and female HCC subgroups (table3), SIP was correlated positively with adiponectin (

p = 0.02, 0.002), total testosterone (p = 0.04, p < 0.001, FAI(p = 0.01, 0.01) BAT(p = 0.03, p = 0.001) and negatively with E/T ratio (p = 0.02, p = 0.001) respectively. SIP was positively correlated to SHBG only among female HCC(r = 0.56, p < 0.001). Serum adiponectin was correlated negatively with testosterone, SHBG, BAT and FAI and positively with estradiol and E/T ratio among HCC group (r= -0.46, r= -0.59, r= -0.43, r= -0.44, r = 0.59, r = 0.50 respectively, p < 0.001 for all). In contrary, adiponectin was positively correlated with testosterone, BAT and FAI and negatively correlated with E/T ratio in female- HCC patients with p < 0.001 for all. S1P correlated positively with age among male-HCC (r = 0.42, p = 0.007) and positively with HB level in male and female subgroups (r = 0.46, p = 0.003; r = 0.35, p = 0.02) but didn't show any correlation with liver enzymes nor severity of liver disease among HCC subgroups .

Table (3):Spearman's correlation coefficients of SIP and adiponectin in male and female HCC subgroups

Correlation of SIP and adiponectin with sex hormone							
variables	Adiponectin (mg/l)	totalT (ng/ml)	SHBG (nmol/l)	BAT (ng/ml)	FAI (ng/ml)	Estr (pg/ml)	E/T $x10^{-3}$
S1P	**0.36* (0.47**)**	**0.31*(0.66***)**	**0.05(0.56***)**	**0.34*(49**)**	**0.39*(0.4*)**	-0.24(-0.04)	**-0.36*(-0.49**)**
Adiponectin	———	0.22 **(0.67***)**	-0.03(0.17)	0.22**(0.66***)**	0.15**(0.64***)**	0.11(0.14)	-0.06 **(-0.56***)**
Correlation of SIP with liver markers							
Liver markers	AST(U/l)	ALT(U/l)	Albumin (gm/dl)	Bilirubin (mg/dl)	INR	Child score	Meld score
S1P(ng/l)	-0.24 (0.06)	-0.21 (0.16)	0.17 (0.11)	-0.07 (-0.08)	-0.05 (-0.24)	-0.06(-0.33)	0.11(-0.13)

Correlation coefficients are written first y for male HCC followed by correlation coefficients in female HCC are shown within parentheses).HCC = Hepatocellular carcinoma ;S1P = Sphingosine1phosphate; T = testosterone; SHBG = Sex hormone binding globulin; BAT = Bioavailable testosterone ; FAI = Free androgen index; Estr = Estradiol ; E/T = Estradiol to testosterone ratio; ; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; INR = International normalized ratio; MELD: Model of End stage Liver Disease. Significant correlation coefficients are given in bold. ***=p < 0.001, **=p < 0.01,*=p < 0.05.

Secondly, association of S1P, adiponectin and sex hormones with tumor morphological features and TNM Staging was done table (4). Among male HCC, presence of PVT was associated with significant lower SHBG and those with TNM stage II had higher total testosterone and BAT compared with stage I respectively). Female HCC patient with large tumor size and advanced TNM staging in comparing stage IIIa to stage II had significant higher adiponectin and testosterone forms(total, BAT, FAI), and significant lower E/T ratio meanwhile presence of multiple lesions and PVT were associated with higher estradiol and BAT respectively. TNM stage II was associated with significant higher estradiol while stage IIIa was associated with higher S1P, total testosterone and estradiol and significant lower E/T ratio in comparison to stage I among female HCC patients.

The diagnostic performance of S1P for diagnosis of HCC among all HCC patients, male HCC and female HCC was assessed by ROC analysis, the AUCs were was 0.79, 0.78, 0.81, the 95% confidence interval CI were (0.70–0.88), (0.65–0.92) and (0.69–0.93) and P < 0.001 for all respectively(Fig. 3) when the cut-off value was set ≥ 113 ng/l as a screening test, the sensitivity was 95% for HCC group and subgroups while specificity was 56%, 52% and60% among all HCC patients, female HCC and male HCC respectively. When the cut-off value was set ≥ 125ng/l as a diagnostic test, the sensitivity and specificity were 72% and 70% among all HCC patients and 60% and 72% among male HCC 85% and 64% among female HCC.

