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Research Article

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Obesity effect on xanthine oxidoreductase activities in gallstone patients

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ABSTRACT

Xanthine oxidoreductase enzyme is part of a group of enzymes known as the molybdenum iron-sulfur flavin hydroxylases, it widely distributed throughout various organs including the liver, kidney, gut, lung, heart, brain and plasma with the highest levels found in the gut and the liver. Cholelithiasis or Gallstone disease (GD), is one of the most prevalent gastrointestinal tract diseases, with a substantial burden to healthcare systems and it is abnormal masses of a solid 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, however obesity is a firm risk factor for gallstone disease. Total of (133) individual samples were included in the present study the control group consist of (57) apparently healthy individual samples, while the gallstone patients were (76) individual samples. The studied samples were classified in to two groups according to gender and Body Mass Index (BMI) for each control and patient groups, xanthine oxidoreductase and other biochemical parameters were measured by colorimetric tests. The results showed that there were significant differences in the mean xanthine oxidase activity and its specific activity and the mean xanthine dehydrogenase activity and its specific activity of patients when compared to control group and between another parameters according to (BMI). The present study suggests that there is a correlation between the obesity and xanthine oxidoreductase enzyme.

Keywords: Gallstone, Xanthine oxidoreductase enzyme, obesity.

INTRODUCTION

Gallstones (GS) are abnormal masses of a solid 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 (from the Greek: chol, "bile" + lith-, "stone" + iasis-, "process") [2] Cholelithiasis or Gallstone disease (GD), is one of the most prevalent GIT diseases, with a substantial burden to healthcare systems [3]. Autopsy reports have shown a prevalence of cholelithiasis from 11% to 36 % [4], the female is three times more likely to develop cholelithiasis than male, and first-degree relatives of patients with cholelithiasis have a twofold greater prevalence [5].There were some causes and risk factors for this disease which were: age [6],Gender [7],Obesity[8],Weight loss[9, 10],Genetics[11],Pregnancy[12],and another parameters.

Body Mass Index (BMI):It was obtained from measuring weight in (kg) & height in (m) by using suitable scales and applied the following equation [13]:

$$\text{BMI} = \frac{\text{weight (kg)}}{\text{Height (m)}^2}$$

Xanthine oxidoreductase (XOR) is an evolutionarily conserved housekeeping enzyme, with a principal role in purine catabolism by catalysing the two last steps in purine catabolism, forming uric acid from hypoxanthine and

xanthine. Also, it is necessary for detoxification and the organization of the cellular redox potential. It is Interesting; the same protective functions are involved in multiple features of the innate immune system, suggesting that XOR is a central molecule in the evolution and function of this ancient defence system [14].Xanthine oxidoreductase exists in two distinct functional but interconvertible forms: xanthine oxidase (XO; xanthine- oxygen oxidoreductase; EC (1.17.3.2), and xanthine dehydrogenase (XDH; xanthine- NAD oxidoreductase; EC (1.17.1. 4).

EXPERIMENTAL SECTION

Total of (133) individual samples were included in the present study,the control group consist of (57) apparently healthy individual samples, while the gallstone patients were (76) individual samples. The studied samples were classified in to two groups based on the gender(male,female) and then divided into further subgroups according to the differences in BMI [Overweight (25 - 29.9) and obese (30 - 34.9)] for each control and patient groups **,** all were subjected to a personal interview using especially designed questionnaire 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. The remainder of the sera was stored at (-20°C), to be used for other parameters estimation.lipid profile (TC, TG, LDL-c, and VLDL), [15-16] were measured by spectrophotometrically methods using commercial kits. Xanthine oxidase activity (XO) was determined by the method of Ackermann and Brill [17], while dehydrogenase (XDH) activity of xanthine was determined by Fried et al. method [18]. Flame atomic absorption spectrophotometer is the recommended technique to determination of iron (Fe) and flameless for molybdenum (Mo) in serum. This method is sensitive and rapid to determine the numerous elements [19].

