Full Length Research Article

Evaluation of Spermine and Spermidine levels in Gallstone patients

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Sahar Mohammod Eedan[1], Jwan Abdulmohsin Zainulabdeen[1],*

1. Department of Chemistry, College of Sciences, University of Baghdad, Baghdad, Iraq

* Jwanbio2016@gmail.com

ABSTRACT

B ackground: Gallstones or cholelithiasis are a major public health issue, which often remain asymptomatic, although they can sometimes cause discomfort by obstructing the digestive tract. Spermidine (SPD) and Spermine (SPE) are two polyamines (PAs) that regulate the growth of hepatocytes in the liver. The PAs play two functions in preserving cellular oxidative equilibrium by generating reactive oxygen species (ROS) and providing protection against free radical damage when serving as an enzyme substrate. Therefore, levels and metabolism of PA serve as significant markers of neoplastic alterations in the liver.

Methods: Gallstone patients from multiple internal medicine hospitals were surveyed, and samples were taken and evaluated from them. Similarly, samples from healthy individuals were also taken. Under regular laboratory circumstances, the patient samples were characterized using biochemical tests, and their data was documented.

Result: The levels of polyamines (PAs) and the common oxidative stress (OS) biomarker malondialdehyde (MDA) were determined. The main liver function tests and lipid profile levels showed no significant differences between the two studied groups. Whereas malondialdehyde (MDA), SPE, and SPD levels were significantly higher in patients than in the control group.

Conclusion: The results of the study led to an association between polyamine levels and gallstone disease. An increase in PA levels was observed in cholelithiasis patients. It is concluded that PAs are associated with cholelithiasis and may be considered as potential predictors of this disease.

INTRODUCTION

The positively charged amines, known as polyamines (PAs), are organic polycationic alkylamines produced from L-ornithine or by decarboxylating amino acids [1]. Examples of PAs include spermine (SPE) and spermidine (SPD). These molecules are widely distributed throughout the body and may have the ability to nucleate cholesterol in both human and model bile. However, there is not enough information available about the specific types of PAs found in human bile [1]. The three processes that maintain the body's PA pool are exogenous nutritional supply, endogenous de novo generation, and intestinal microorganisms. These processes regulate the production, transport, and catabolism of PA in addition to its intracellular concentration. They are produced in the cytoplasm of cells from all tissues using the amino acids arginine, ornithine, and methionine [1]. While ornithine decarboxylase is the rate-limiting enzyme in polyamine biosynthesis. However, the exogenous diet provides the most PAs in comparison to endogenous production. Therefore, since PA metabolic anomalies can lead to a variety of disorders, dietary polyamines (PAs) in food are crucial for maintaining PA biosynthesis. Numerous foods have the necessary levels of PAs, with plant-derived foods mostly containing Putrescine (PUT) and SPD, while animal products primarily contain SPE, and dairy products contain SPD and PUT [2,3]. These PAs are especially necessary for OS regulation, DNA and RNA stabilization, and are crucial in controlling the molecular pathways that support cellular development, differentiation, and proliferation. Importantly, excessive polyamine catabolism can lead to a prominent source of OS, which means an imbalance between pro-oxidants and antioxidants, leading to a decline in antioxidant defense mechanisms, which is caused by elevated levels of ROS and/or reactive nitrogen species (RNS) [4,5]. This raises the inflammatory response and is believed to play a role in a number of illnesses, such as cancer, neurological disorders, liver disease, stroke, and renal failure [6]. On the other hand, one of the most common gastrointestinal conditions and a frequent reason for urgent abdominal surgery is gallstone disease (GS) or Cholelithiasis. The term cholelithiasis (from the Greek: chole, "bile" + lith-, "stone" + iasis-, "process") is used to describe stones in the gallbladder [7]. Gallstones are referred to as "silent stones" since they are nonspecific and do not need to be treated because they do not exhibit any symptoms, even for years. Nonspecific symptoms due to gallstones occur in a minority of individuals [8]. This condition has a number of causes and risk factors, including pregnancy, age, gender, obesity, weight loss, and heredity [9]. The majority of studies indicate that the prevalence of GS rose twice to thrice in women, making GS the most common and well-established risk factor in women. As people age, the gap between men and women narrows, and by the fifth decade of life, infection rates are about equal [10]. Comparing GS patients to those without gallstones, there is strong evidence that they have different gallbladder functions in terms of secretion, absorption, and mucus membrane inflammation. Increased levels of ROS and toxic degradative products of lipid peroxidation have been reported in the plasma of individuals with gallstones [9]. Numerous studies have revealed that OS level is linked to a number of illnesses in Iraq [11,12]. It is significantly increased in patients with cholelithiasis when compared with healthy individuals [13]. This study aims to evaluate the levels of PA with the common oxidative stress biomarker malondialdehyde (MDA) concerning pathological changes in the gallbladder in sera of non-obese cholelithiasis patients (n = 50) in comparison with fifty healthy control individuals. The study also aims to develop a relation between three axes (oxidative stress, cholelithiasis, and PAs) and assess the possible uses of either SPE or SPD as a predictor of this disease.