Discussion

Our study was the first to demonstrate significant elevated SIP when HCC and cirrhotic patients related HCV etiology were compared to healthy subjects and when HCC patients were compared to cirrhotic either among entire group or female and male subgroups. Moreover, we recommended serum SIP ≥ 113ng/l as a screening test with high sensitivity (95% for male and female) but unfortunately lower specificity 56% for diagnosis of HCC being more specific in male than female (60% vs. 52%). On the other hand, with S1P ≥ 125ng/l as a diagnostic test, the sensitivity and specificity were 72% and 70% among all HCC patients respectively. Notably, SIP was more sensitive in female (85% vs 69%) but more specific in male (72% vs 64%). S1P and adiponectin showed gender disparity in respect to HCC patients being significant higher among women than men. Compared to sex respective cirrhotic patients, higher adiponectin, estradiol and E/T and lower SHBG among female HCC while inverse profile among male HCC were observed. Our study was the first to describe cross talk S1P, adiponectin and sex hormone in HCC that was described by previous molecular studies. Although S1P had significant positive correlation with adiponectin, testosterone forms and negatively with E/T ratio among both male and female HCC subgroups, adiponectin relations showed marked gender disparity: negative association with testosterone forms and SHBG and positive association with estradiol and E/T ratio among entire HCC group; no association among male-HCC; and reverse relations among female- HCC versus to those of entire HCC group. Also, only among female HCC, larger tumor size was associated with higher adiponectin, testosterone forms and significant lower aromatization (lower E/T ratio); multiple lesions with higher estradiol and PVT with higher BAT. Advanced TNM staging was associated with higher testosterone in both sex while with SIP, adiponectin, estradiol and E/T ratio in female only.

Higher S1P among cirrhotic patient than healthy subjects was consistent with its enhanced tissue expression in hepatic fibrosis as it induces Kupffer cell infiltration which increases collagen and α-smooth muscle actin expressions (33, 34). As regard sparse previous researchers address its relation to HCC, significant higher level in HCC than cirrhotic in previous three studies was restricted either to very small number (n = 10 for each group) with lacking clinical, morphological and laboratory data in the first study (35) or non- comparable cirrhotic group as regard etiology, age, severity of liver disease and haemogram with latter two variables were correlated to S1P level in this second retrospective study that might be argued us to accept their results (36) and recent Chinese study that promoted its elevation and diagnostic role for HCC patients mainly of HBV etiology with sensitivity and specificity of 79.2% and 78.7% respectively (24). Two Chinese studies reported conflict data about its up-regulation among HCC patient mainly of HBV compared to healthy subjects (22, 23).

Our study overcame these obstacles and provided different population design, etiology, region, ethnicity and methodology. We studied the effect of gender by constrict equal number of old male and postmenopausal female participates in sex stratified HCV related HCC subgroups that were comparable regard age, severity of liver disease and clinic-pathological data and staging of HCC among both cirrhotic and HCC patients as average age of HCC diagnosis is approximately 65 years (1) and association of sphingosine with chronic hepatitis differs whether HCV or HBV infection (25, 26) .We used standard antibody- based ELISA method to measure S1P as it is more sensitive than available conventional spectrometry in our locality but it was higher cost and may showed cross-reactivity. Mentioned studied used high performance liquid chromatography tandem mass spectrometry.

Consistency with our result of higher S1P with higher T class or TNM staging only among female HCC, animal and molecular studies suggested enhancing effect of SIP on initiation and progression of HCC (19). This gender diversity of SIP was similar to its association with cardiac autonomic neuropathy only among diabetic women not men (37). In our study, S1P correlated positively with age among male HCC and positively with HB level in male and female subgroups but not correlated to liver disease markers and severity scores, tumor size and multiplicity or presence of PVT. This was to some extent agreeing with (24, 36).Elevated S1P level among female HCC compared to either male-HCC or female- cirrhotic may be attributed to higher adiponectin and estradiol levels in female HCC and its positive association with adiponectin and estradiol among entire HCC. This association was in line with molecular studies; adiponectin receptors have intrinsic ceramidase activity that is markedly increased by adiponectin binding with increased