RESULTS AND DISCUSSION

Recently some biochemical parameters were evaluated in sera of Iraqi patients with gallstones and the effect of gender on selected biochemical parameters was also studied [20-21] , in the present study, the patient with GS under study were classified into two groups depended on their obesity factor (BMI: kg/m^2) above thirty obese and under thirty overweight, table (1) ,there was significant difference between all studied groups.

Table 1. Mean serum cholesterol in control and patients groups according to BMI

Groups	No.	Cholesterol [mg/dl] Mean ± SD	Comparison of Sig.	
			p value	Sig
Control M1	17	$127.997^{a,c}$ ± 33.064	< 0.05	S
Patient M1	18	165.444 ± 44.113		
Control M2	12	148.901 ± 25.697		
Patient M2	17	166.941 ± 42.378		
Control F1	15	156.227^{a} ± 23.586	< 0.05	S
Patient F1	20	193.300 ± 45.102		
Control F2	13	139.767^{d} ± 38.154		
Patient F2	21	173.143 ± 44.894		

(a): indicated significant difference between groups (CM1),(CF1) and (PM1),(PF1).
(b): indicated significant difference between groups (CM2), (CF2) and (PM2), (PF2).
(c): indicated significant difference between groups (CM1), (CF1) and (PM2), (PF2).
(d): indicated significant difference between groups (CM2), (CF2) and (PM1), (PF1).
(e): indicated significant difference between groups (CM1), (CF1) and (CM2), (CF2).
(f): indicated significant difference between groups (PM1), (PF1) and (PM2), (PF2).

Tables (2-5) summarized the lipid profile levels in gallstone patients and controls according to BMI. There were significant differences between all studied groups, meanwhile there were non- significant differences of HDL - Cholesterol levels between all groups for female according to BMI.,

Table 2. Mean serum triglyceride in control and patients groups according to BMI

Groups	No.	Triglyceride [mg/dl] Mean ± SD	Comparison of Sig.	
			p value	Sig
Control M1	17	$106.559^{a,c}$ ± 41.287	< 0.05	S
Patient M1	18	179.833 ± 29.035		
Control M2	12	$123.456^{b,d}$ ± 30.880		
Patient M2	17	178.176 ± 22.302		
Control F1	15	$118.245^{a,c}$ ± 29.129	< 0.05	S
Patient F1	20	161.750 ± 44.195		
Control F2	13	$116.119^{b,d}$ ± 40.089		
Patient F2	21	167.667 ± 22.315		

Table 3. Mean serum HDL - Cholesterol in control and patients groups according to BMI

Groups	No.	HDL - Cholesterol [mg/dl] Mean ± SD	Comparison of Sig.	
			p value	Sig
Control M1	17	38.685 [a] ± 8.627	< 0.05	S
Patient M1	18	30.722 ± 3.937		
Control M2	12	33.341 ± 8.932		
Patient M2	17	32.882 ± 5.170		
Control F1	15	41.750 ± 11.926	> 0.05	N.S
Patient F1	20	35.200 ± 5.709		
Control F2	13	39.174 ± 16.762		
Patient F2	21	35.143 ± 7.087		

Table 4. Mean serum LDL - Cholesterol in control and patients groups according to BMI.

Groups	No.	LDL - Cholesterol [mg/dl] Mean ± SD	Comparison of Sig.	
			p value	Sig
Control M1	17	62.329 [a,c] ± 26.935	< 0.05	S
Patient M1	18	89.556 ± 9.037		
Control M2	12	77.659 ± 19.863		
Patient M2	17	89.118 ± 13.313		
Control F1	15	90.828 [a] ± 20.924	< 0.05	S
Patient F1	20	123.210 ± 41.473		
Control F2	13	77.369 [d] ± 31.645		
Patient F2	21	101.609 ± 35.685		

Table 5. Mean serum VLDL - Cholesterol in control and patients groups according to BMI.

