Sex hormones profiles in Iraqi males patients with symptomatic cholelithiasis before cholecystectomy

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Abstract: Gallstone disease (GD) is a worldwide disease and it remains to be one of the most common health problems leading to surgical intervention. GB stone formation is multifactorial, and many theories have been put forward to explain the mechanism of stone formation. It is not fully clear if symptomatic gallstone disease is associated with some hormonal abnormalities in the serum of male with symptomatic gallstone. The objective is to find the relationship between serum levels of sex hormones (free testosterone, SHBG, dihydrotestosterone (DHT), estradiol (E2), and progesterone) and gallstone formation in men. This cross-sectional study was carried on 50 males' patients and 37 healthy males as control group. After 12 hrs fasting, blood samples were collected from all subjects to evaluate the serum levels of these hormones (free testosterone, SHBG, dihydrotestosterone (DHT), estradiol, and progesterone). There was a significant decrease (P<0.05) in serum free testosterone & DHT. The study also showed that there was a significant decrease (P<0.05) in serum SHBG & estradiol (E2) of patients with symptomatic cholelithiasis compared to healthy controls. While there was no significant change (P>0.05) in serum progesterone between patients and controls groups. Aging, over weight and obesity, physical inactivity, smoking, cardiovascular diseases, DM and renal stones were all associated with cholelithiasisin men.

Conclusions: Elevation of SHBG & estradiol (E2) levels, lower free testosterone & DHT levels in the patient's serum compared to controls, which conclude that males patients with symptomatic gallstone disease was associated with some hormonal abnormality that may be the cause or the result of gallstone formation. The present study could be vital in achieving a better understanding of the pathogenesis of gallstones formation.

Keywords: Gallstone disease, sex hormones, males' patients.

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Abbreviations

I. Introduction
Cholelithiasis common with the incidence ranging from 10% to 20% of the world population, 11% of the general population of the US (1). In the US, gallstone disease has the most common inpatient diagnosis among gastrointestinal and liver diseases (2) and stands for $5.8 billion direct costs, exceeded only by gastroesophageal reflux disease (3). Gallstones (GS) are abnormal masses of a solid mixture of cholesterol crystals, mucin, calcium bilirubinate, and proteins that have affected people for centuries (4). Presence of stones in the gallbladder is referred to as cholelithiasis (from the Greek: chol-, "bile" + lith-, "stone" + iasis, "process") (5). GB stone formation is multifactorial, and known risk factors are advancing age, female gender, genetics/ethnicity, obesity, rapid weight loss, diet, and drugs (6). The association of GB stone disease with metabolic abnormalities such as diabetes, dyslipidemia, obesity, and hyperinsulinemia has supported the hypothesis that GB stone formation is a type of metabolic syndrome (7,8). Gallstone is common in southern Iraq because high calorie intaken food by population of this area (9). Clinically, the incidence of gallstone disease has been increasing coincident with the rise in calorie and fat consumption, decrease in fiber intake, and increased prevalence of the sedentary lifestyle in the Asian population (10). GB stone formation is more common in women than in men (11). Endogenous estrogen secretion may explain the observed gender differences in gallstone development (12). Estrogen in particular stimulated the HMG-Co-A reductase enzyme causing increased synthesis of cholesterol and thus putting women at an increased risk of supersaturation (13) Women have smaller total bile acid pools and increased

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biliary cholesterol content that predisposes them to super-saturation of cholesterol in the bile pool, resulting in gallstone formation (14). Progesterone may also contribute to gallstone disease by inhibiting gallbladder contraction and promoting hypomotility and gallbladder stasis (13). The effects of testosterone (T) or its active metabolite 5α dihydrotestosterone (DHT) on human gallbladder are not well studied. There is study investigates the effect of T and DHT on contraction in guinea pig gallbladder strips. It is found that T and DHT inhibits gallbladder motility rapidly by multiple pathways that include inhibition of intracellular Ca(2+) release, inhibition of extracellular Ca(2+) entry, and the actions of a Protein kinase C (PKC) may mediate this effect (15).

II. Materials And Methods

The study was carried out on patients with clinical and imaging features confirming symptomatic cholelithiasis admitted to the surgery unit in Baghdad, Medical City Teaching Hospital from November 2011 to June 2012. A total of 50 males patients were included in this study, their age ranged between 15-71 years and mean age ±SEM (44.14 ± 1.92). These were compared with 37 healthy males as control group, their age ranged between 23-72 years and mean age ±SEM (39.51 ± 1.89). The diagnosis of symptomatic gallstones depends on the presence of typical symptoms and the demonstration of stones on diagnostic imaging. An abdominal ultrasonography is the standard diagnostic test for gallstone detection (16).