SIP production (17). In parallel, either overexpressing adiponectin receptors or adiponectin agonist administration in hepatocyte increase S1P levels (38). This may be an oncogenic mechanism of adiponectin. In vitro and molecular studies demonstrated that estradiol markedly improves S1P synthesis and export by activating SphK1 in normal and breast cancer cells .Compared to men, higher level was reported in childbearing women in one study while irrelevant to sex but higher in menopausal women in another study (27,28,39). The positive association of SIP with various testosterone forms in male and female HCC was in one hand with previous experimental finding of T deprivation down-regulated SphK1 expression but up-regulated SphK2 Hence, SphK1 is more contributor to S1P synthesis than SphK2 (40). SIP was correlated to estradiol in entire HCC only but was correlated to testosterone and E/ T in only stratified male and female HCC. This may be attributed to different wide and narrow ranges of studied hormonal levels)

Adiponectin showed complex dual tumor promoter or suppressor effect in hepatocarcinogenesis (5). Tissue or serum adiponectin has been reported to be either elevated or decreased or even not associated with HCV related HCC development in global and Egyptian studies as compared to cirrhotic patients (4, 41, 42). In our study, we demonstrated significant decrease adiponectin level in HCC and cirrhotic patients compared to healthy subjects in entire groups and sex stratified subgroups. Adiponectin showed gender disparity being elevated in female HCC compared to cirrhotic females but Sadik et al., 2012 reported similar finding in both sex. Secondly, we reported higher level in female than male HCC similar to Shen et al 2016(5, 41). This can be explained by higher estradiol and E/T ratio and lower testosterone and SHBG levels compared to either HCC men or cirrhotic women. In this respect, we were pioneering in reporting significant correlations of adiponectin positively with estradiol and E/T ratio and negatively with testosterone and SHBG among HCC patients. Our findings were supported by previous data. Manieri et al., 2019 reported physiological higher adiponectin in women than men and observed faster growth of HCC allografts in male mice than in female, but not in castrated or adiponectin knockout mice. Adiponectin activates AMPK and p38activation in HCC cells through an adiponectinR2–dependent pathway which is predominantly expressed in the liver as protective mechanism of adiponectin against HCC. They suggested that testosterone enhance tumor growth through reduction of adiponectin production via activation JNK in human and mouse in adipocytes preventing AMPK and p38 activation in HCC cells through an adiponectinR2–dependent pathway(6). However, adiponectin had opposite relation to sex hormones within female HCC and no relation was detected in male HCC that may attributed to narrow and different range or menopausal state .In line with us, a higher androgen to estrogen ratio is associated with lower adiponectin in both gender while estrogen administration in postmenopausal women decrease adiponectin level (3). In contrary to our findings, adiponectin increases hepatic SHBG production in men while endogenous estrogen reduction causes a drop in adiponectin in postmenstrual women (3, 43).The conflict may be attributed different behavior in HCC pathology and normal physiology.

In our study, higher adiponectin was correlated to large tumor size and advanced staging only in female HCC. This matched with previous reported in HCC tissues (44). In our study, HCV related cirrhosis in both sex was accompanied by significant lower estradiol and insignificant lower SHBG than corresponding healthy subjects but these parameters showed gender disparity with HCC development: male HCC patients had significant lower estradiol and E/T ratio and significant higher SHBG while female HCC patients showed the opposite changes compared to sex cirrhotic respective subgroups, an aspect not studied before.

We reported lack of association of various testosterone forms with HCC development in cirrhotic subgroups (except total testosterone in HCC female). Most previous studies were longitudinal and involved males and showed conflict results may be attributed to ethnicity. testosterone was reported to be not predictor, positive or negative predictor to HCC occurrence among healthy European, HBV cirrhotic Asian and cirrhotic Egyptian respectively (12, 13, 15, 16). Among male and female in our study, testosterone forms enhance tumor growth being associated with tumor size and staging while BAT was associated with PVT in female only. This was similar to previously described in ovariectomized mice that showed accelerated hepatocarcinogenesis upon testosterone administration owing to up-expressed cell cycle regulators and down-expressed apoptotic factors (45). It is worth emphasizing that androgenic receptors overexpressed in HCC and showed “vicious circle” of androgenic signaling and tumor growth and decline of HCC malignancy after androgenic receptors antagonism treatment (7).