METHODS

One hundred individual samples were included in this study; fifty healthy (AH group) samples of 26 women and 24 men made up the control group, and fifty newly diagnosed gallstone patients (Pn group) constituted the group of 27 women and 23 men. All of the groups, whose ages ranged from 40 to 65, underwent in-person interviews utilizing a specially created questionnaire format that included a thorough history and comprehensive details. The body mass index (BMI) was calculated using height in meters squared (m^2) and weight in kilograms (kg); underweight is defined as having a BMI of less than 18.5 kg/m^2, normal is defined as having a BMI of 18 to 24.9 kg/m^2, overweight is defined as having a BMI of 25 to 29.9 kg/m^2, and obese is defined as having a BMI of 30 kg/m^2. After letting the blood samples clot, the sera were separated using centrifugation at 3000 rpm for 10 minutes at 25°C. The first part of the serum was used on the same day for the lipid, liver function, and MDA determination (spectrophotometrically) tests. To be used for estimating other parameters, the remaining sera were kept at -20°C. Two PAs (SPD as well as SPE) were determined by using high-performance liquid chromatography (HPLC-UV) [14]. Correlation and receiver operating characteristic (ROC) were assessed by the SPSS program.

RESULTS

Fifty cholelithiasis patients and fifty controls were considered for the current study. Their age and BMI mean ± SD values and gender distribution were shown in Table 1. The two groups were classified based on the information of mean and standard deviation.

Lipid profile

Table 2 displays the groups' lipid profile and liver function test results. The comparison of the lipid profile and LFT values in the GS patients and the control samples revealed no significant differences (p ≤ 0.05). The serum did not differ significantly (p ≤ 0.05) in levels of TC, TG, HDL, LDL, and VLDL between the two groups. To assess and screen for lipid abnormalities, a lipid profile is performed, which is a set of tests in which TC, TG, HDL, LDL, and VLDL are typically ordered together. Low HDL and high blood levels of TC, TG, and LDL are signs of hyperlipidemia. Some researchers suggest that GSs and hyperlipidemia are connected.

Liver Function

Liver function tests are insufficient to track early pathological alterations in the liver, like nonspecific reactive hepatitis, which is brought on by a blockage in the bile flow. This is due to the possibility of bile flow obstruction.

Polyamines (Spermidine & Spermine)

Using high-performance liquid chromatography (HPLC-UV), polyamines (PAs) (SPD & SPE) were identified [14]. The results of Table 3 showed that the patient group's serum concentrations of SPD, SPE, and MDA were significantly higher ($p < 0.05$) than those of the control group.

Table 4 and Figure 1 display the SPD and SPE receiver operating characteristic (ROC) curve analysis for the control and cholelithiasis groups. According to the p-value of less than 0.001, SPD's area under the curve (AUC) is 0.943 with a standard error of 0.027, making it statistically significant at a 95% confidence level. With a sensitivity of 95.6% and a specificity of 90.9%, 4.20 is found to be the ideal cut-off value for SPD. The p-value for SPE is less than 0.001, indicating that the AUC of 0.969 with a standard error of 0.018 is statistically significant at a 95% confidence level. With a sensitivity of 97.8% and a specificity of 94.5%, 6.80 was found to be the ideal cut-off value for SPE. The receiver operating characteristic (ROC) is displayed in Figure 1.