Groups	No.	VLDL - Cholesterol [mg/dl] Mean ± SD	Comparison of Sig.	
			p value	Sig
Control M1	17	21.312 [a,c,e] ± 8.257	< 0.05	S
Patient M1	18	35.967 ± 5.807		
Control M2	12	24.691 [b,d] ± 6.176		
Patient M2	17	35.635 ± 4.460		
Control F1	15	23.649 [a, c] ±5.826	< 0.05	S
Patient F1	20	32.350 ± 8.839		
Control F2	13	23.224 [b, d] ± 8.018		
Patient F2	21	33.533 ± 4.435		

The results showed that there were significant differences in the concentration of cholesterol, TG, HDL, VLDL, and LDL in obese group comparing with overweight subjects. Obesity is a major risk factor for GS [20], the most important factors that influence excretion and concentration of lithogenic and inhibitory substances are diets and related metabolic disorders. Increasing incidence of urolithiasis in world countries in the last decades is due to changes in lifestyle. Factors raise particular attention to dietary habits and nutritional status of stone formers, therefore larger body size (BMI) was suggested to be associated with a higher risk of stone formation [22]. A risk factor for the development of recurrent stones may be overweight or obesity and associated dietary pattern. However, the mechanisms for this effect are still unclear and a large clinical study showed that being even moderately overweight increases the risk for developing GS [23], the most likely reason is the amount of bile salts in bile is reduced, resulting in more cholesterol [24]. Because obesity is a risk factor, the people should aim to maintain an ideal body weight. Otherwise there is no specific diet for GS disease. Very obese individuals who are attempting drastic weight reduction are at risk for developing GS. They should lose weight under medical supervision. In conclusion, cholelithiasis was associated with lipid profile abnormality that is the cause or the effect of GS formation. Meanwhile these findings should be taken into consideration while treating GS patients. A previous study described a decrease in HDL in GS patients, and there will be a return to the normal condition after GS removal. The results of the present study indicated that GS disease (cholelithiasis) is associated with the elevation of lipid profile (except HDL) when compared to control, that may be the cause or the result of GS formation.

Activities and specific activities of both XO and XDH were summarized in Tables (6 and 7, respectively), the results appeared significant differences between all studied groups.

Table 6. Mean serum xanthine oxidase activity (XO) and its specific activity in control and patients groups according to BMI

Groups	No.	XO [U/L] Mean ± SD	S.A. XO [U/g] Mean ± SD	Comparison of Sig.	
				p value	Sig
Control M1	17	22.271 $^{a, c}$ ± 8.876	0.301 $^{a, c}$ ± 0.121	< 0.05	S
Patient M1	18	72.979 ± 30.505	1.041 ± 0.415		
Control M2	12	20.505 $^{b, d}$ ± 7.081	0.283 $^{b, d}$ ± 0.099		
Patient M2	17	88.0995 ± 35.057	1.283 ± 0.532		
Control F1	15	22.604 $^{a, c}$ ± 7.067	0.311 $^{a, c}$ ± 0.098	< 0.05	S
Patient F1	20	74.342 ± 29.791	1.042 ± 0.408		
Control F2	13	21.983 $^{b, d}$ ± 7.337	0.299 $^{b, d}$ ± 0.101		
Patient F2	21	84.923 ± 31.005	1.206 ± 0.438		

Table 7. Mean serum xanthine dehydrogenase activity (XDH) its specific activity in control and patients groups according to BMI

Groups	No.	XDH [U/L] Mean ± SD	S.A. XDH [U/g] Mean ± SD	XO/XDH ratio Mean ± SD	Comparison of Sig.	
					p value	Sig
Control M1	17	3.054^{a,c} ± 2.421	0.041^{a,c}±0.033	10.659^{a,c} ±7.684	< 0.05	S
Patient M1	18	1.534 ± 0.814	0.022±0.011	66.485±54.510		
Control M2	12	2.450^{b,d} ± 1.803	0.034±0.025	11.772^{b,d} ±11.306		
Patient M2	17	1.311 ± 0.541	0.019±0.008	92.789±77.699		
Control F1	15	1.978^{a,c} ± 0.545	0.028^{a,c} ±0.008	12.559^{a,c} ±6.329	< 0.05	S
Patient F1	20	1.514±0.509	0.021±0.007	55.570±31.044		
Control F2	13	2.141^{b,d} ±0.666	0.029^{b,d} ±0.009	11.413^{b,d}±5.367		
Patient F2	21	1.716±0.564	0.024±0.008	57.224±37.293		