In our study, valid characterization of the connection of testosterone with HCC needs a concurrent measurement SHBG being synthetized in liver and to calculate FAI and BAT. SHBG synthesis may increase or decrease with liver damage depending on sex and etiology e.g. increased among HCV cirrhotic male but decreased among decompensated non-alcoholic cirrhotic men and HCV-related cirrhotic post-menopausal women (10, 46, 47). We recognized gender disparity of SHBG with respect HCC and its relation with PVT in male that developed mainly subsequent to tumor invasion. Recent studies reported role of SHBG in enhancing prostate and ovarian cancer but protective in breast and endometrial cancers and may even expressed by tumor cell (48). Little data mainly in men was available regard role of SHBG and HCC. Elevated SHBG may be predict or not HCC development in longitudinal cirrhotic males either post alcoholic or post HCV respectively (12, 13). Similar, it was predictor among healthy subjects even with sex stratified groups and associated with IGF-1 and liver damage markers in one study but not in cross section Greece healthy male (14, 15). Elevated SHBG may have direct or indirect effect. The indirect effect via decrease BAT which either subsequently inhibits tumor growth and invasion to portal vein as in our male HCC or enhancing effect via enhanced fibrosis, steatosis and insulin resistance in HCV cirrhotic males. On the other hand, reduced SHBG in female lead to increased free testosterone that enhance IR and tumor growth in females (3, 10).

Protective effects of estrogen against HCC through IL-6 restrictions and STAT3 inactivation via binding to wild type estrogen receptor (ER) is well recognized (7). In line with us, previous study of Farinati et al., 1995 who reported lower estradiol among viral related HCC than cirrhotic in male predominant study but others reported the reverse among HBV or alcoholic men (11, 14). We partly agreed with Egyptian study that reported reducing estradiol in cirrhotic and HCC patients of both sex (16). Moreover, higher estradiol was associated with mutilpe focal lesion and advanced TNM staging among HCC female. No mechanistic conclusive evidence links local estrogen production to HCC growth but the enhanced expression and activity of aromatase was observed in Heptocyte G2 cells and human HCC tissue. This aromatization associated with degree of malignancy of the liver tissue/cell

line and enhanced HCC tissue expression of oncogenic ER called variant ER that could induce tumor growth t in contrast to wild type ER (7). This may contradicts the suppressive role of estrogen and oncogenic task of androgen, and further studies are required to verify this observation. Gender disparity of estradiol and E/T ratio in HCC may be explained by sex differences in ER α expression in normal livers and altered subtype expression in HCV-related cirrhotic male progress to HCC (49). However, the role of increased estrogen in female HCC as compensatory mechanism to protect and decrease morbidity and mortality in women cannot be excluded.

Limitation of our study: Cross section not enough to describe causal relationship, relatively small number. We studied elderly HCC not young age groups. Further studies may address this issue. We used ELISA method for quantitative measurement of SIP not using high-performance liquid chromatography-tandem mass spectrometry mass spectroscope that allows quantification of many serum sphingolipid members. Despite these limitations, this study has much important strengths. First and foremost, this was the first study to examine gender disparity of SIP in respect to HCV related HCC, to support ceramidase activity of adiponectin in both sex and describe gender disparity of cross talk of adiponectin(but not S1P) with sex hormones. Secondly, male and female HCC were comparable as regard age, severity of liver disease, clinic-pathological and tumor staging. Also cirrhotic and HCC (entire or sex stratified groups) were matched for clinical and laboratory data as well as severity of liver disease. lastly choose of elderly HCC patients as the commonest age for its incidence .

