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Effect of Gender on Some Biochemical Parametersin Iraqi Cholelithiasispatient's.

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Abstract: Gallstones (GS) are abnormal solid lumps of a mixture of cholesterol crystals, mucin, calcium bilirubin, it is the most common problems affecting the digestive tract, the present study enable us to classify the studied gallstone patients into gallstone patients without any complication (cholelithiasis) . The aim of the current study is to evaluate the level of the xanthine oxidase(XO) and dehydrogenase(XDH) activity, and several biochemical factors in sera of cholelithiasis patients according to the gender (male and female). Thirty-five of cholelithiasis patients were male and forty-one were female, for comparison, apparently fifty-seven healthy were enrolled as control; twenty-nine of them were male and twenty-eight were female.The age of these groups ranged from 41 to 50years.The resultswere appeared that XO activity and its specific activity(S.A.XO) were significantly increase in female patient groups when compared to male and female patient groups, on the other hand the mean levels of XDH activity and S.A.XDH showed high significant decreases, that's meanfemale gender is the highlights and proven risk factor for gallstone formati

Keywords: Cholelithiasis, Xanthine dehydrogenases enzymes, LiverFunction Tests, Lipid Profi

I. Introduction

The gallbladder (GB) is a part of the gastrointestinal tract (GIT), one of the gallbladder diseases is Gallstones (GS)which were solid lumps masses of a mixture of cholesterol crystals, mucin, calcium bilirubinate, and proteins that have affected people for centuries; it is the most common problems affecting the digestive tract[1]. The presence of stones in the GB is referred to as cholelithiasis with a substantial burden to healthcare systems [2]. Autopsy reports have shown a prevalence of GS from 11% to 36 % , the female is three times more likely to develop GS than male, and first-degree relatives of patients with GS have a twofold greater prevalence. Female gender is the highlights and proven risk factor for GS formation, the most studies showing the incidence of GS increased two to threefold in females.This difference between men and women decreasing with increasing age, with infection rates becoming essentially equal after the fifth decade of life[3]. Gallstone (GS) can vary in number (may be single large stone or many smallerstones) ,inshapeand in size from as small as a grain of sand to as large as a golf ball, also they classified by their cholesterol content as either cholesterol stones or pigment stones.There were some causes and risk factors for this disease such as: Age,Gender,Obesity, Weight loss,Genetics, Pregnancy,and Die[4]. Gallstones cause no symptoms, even for years; therefore, GS are called "silent stones" nonspecific GS-associated symptoms occur[5,6]Onset more than an hour after meals support this diagnosis, meanwhile, symptoms of GS may include:[7]pain in the abdomen and back is infrequent but severe, the increase in abdominal pain after eating a fatty meal, fever and pain, if the GB or bile duct becomes infected, and jaundice.The number and the exact size of stones have not been specified by ultrasound, but have an approximate diagnosis where it stated in the report GS single or multiple grit size, gallstones treatment depends on the size and position of the GS, and may include:[8]dietary modifications,medications, lithotripsy, and surgical treatment.

Xanthine oxidoreductases (XOR) is aconserved housekeeping enzyme, with a main role in purine catabolism by catalysing the two last steps in purine catabolism, converts hypoxanthine into xanthine and xanthine into uric acid.This enzyme is part of a group of enzymes known as the molybdenum iron-sulfur flavin hydroxylases, it exists in two special functional but interconvertible forms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH).XOR under normal conditions, exists in dehyrogenase form (XDH) and uses NAD^+ and there is no or very little production of superoxide anion.[9] The wider consideration of XOR in human pathology involves the ability of the enzyme to generate ROS, via both the production of superoxide and peroxide, when using O_2 as its terminal electron acceptor. XO may play a beneficial role by producing either superoxide radical or uric acid. Superoxide radicals derived from XO may be involved in the differentiation and/or proliferation of the epithelial cells.More relevant to human pathology is the conversion of XDH to XO by thiol oxidation or limited proteolysis, yielding an oxidase form that is unable to reduce NAD^+. The ability of XDH to utilize NAD^+ or O_2 while XO is only able to utilize O_2 stems from modifications at the flavin site the conversion of XDH to XO[10].The aim of the current study is to investigate the effect of gender differences in

the levels of the xanthine oxidoreductasesactivitiesand several biochemical factors in sera of cholelithiasispatients.