In present study, a highly significant increase has been showed in activities and specific activities of XO, in contrast a highly significant decrease in the activities and specific activities of XDH were found in sera of cholelithiasis patients group in comparison to control group. Alsothe results of our study show 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 proposed to be involved in the generation of oxygen free radicals but XO has been shown to be a major source of free radical generation under ischemic conditions.

Our results were in agreement with many other studies [25-26] which suggested that the overall purine enzymatic pattern confers selective advantages to disease cells by making them more efficient for retention and production of precursors for synthesis of purine and pyrimidine nucleotides and subsequently, for RNA and DNA biosynthesis, 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. The high XO activity may be an attempt to lower salvage pathway activity for purines, which is vital for rapid DNA synthesis. Congenital diseases may also give rise to hyperuricemia, recessive disorders involving the overproduction of uric acid due to complete or partial lack of hypoxanthine phosphoribosyl-transferase (HPRT), which acts to salvage purines from degraded DNA, taking intracellular hypoxanthine to inosine monophosphate (IMP) and xanthine to xanthine Monophosphate (certain isozymes), and a deficiency or absence of this enzyme results in elevated concentrations of XOR substrates in the cell [27].

The results of our study indicated that high significant increase in Mo levels in patients group when compared to control group, table (8) that may be because increasing the activity of XO which is directly proportional to the amount of Mo in the body [28].

Table 8. Mean serum trace elements in control and patients groups according to BMI

Groups	No.	Fe [µg/ml] Mean ± SD	Mo [µg/ml] Mean ± SD	Comparison of Sig.	
				p value	Sig
Control M1	17	2.678 ^{a,c} ± 0.795	0.010 ^{a,c} ± 0.002	< 0.01	S
Patient M1	16	7.152 ± 1.040	0.025 ± 0.009		
Control M2	12	2.925 ^{b,d} ± 0.530	0.012^{b,d}± 0.002		
Patient M2	13	6.813 ± 1.282	0.024 ± 0.008		
Control F1	15	2.206^{a,c}± 0.869	0.012 ^{a,c}± 0.006	< 0.01	S
Patient F1	16	6.552 ± 0.477	0.034 ± 0.011		
Control F2	13	2.299^{b,d} ± 0.773	0.012 ^{b,d} ± 0.005		
Patient F2	16	6.345 ± 1.993	0.032 ± 0.010		

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 , and that may also prove the increase the rate of conversion of XDH to XO in this disease.

Iron is abundant in biology, Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans [29]. Iron-containing enzymes and proteins, often containing heme prosthetic groups, participate in many biological oxidations and in transport. Examples of proteins found in higher organisms include hemoglobin, cytochrome P450 and catalase [30].Most of the iron (Fe^{2+}) is oxidized to (Fe^{3+}) by the ferroxidase activity of ceruloplasmin and /or spontaneous oxidization 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 ferrous ion to be oxidized to ferric ion: 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 ferrous iron 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 ferrous ion to ferric ion will increase so that more (Fe^{3+}) can be formed, as well as, generate a large amount of (ROS) [31]. The results of our study indicated that high significant increase in Fe levels in patients group when compared to control group that may be because increasing the activity of XO as it is as well as Mo components of enzymes.

CONCLUSION

The present study suggests that the activities of xanthine oxidase and xanthine dehydrogenase were affected by gallstones in cholelithiasis patients, meanwhile obesity is a firm risk factor for GS disease therefore there were an effect of BMI in the most of the studied biochemical parameters.

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