Perspectives and significance

In conclusion, our study described gender disparity of SIP, adiponectin, sex hormones (estradiol, total and free testosterone forms, SHBG, E/T ratio) in respect to HCC development among HCV- cirrhotic patients, and its clinic-pathological feature and staging. The cross talk of adiponectin with sex hormones parameters show gender dimorphism but this of S1P was similar in both sex. SIP may be used as novel screening and diagnostic biomarkers test for diagnosis of HCC among cirrhotic and healthy subjects with cut-off diagnostic value that was more sensitive in female but more specific in male .. The present study suggested enhanced ceramidase activity of adiponectin in HCC as a mechanism that may explain oncogenic role of adiponectin Further Mechanistic delineation of S1P, adiponectin, sex hormones using systems approach will be needed to shed light on HCC and their gender disparity. Further studies among young men and fertile women will be recommended. This described gender disparity may impact in development, pathogenesis, progression and staging of HCC as well as may identify treatment modality with response or relapse.

Declarations

Acknowledgements

None

Authors' contributions

Ms. Matta designed the study and protocol and conducted the literature review,

recruited the participants, conducted the statistical analyses and wrote the first draft of

the manuscript. Prof **shatat** followed up the work , Mr.**Sedik** shared the collection of the cases and performing the examination and follow them during investigation, collected data . Dr. **Abu Elela** did the laboratory work of this study.

The author(s) read and approved the manuscript.

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Availability of data and materials

The datasets generated and analyzed during the present study are not Publicly accessible due to concerns of participates confidentiality but are offered by the corresponding author on realistic request.

Ethics approval and consent to participate

All procedures performed in our clinical study including human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration. All individual participants gave informed consent to be included in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING STATEMENT

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Tables

Table (2): Clinical and laboratory Data of the Studied Subgroups

Variables	Male subgroups			Female subgroups		
	HCC (n=40)	Cirrhotic (n=30)	Healthy (n=25)	HCC (n=40	Cirrhotic (n=30)	Healthy(n=25)
Age(years) [a]	62. 8 ± 10.5	60.1±7.5	61.9±7.3	59.9 ±10.9	60±7.7	59.5±8.9
Hb (gm/dl) [a]	12.7±2.2**££	13.3±2.4£££	14.2±1.1£££	10.7±2.2*	10.4±2.3**	12.1±0.4
WBCs(x103) [b]	6.7(5.4-8) ‡‡‡	5.5(4.5-6.1) **	6.9(5.6-7.9)	5.9(5.2-10.3)	5.6(4.5-7.7)	6.2(4.9-7.8)
Plat(x103)[b]	162(141-240)	160(140-179) **	207(173-243)	147(108-210) **	166(150-247) **	237(176-290)
ALT(U/l l)[b]	48(30-60) **£	39(24-75) *££	31(26-35)	53(38-70) ‡‡‡***	29(16-35)	29(22-32)
AST(U/l)[b]	49(39-68) ‡ ***	73(29-58) **	26.5(20-38)	49(29-88) ‡‡ ***	33(20-53)	24(22.7-31)
Album(gm/dl)[a]	3.4±0.7**	3.4±0.8**	4± 0.4	3.2±0.6***	3.2±0.9***	4.1±0.5
Bil(mg/dl)b	1.5(0.9-2.3)***	1.1(0.8-1.8) ***	0.58(0.4-0.7)	1.3(0.9-1.9)***	1.1(0.9-1.4) ***	0.5(0.3-0.7)
INR [a]	1.3 ± 0.2**	1.4 ± 0.3***	1.1 ± 0.2	1.3 ±0.3***	1.4 ±0.3***	1 ±0.1
RBS(mg/dl) [a]	132±16	127±18	127±15	128±18	128±18	121±15
Crea(mg/dl) [a]	1 ± 0.31	0.97 ±0.21	0.91 ±0.21	0.96 ±0.27	0.94 ±0.31	0.95 ±0.25
Child class n A/B/C %	12/14/14 30/35/35	11/9/10 36.7/33.3/30	——	12/14/14 30/35/35	10/10/10 33.3/33.3/33.3	——
Child score [a]	9(6-11)	8(5-11)	———	8(6-11)	8(6-11)	———
MELD score [a]	12.5 (9-15)	12(10-18)	———	13(8-14)	11(9-17)	———
S1P(ng/l) [a]	134.3±22.1‡***££	124.8±19.6***	36.7±15.7	157.5±33.7‡‡***	134.8±24.7***	31.5±12.1
adipon(mg/l) [b]	2.6(2.4-3.2) ‡ ***£££	3.3(2.5-4.1) ***	5.1(4.5-7) £££	5.6(3.5-8.3)‡‡‡***	2.7(2.4-4.1) ***	12.2(9.8-14.5)
Test(ng/ml) [b]	3.8(2.9-5.1) *** £££	3.4(1.4-5.9) **£££	8.7(8.2-10.2) ££	0.51(0.34-0.82) ‡	0.66(0.49- 1.7)	0.48(0.17-0.82)
SHBG(nmol/l)[b]	29.4(21.8-32.8)‡‡‡**£££	19.8(14.7-24.8) £££	23.1(19.8-24.7) £££	10.65(8.7-12.3) ‡‡*	12.7(9.9-14.2)	15.2(8.6-20.4)
BAT(ng/ml) [b]	1.7(1.12- 2.6) *** ££ £	1.6(0.97-3.8) *** ££ £	5.8(4.8-6.3) £££	0.3(0.7-0.55)	0.36(0.31-0.7) *	0.26(0.9-0.5)
FAI (ng/ml) [b]	0.09(0.06-0.13) **££ £	0.09(0.05-0.19) *** £££	0.25(0.22-0.3) £ ££	0.02(0.01-0.03) *	0.02(0.01-0.03) **	0.01(0.004-0.02)
Estr (pg/ml) [b]	18(13-23) ‡ ‡***£££	24(17.9-27.8) ***££ £	53(45.5-65)	52(45-64) ‡‡‡*	33(28.1-42.7) ***	63(52-67)
E/T x10-3 [b]	5.3(2.3-7.8) ‡ £££	6.6(4.35-11.2) * £££	5.6(2.4-6.7) £££	100(66-153) ‡‡‡	45(32.9-77.4) ***	185(47-393)