II. Materials and Methods

In the present study total of (133) individual samples were included, the control group consist of (57) apparently healthy individual samples; twenty nine of them were male and twenty eight were female, while the gallstone patients were (76) individual samples**.**thirty five of them were male and forty one were femaleThe age of these groups ranged from (41 year to 50) years, all were subjected to a personal interview using chieflypreparedquestionnaire format full history with detailed information**.**The blood samples were allowed to clot and then sera were separated by centrifugation at 3000 rpm for 10 min at room temperature. The serum was divided into two parts the first were used in the same day for the enzymatic activity assays, lipid profile and protein determination. The remainder of the sera was stored at (-20°C), to be used for other parameters estimation.

Total protein concentration was determined by Biuret method[11], and serum albumin was determined by dye-binding method using kit manufactured by Randox.[12], while serum globulin was calculated mathematically from the subtraction albumin concentration from theconcentrationof total protein,also the concentration of albumin divided by the concentration of globulin was expressed as albumin to globulin ratio (A/Gratio). By colorimetric method serum creatinineand urea concentrationsweredetermined[13]. Common liver function tests:transaminases(GPT, GOT), alkaline phosphatase (ALP), bilirubin (total and direct;TSB and DB) were estimated using commercial kits[14,15].Lipid profile:Triglycerides (TG) ,Total cholesterol(TC), High-density lipoprotein (HDL-c), Low-density lipoprotein (LDL)and veryLow-density lipoprotein (VLDL), as well as uric acid were measured by spectrophotometrically methods using commercial kits[16,17,18]. xanthine oxidase activity(XO) was determined by the method of Ackermann and Brill[19], whereas dehydrogenase (XDH) activity of xanthine was determined by Fried *et al.* method[20].Flameless atomic absorption spectrophotometer is the recommended technique to determination of molybdenum (Mo) in serum, this method is sensitive and rapid to determine the numerous elements[21].

III. Results

The mean serum levels of total protein, albumin, and A/Gratiowere showedsignificant decreases ($p< 0.05$)inmale and female patients group when compared to male and female control group, while globulin showed significant increases ($p< 0.05$), in male and female patients group when compared to male and female control group., also there were significant differences between all groups(Table 1).

Table 1: The mean totalserum protein, albumin, globulininsera of control and patients groups according to gender.

Parameter	Control male (n=29) Mean ± SD	Patient male (n=35) Mean ± SD	Control female (n=28) Mean ± SD	Patient female (n=41) Mean ± SD
S. Protein (g/dl)	7.358 ± 0.369[a]	6.937 ± 0.381[f]	7.311 ± 0.387 [b]	7.081 ± 0.400 [e]
S. Albumin (g/dl)	4.511 ± 0.235[a]	3.815 ± 0.400 [f]	4.496 ± 0.241 [b]	3.839 ± 0.435 [e]
S. Globulin (g/dl)	2.848 ± 0.491[a]	3.122 ± 0.524 [f]	2.815 ± 0.482 [b]	3.242 ± 0.660 [e]
A/GRatio	1.648 ± 0.401[a]	1.273 ± 0.330 [f]	1.661 ± 0.395 [b]	1.263 ± 0.420 [e]

Correlation is significant at the 0.05 level (2-tailed).
(a): indicated significant difference between groups (CM) and (PM).
(b): indicated significant difference between groups (CF) and (PF).
(c): indicated significant difference between groups (CM) and ((CF).
(d): indicated significant difference between groups (PM) and (PF).
(e): indicated significant difference between groups (CM) and (PF).
(f): indicated significant difference between groups (CF) and (PM).

The mean liver function tests (GPT, GOT, ALP, TSB and DB) were appeared no significant differences ($p>0.05$) in male and female patients group when compared to male and female control group. The results of mean creatinine and urea wereshowednon-significant differences ($p>0.05$) in male and female patients group when compared to male and female control group(Table 2).

Table 2: The mean liver function tests, creatinine and urea in in sera of control and patients groups according to gender.