Figures

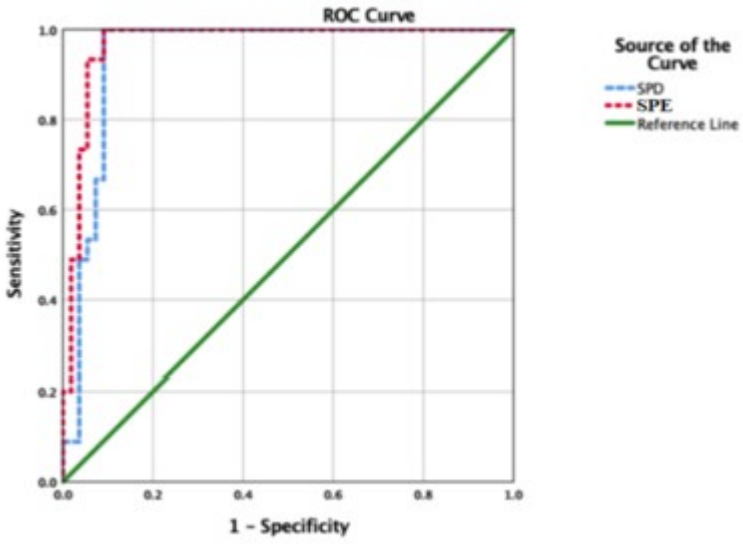

Figure 1: The receiver operating characteristic (ROC), sensitivity, and specificity for SPD and SPE across cholelithiasis and control groups.

Tables

Parameters	AH group (No. = 50) Mean ± SD	Pn group (No. = 50) Mean ± SD	Comparison of Sig.	
			p-value	Sig.
Age (years)	52.10 ± 7.08	53.44 ± 7.67	0.48	N.S
BMI (kg/m^2)	26.35 ± 3.86	25.93 ± 3.99	0.63	N.S
Gender	52% : 48%	54% : 46%	-	-

*Table 1: The age and BMI mean and standard deviations for each of the groups under study. *Sig. = Significance*

Parameters	AH group Mean ± SD	Pn group Mean ± SD	Significance (p-value)	
			p-value	Sig.
Cholesterol (mg/dl)	202.46 ± 22.67	207.40 ± 40.00	> 0.05	N.S
Triglyceride (mg/dl)	159.50 ± 52.29	153.80 ± 65.91	> 0.05	N.S
HDL (mg/dl)	42.38 ± 4.61	42.04 ± 4.82	> 0.05	N.S
LDL (mg/dl)	128.26 ± 10.43	133.62 ± 19.56	> 0.05	N.S
VLDL (mg/dl)	31.82 ± 10.43	30.40 ± 12.01	> 0.05	N.S
GOT (U/L)	28.16 ± 3.38	32.56 ± 5.91	> 0.05	N.S
GPT (U/L)	29.80 ± 3.38	34.02 ± 5.72	> 0.05	N.S
ALK. Ph. (U/L)	52.90 ± 6.54	68.96 ± 7.24	> 0.05	N.S
TSB (mg/dl)	0.784 ± 0.07	0.86 ± 0.099	> 0.05	N.S

*Table 2: Lipid profile & function tests parameters for control and cholelithiasis patient groups. *Sig. = Significance using T-test at 0.05 level of significance*

Parameters	AH group Mean ± SD	Pn group Mean ± SD	Significance (p-value)	
			p-value	Sig.
SPD (ppm)	1.68 ± 0.11	5.68 ± 0.35	S	< 0.001
SPE (ppm)	0.69 ± 0.13	3.65 ± 0.28	S	< 0.001
MDA (nmol/L)	11.11 ± 2.11	13.56 ± 1.88	S	< 0.001

*Table 3: The mean SPD, SPE, and MDA levels in the Cholelithiasis patient group and control group. *Sig. = Significance*

Parameter	AUC	Std. Error[a]	P Value	Sensitivity	Specificity	Cut-off value	Asymptotic 95% C.I.	
							L.B.	U.B.
SPD (ppm)	0.943	0.027	< 0.001*	95.6%	90.9%	4.20	0.891	.995
SPE (ppm)	0.969	0.0018	< 0.001*	97.8%	94.5%	6.80	0.934	1.000