HCC= Hepatocellular carcinoma; HB= Hemoglobin; WBCs=White blood cells; Plat=platelet ,ALT=Alanine aminotransferase;AST= Aspartate aminotransferase; ALB= Albumin; Bil = Bilirubin; INR= International normalized ratio; RBS=Random blood sugar; crea = creatinine ; MELD: Model of End stage Liver Disease, S1P= Sphingosine 1- phosphate ;adipon= Adiponectin; Test= Testosterone; SHBG=Sex hormone binding globulin; BAT= Bioavailable testosterone ; FAI = Free androgen index; Estr = Estradiol ; E/T = Estradiol to testosterone ratio.a= normally distributed quantitative data are expressed as mean ± standard deviation and compared using independent sample T test between each two group.b= not- normally distributed quantitative data are expressed as median and interquartile (25%-75%) and compared using Mann Whitney U test between each two groups. Child

class is expressed as number (percentage) and compared by Chi square test P < 0.05 is considered to be statistically significant. Statistical significance vs. sex- respective matched cirrhotic patients ‡=P <0.05, ‡‡=P<0.01, ‡‡‡= p<0.001; Statistical significance vs. sex - respective matched healthy subjects *=P<0.05, **=p<0.01 , ***= p<0.001; Statistical significance vs. corresponding group of females £=p<0.05,££= p<0.01 , £££= p<0.001

Table (4): Association of Sphingosine 1 Phosphate, adiponectin and Sex Hormones with Tumor Clinicopathological status and TNM Staging among female HCC subgroup