Parameter	Control male (n=29) Mean ± SD	Patient male (n=35) Mean ± SD	Control female (n=28) Mean ± SD	Patient female (n=41) Mean ± SD	Comparison of Sig.	
					*p*value	Sig
GPT [U/L]	10.610 ± 4.880	12.229 ±5.662	12.471 ± 7.400	12.463 ±4.336	> 0.05	NS
S.A.GPT [U/g]	0.144 ± 0.060	0.176 ± 0.080	0.171 ± 0.100	0.177 ± 0.064	> 0.05	NS
GOT [U/L]	17.673 ± 3.714	19.20 ± 2.898	19.515 ± 6.359	18.293 ± 3.723	> 0.05	NS
S.A.GOT[U/g]	0.240 ± 0.049	0.283 ± 0.056	0.268 ± 0.087	0.259 ± 0.051	> 0.05	NS
ALP[U/L]	59.465 ± 14.850	66.20 ± 11.30	72.175 ± 13.060	66.293 ± 14.384	> 0.05	NS
S.A. ALP [U/g]	0.811 ± 0.209	0.959 ± 0.181	0.987 ± 0.169	0.939 ± 0.214	> 0.05	NS
TSB [mg/dl]	0.513 ± 0.266	0.608 ± 0.196	0.489 ± 0.248	0.509 ± 0.202	> 0.05	NS
DB [mg/dl]	0.179 ± 0.119	0.411 ± 0.566	0.136 ± 0.075	0.200 ± 0.246	> 0.05	NS
Creatinine[mg/dl]	0.912 ± 0.062	0.909 ± 0.200	0.748 ± 0.062	0.707 ± 0.170	> 0.05	NS
Urea [mg/dl]	26.721 ± 5.93	23.857 ± 10.98	27.711 ± 9.790	24.098 ± 10.340	> 0.05	NS

The present study was found that there were significant increases in serum: TC, TG, LDL-c, and VLDL-c levels, whilethere were significant decreases in serum HDL of male and female patients when compared to the controls.also there were a significant differences between all groups(Table 3).

Table 3: The mean lipid profile, incontrol and patients groups according to gender.

Parameter	Control male (n=29) Mean ± SD	Patient male (n=35) Mean ± SD	Control female (n=28) Mean ± SD	Patient female (n=41) Mean ± SD	Comparison of Sig.	
					*p*value	Sig
Cholesterol[mg/dl]	136.647 ± 31.52 [a]	166.171 ± 42.64	148.585 ± 31.707[b]	182.976 ± 45.628[e]	< 0.05	S
Triglyceride[mg/dl]	113.551 ± 37.86 [a]	179.029 ±25.62[f]	117.258 ± 33.991[b]	164.780 ± 34.388[e]	< 0.05	S
HDL – C [mg/dl]	36.474 ± 9.002[a,c]	31.771 ± 4.640[f]	40.554 ± 14.154[b]	35.171 ± 6.371	< 0.05	S
LDL – C [mg/dl]	68.673 ± 23.939[a]	89.343 ±11.149[d]	84.579 ± 26.870[b]	114.849 ± 44.018[e]	< 0.05	S
VLDL - C [mg/dl]	22.710 ± 7.537[a]	35.806 ± 5.124[f]	23.298 ± 6.502[b]	32.956 ± 6.878[e]	< 0.05	S

The mean levels of uric acid were showed significant increase in both gender (male and female) patients groups when compared to control groups, the mean values of XO activity , specific activity of XO (S.A. XO), (XO/XDH) ratio, were revealed significant increases (p<0.05) for patients both groups when compared to control group, in contrast XDH activity and specific activity of XDH (S.A XDH) were found to be significant decreases (p< 0.05) for patients male and female group in comparison with control, and the mean levels of trace elements (Fe, Mo) showed significant increases (p< 0.05) in male and female patient groups when compared to male and female control groupsandalso there were a significant differences between all groups.(Table 4).

Table 4: The mean uric acid, Activities and Specific Activities of Serum Xanthine Oxidase, Xanthine Dehydrogenase, Uric Acid and trace elements (Fe & Mo) ,incontrol and patients groups according to gender.