*Table 4: The sensitivity, specificity, and receiver operating characteristic (ROC) for SPD and SPE in cholelithiasis and control groups. Confidence Interval = C.I. L.B. = Lower Limit Upper Bound = U.B. *significant under p-value < 0.05*

DISCUSSION

The most frequent conditions affecting the digestive tract are gallstones (GS), aberrant masses containing a solid combination of proteins, mucin, calcium bilirubinate, and cholesterol crystals that have plagued people for ages. The BMI of a person is an anthropometric measurement based on the weight and height that aids in assessing their obesity status. The individuals for the two groups were non- obese (Table 1), so an important risk factor (obesity) for many diseases, including the studied disease, causing premature death and substantial disability, was excluded [15,16]. The gender effect of some oxidative status parameters was studied previously by Zainulabdeen (2016), and as no significant difference was observed in that study and the contemporary study, so gender factor was not considered in the present study [17].

The mean levels of L.F.T. parameters (GPT, GOT, ALP and TSB) showed non-significant increase ($p > 0.05$) in gallstone patient groups when compared to healthy group because the patients were newly diagnosed and the disease was in its earlier stage (with no complication), therefore the liver was not affected.

In parallel, non-significant differences were found in serum levels of TC, TG, LDL-c, and VLDL-c for cholelithiasis patients compared with controls. In addition to the fact that before the blood tests, patients' food intake was decreased at the beginning of the disease, and dietary convergence among participants may also be the cause of such results. It is difficult to accurately reflect OS status using the same biomarkers in a variety of diseases due to a decline in antioxidant defense mechanisms, due to an imbalance of antioxidants and pro-oxidants.

OS typically plays different roles and activates different signaling pathways in different diseases [18,19]. One typical aldehyde that results from OS is MDA, a stable substance that is thought to be a sign of lipid peroxidation and is created when polyunsaturated fatty acids in the cell membrane oxidize [20]. Numerous chronic disorders in humans have been linked to elevated MDA levels [21,22]. The results indicated that the patient group's serum MDA concentration was high (a significant increase; $p < 0.05$) in comparison to the control group.

In comparison with endogenous production, the exogenous diet offers the greatest number of PAs. As a result, PAs in food (dietary PAs) are essential for preserving PA biosynthesis since PA metabolic abnormalities can result in a number of diseases [23,24]. In tissues, an increase in concentration of SPD was found; the current work on serum found a significant increase in both SPD and SPE concentrations in the patient group, which supports an association of the disease with OS. Also, excessive accumulation of SPD and SPE within the cells leads to toxic effects on cells because these molecules are considered a substrate for a certain type of enzyme that includes copper-containing amine oxidases, mono-diamine oxidases, and Polyamine oxidases (PAOs). These enzymes lead to the consumption of these molecules to give toxic molecules like aldehyde, hydrogen peroxide, and acrolein. Hydrogen peroxide, which is one of the major *in vivo* oxidants, has the ability to penetrate the inner membrane of the mitochondria and interacts with endogenous molecules, leading to OS [25]; this may predict an increase in OS. Correlations are typically written with two key numbers (r and p values). The correlation of Pearson (r) measures the direction and strength of linear relationships between two continuous variables and ranges between -1 and 1. Results indicated that r is +0.338 between SPD and SPE, which indicated a moderate correlation between them with a significant p-value (0.018). The ROC curve (Table 4 and Figure 1) was employed to compare the results of two or more diagnostic tests and evaluate the two PAs' overall diagnostic performance. Additionally, it is employed to choose the best cut-off value for identifying whether cholelithiasis is present or not. The AUC value was closer to 1 for both PAs (Spermidine and Spermine).

According to the aforementioned findings, free radicals are implicated in cholelithiasis, and the elevated levels of PAs play a noteworthy part in the production of free radicals, which lead to an imbalance between cellular oxidants and antioxidants and, as a result, an increase in OS in cholelithiasis patients. It is advised to consider SPD or SPE as candidate biomarkers associated with this illness.

CONFLICT OF INTEREST

There is no conflict of interest disclosed by the authors in the publication of this manuscript.

AUTHOR CONTRIBUTIONS

Sahar Mohammod Eedan and Jwan Abdulmohsin Zainulabdeen equally contributed to the experimental design, data collection, editing, and finalization of the manuscript.

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