Clinicopathological and TNM staging		n	Sphigosine 1 phosphate	**Adiponectin**	Total testosterone	Sex hormone binding globulin	Bioavailable testosterone	Free androgen index $x10^{-3}$	estradiol	Estradiol to testosterone ratio($x10^{-3}$
Tumor diameter (cm)	<5	14	130(120-192)	3.7(3.4 -5.1)	0.38(0.34-0.48)	11.4(9.5-2.8)	0.22(0.21-.21)	12(11-17)	63 (48-73)	152(102-165)
	≥5	26	170(130-181)	7.5 (5 - 8.4)	0.75(0.37-0.83)	9.3(8.2-12)	0.48(0.23-0.57)	25(14-31)	51(42-59)	79(61-139)
P-value			0.21	**0.002**	**0.04**	0.10	**0.048**	**0.003**	0.07	**0.005**
Number of	S	18	131(128-176)	4.9(3.4-8.8)	0.36(0.32-0.79)	9.3(8.2-1.9)	0.20(0.22-0.54)	15(12-27)	51(37-53)	102(67-147)
Focal lesion	M	22	170(126-208)	5.8(3.9-8.3)	0.59(0.38-0.83)	12(8.9-12.3)	0.23(0.33-0.56)	25(12-29)	63(48-70)	98(62-165)
			0.14	0.67	0.21	0.23	0.26	0.56	**0.003**	0.88
Portal vein thrombosis.	no	10	176(127-194)	6.8(4.5-8.6)	0.4(0.34-0.81)	11.4(8.9-12.3)	0.27(0.21-0.52)	14(11-29)	51(43-66)	137(62-165)
	Yes	30	150(128-176)	5.5(3.4-8.1)	0.75(0.49-0.84	9.3(7-12.8)	0.53(0.36-0.57	25(19-27)	63(48-65)	79(70-101)
P-value			0.47	0.41	0.08	0.86	**0.04**	0.19	0.46	0.30
TNM	I	12	130(128-144)	5.5(3.4-12.8)	0.34(0.29-0.78	9.4(8.6-11.7)	0.21(0.19-0.57)	13(11- 35)	45(36- 53)	120(65-155)
	II	16	157(122-188)	4(3.4-5.7)	0. .39(0.34-0.52	10.6(8.5-12.6	0.25(0.22-0.37)	14(11- 19)	65(49- 72)	148 (88-179)
	IIIa	12	184(170-208)	8.1(6.5-8.3)	0. .82(0.75-.083	12.1(8.9-12.3)	0.52(0.48-0.65)	29(25-30)	54(48-65)	71(57- 86)
P-value		IvsII	0.3	0.32	0.42	0.47	0.2	1	**0.004**	0.2
		IvsIII	**0.006**	0.38	**0.02**	0.17	0.17	0.17	**0.03**	**0.03**
		IIvsIII	0.07	**<0.001**	**<0.001**	0.8	**0.002**	**<0.001**	0.14	**0.001**

HCC =hepatocellular carcinoma; S= solitary, M=multiple Data are expressed as median (25-75%) quartile range and compared by Mann Whitney test.

Bold values indicate statistically significant results.

Figures

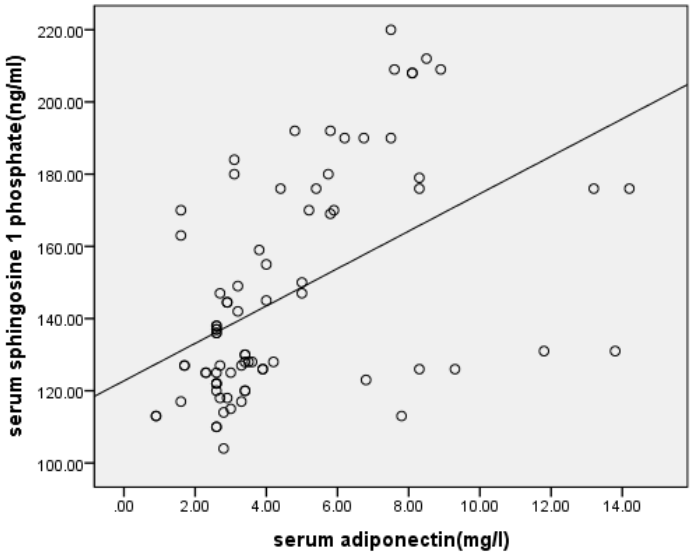

Figure 1

Correlation of serum sphingosine 1-phosphate with serum adiponectin in all HCC patients ((r= 0.56, p<0.001)