Parameter	Control male (n=29) Mean ± SD	Patient male (n=35) Mean ± SD	Control female (n=28) Mean ± SD	Patient female (n=41) Mean ± SD	Comparison of Sig.	
					p value	Sig
Uric acid(mg/dl)	4.447 ± 1.200[a]	5.089 ± 1.260[f]	4.425250 ±1.10[b]	5.053659 ±1.25[e]	< 0.05	S
XO [U/L]	21.540 ± 8.093[a]	74.375± 30.485 [f,d]	22.316 ± 7.066 [b]	86.674± 25.669[e]	< 0.05	S
S.A. XO [U/g]	0.294 ± 0.111 [a]	1.0780±0 .454 [f,d]	0.3056 ± 0.098 [b]	1.222± 0.359 [e]	< 0.05	S
XDH [U/L]	2.804 ± 2.172[a,c]	1.884± 0.665 [f,d]	2.054 ± 0.598[b]	1.286± 0.526 [e]	< 0.05	S
S.A. XDH [U/g]	0.038 ± 0.030[a,c]	0.027± .009[d,f]	0.028 ± 0.009[b]	0.018± 0.0078[e]	< 0.05	S
XO/XDH ratio	11.120 ± 9.180[a]	41.551±22.787[d,f]	12.027 ± 5.823[b]	76.864± 31.418[e]	< 0.05	S
Fe [µg/ml]	2.780 ± 0.698[a]	6.999 ± 1.146[d,f]	2.249 ± 0.812[b]	6.448 ± 1.430[e]	< 0.05	S
Mo [µg/ml]	0.011 ± 0.002[a]	0.025 ± 0.008[d,f]	0.012 ± 0.006[b]	0.033 ± 0.010[e]	< 0.05	S

IV. Discussion

Protein was indicated many diseases at an early stage in defined cellular injury that could be of substantial clinical value for the development of strategies for early detection and or treatment of diseases.In this study the values of total serum proteins and albumin in sera of male and female patients with gallstone lowered in comparison to that of their corresponding healthy control. On the other hand, the results obtained in the present study for serum globulin show significant increase among the groups of patients and control involved in the present study.[22]

In general terms, variations in plasma protein concentrations can be due to any of three changes: in the rate of protein synthesis, the rate of their removal, and in the volume of distribution .So, the differences in serum total proteins pattern may be explained mainly by the differences in serum albumin concentrations, and synthesis of albumin was reported to be reduced in case of hereditary defects, liver diseases, malnutrition and another disease.

There may be a decreased protein synthesis due to malnutrition and malabsorption in patients. It is widely reported that hypoproteinemia is one of the features of patients with inflammatory bowel disease. Different molecules behave differently during an inflammatory phase; albumin synthesis decrease, while other inflammatory globulins rise.[23]The mean liver function tests (GPT, GOT, ALP, TSB and DB), were showed no significant differences ($p>0.05$) in male and female patients group when compared to male and female control group.This means that all patients in this study have no liver disease to interactions with gallstone.

Serum creatinineandBUN are useful clinical tools in assessing renal function which is becoming a serious health problem.[24]In this study the mean levels of creatinine and BUN were appeared no significant differences ($p>0.05$) in patients group when compared to control group. This means that all patients have no kidney disease to interactions with GS disease.

The present study was indicated significant increases in serum: TC, TG, LDL-c, and VLDL-c levels of patients with cholelithiasis when compared to the controls. seems to play a major contributing role in the pathogenesis of GS in females of up to 45 years age.[25]The elevation of TC and TG levels in patients may be due to that GS patients have abnormal secretory mechanism for bile acids and phospholipids, decrease bile acids and phospholipids (which solubilize cholesterol in the bile) will increase cholesterol precipitation, in addition some of GS patients may present with metabolic syndrome.[26] Which is a cluster of symptoms such as glucose intolerance, high total cholesterol, hyperinsulinemia, increased VLDL and/or total cholesterol, decrease HDL and hypertension who indicate that the hyperlipidemia is strong risk factors in cholelithiasis as estimated previously.[27]There were significant differences in lipid profile levels between male and female in this study, that explain the increased frequency of GS in woman as an effect of female sex hormones on hepatic function, bile secretion and GB function, bile is more saturated during the second and third trimester of pregnancy.Estrogenincreases the biliary of cholesterol secretion and the pathogenicity of bile, there may be stimulation of hepatic lipoprotein receptors and increased hepatic cholesterol uptake.Furthermorethe increased number of pregnancies is associated with an increased risk of GS believe that the progesterone instead of the estrogen is responsible for the changes in biliary lipids,Bile cholesterol increases with age and is raised in women, particularly those taking the contraceptive pill,likewiseoral contraceptive usagestimulates an increased risk of GB disease, that may be associated with an increase in cholesterol saturation[28].

The serum levels of uric acid were reported to be quite variable and higher in males than in female[29]. and this agreeswith our study this increase may be due to the parallel increasing in xanthine oxidase activity observed in gallstone patients reflect the fact that the catabolic pathway is increased, in other words increasing the salvage pathway in which the uric acid biosynthesis.

Recently xanthine oxidoreductases enzymes were studied in sera of Cholelithiasis patients, in the present study, highly significant increaseswere found in activities and specific activities of XO, in contrast highly significant decreases in the activities and specific activities of XDH were found in sera of male and female cholelithiasis patients group in comparison to control group. Also the results of this study were appeared the highest XO/XD ratio in cholelithiasis patients which confirm the idea of increase the rate of conversion of XD to XO in this pathogenic condition in parallel the free radical production increased and so the oxidative stress increase .Several mechanisms have been suggested to be involved in the generation of reactive oxygen species but XO has been shown to be a major source of free radical generation under ischemic conditions.It was suggested that oxidative stress might be increased in abnormal conditions and may affect the course of the disease. On the other hand when the oxidative stress is higher, alteration in some purine metabolizing enzymes was found[30]. The high XO activity may be an attempt to lower salvage pathway activity for purines, which is vital for rapid DNA synthesis.

Molybdenum (Mo) can be utilized as a stably bound, variably coordinated cofactor in proteins, in mammals, Mo is found in three different enzymes: aldehyde oxidoreductase(AOR),sulfiteoxidase (SOX), and xanthine oxidoreductase (XOR). Humans possess each of these enzymes, which differ slightly in the coordination of the Mo -containing cofactor Molybdenum by itself is relatively inert in biological processes, and requires an additional pterin cofactor to be biologically active in molybdo-enzymes. The results of the present

studywere indicatedthat high significant increase ($p<0.01$) in Mo levels in male and female patients group when compared to male and female control group that may be because increasing the activity of XO which is directly proportional to the amount of Mo in the body. However, an extremely high concentration of Mo reverses the trend and can act as an inhibitor in both purine catabolism and other processes. Mo concentrations also affect protein synthesis, metabolism and growth,[31]and that may also prove the increase the rate of conversion of XDH to XO in this disease.

Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans[32]. Iron-containing proteins (such as hemoglobin, cytochrome P450 and catalase) arepredominatingly containing heme prosthetic groups, which participate in many biological oxidations and in transport functions. Most of theferrous ion (Fe^{2+}) is oxidized to ferric ion(Fe^{3+}) by spontaneous oxidizationand /or the ferroxidase activity of ceruloplasmin (Cp) and then bind to transferrin and to be acquired by the cells. However under pathological conditions the loss of Cpferroxidase activity make it impossible for most(Fe^{2+}) to be oxidized to (Fe^{3+}): accordingly, the amount of ferric ion and transferrin – bound Fe^{3+} will decrease, while non- transferrin – bound iron such as citrate –Fe^{2+}, ascorbate –Fe^{2+} and free Fe^{2+}will increase, this will induce oxidative stress and free radical formation, and trigger a cascade of pathological events leading to cell death. It is also possible that the rate of spontaneous oxidization of $Fe^{2+}$to Fe^{3+}will increase so that more (Fe^{3+}) can be formed, as well as, generate a large amount of reactive oxygen species [33]. The resultsofthe present study were showedthat high significant increase ($p<0.01$) in Fe levels in male and female patients group when compared to male and female control group that may be because increasing the activity of XO as it is as well as Mo components of enzymes.

V. Conclusion

Female gender is the highlights and proven risk factor for gallstone formation,cholelithiasis is associated with some biochemical abnormalities [elevation of lipid profile (except HDL), decreases in total protein, albumin, and A/G ratio ,increase in globulin] when compared to control, that may be the cause or the result of gallstone formation.The increase in XO activity observed in these patients reflect the fact that the catabolic pathway of hypoxanthine and xanthine is increased, this increase may confirmed by increasing in uric acid levels.

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