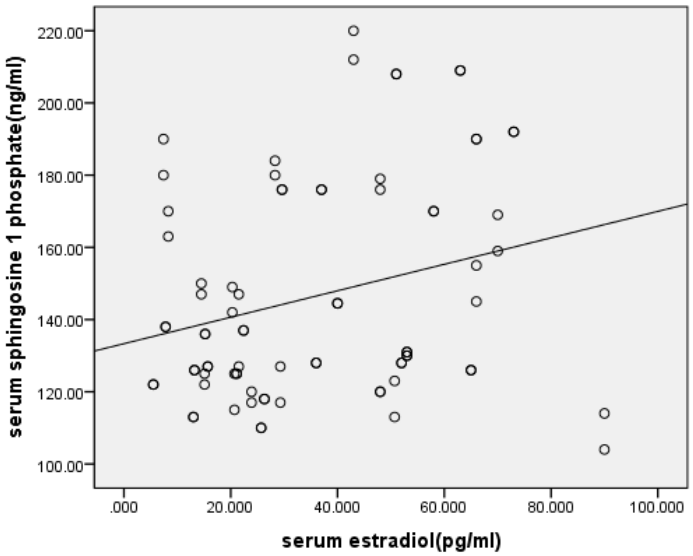

Figure 2

Correlation of serum sphingosine 1-phosphate with Serum Estradiol in all HCC patients(r=0.25, p=0.02)

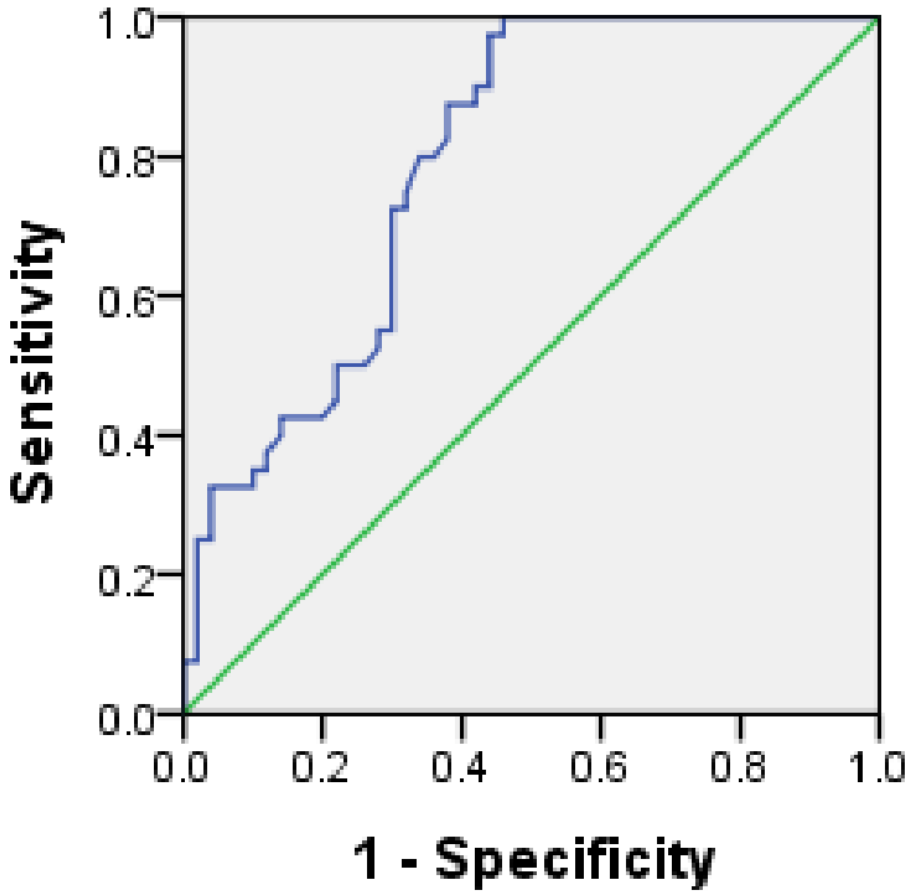

Figure 3

Roc curve analysis of sphingosine 1 phosphate for diagnosis of HCC among cirrhotic patients and healthy subjects. the AUCs were was 0.79, 95% CI (0.70–0.88) with P < 0.001.At cut-off value ≥ 113ng/l as a screening test , the sensitivity and specificity were 95% and 56% among all HCC patients