Patient's exclusion criteria:
1. Female gender patients with gallstone disease
2. Patients with liver cirrhosis, viral hepatitis, renal failure and thyroid diseases.
3. Patients taking antiepileptic drugs, thyroxin, growth hormones, and sex hormones drugs that may interfere with the data obtained.

Subjects were asked to complete a questionnaire that asked for information on name, age, weight, height, occupation, smoking habits, alcohol consumption, any other diseases, drugs used, and duration of gallstone disease. Ultrasound for patients with cholelithiasis and nephrolithiasis were checked to confirm the presence of calculi. All the laboratory investigations were done in Medical City Teaching Hospital Laboratory, Biochemistry and Hormones Departments. After 12 hours fasting, venous blood samples, about 10 ml were collected from patients before laparoscopic cholecystectomy and from healthy volunteers in plain tubes. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 rpm for 15 min to obtain serum. The serum was stored and frozen at -20ºC until analysis was performed. The ELISA kits used for measurement the serum levels of these hormones (free testosterone, SHBG, DHT, estradiol, and progesterone). Accepted normal BMI (Kg/m²) range for men and women is from 18.5 to 24.9 kg/ m² (17). BMI gives evidence about overweight if its value varies from 25 to 29.9 kg/ m² , but obesity can be determined if BMI is greater than 30 kg/ m² (18). The statistical data analysis approaches were done through the Statistical Package of Social Sciences (SPSS) program (version-16) and Excel application. The results were expressed as mean ± standard error of mean (SEM). Student's t-test was used to examine the degree of significance. P values less than 0.05 was considered significant.

III. Results

Demographic presentation of 50 patients & 37 healthy controls was elucidated in table (1-1). Table (1-2) showed that body mass index (BMI) was significantly higher P<0.05 in patients (mean ±SEM 27.58±0.51) than controls (mean ±SEM 25.22±0.38), it also showed that the highest percentage of patients are obese while most of the controls are normal weight. In the present study the age category is divided into 6 subgroups, starting with (< 25yr), (25-34), (35-44yr), (45-54yr), (55-64 yr), ending with (≥ 65 yr) as shown in table (1-3). Table (1-4) shows the distribution of Androgens (free testosterone (FT), dihydrotestosterone (DHT), sex hormone binding globulin (SHBG) levels in the serum of symptomatic gallstone patients in comparison with healthy control group. The present study shows a significant decrease (P<0.05) in the levels of free testosterone (FT) & dihydrotestosterone (DHT), while there is a significant increase (P<0.05) in the level of sex hormone binding globulin(SHBG) in the serum of symptomatic gallstone patients in comparison with healthy control group. Table (1-5) shows the distribution of estradiol and progesterone levels in the serum of symptomatic gallstone patients in comparison with healthy control group. The present study shows a significant increase (P<0.05) in the level of estradiol (E2), While there in no significant change (P>0.05) in the level of progesterone in the serum of symptomatic gallstone patients in comparison with healthy control group.
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Table (1-1): Demographic data of patients & controls.

<table>
<thead>
<tr>
<th>Characters</th>
<th>S.G.S Patients N=50 (57%)</th>
<th>Controls N=37 (43%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smoker</td>
<td>Nonsmoker</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>21 (42%)</td>
<td>29 (58%)</td>
</tr>
<tr>
<td></td>
<td>0 (0%)</td>
<td>37 (100%)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>49 (98%)</td>
<td>37 (100%)</td>
</tr>
<tr>
<td>Occupation</td>
<td>Office worker</td>
<td>Retired</td>
</tr>
<tr>
<td></td>
<td>38 (76%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td></td>
<td>4 (8%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>2 (4%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td></td>
<td>3 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Other diseases</td>
<td>Cardiovascular diseases</td>
<td>Kidney stone</td>
</tr>
<tr>
<td></td>
<td>11 (22%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td></td>
<td>4 (8%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td></td>
<td>Cancer of pancreas</td>
<td>Duodenal ulcer (DU)</td>
</tr>
<tr>
<td></td>
<td>11 (22%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Table (1-2): Percentage of body mass index in patients with symptomatic gallstone & controls.

<table>
<thead>
<tr>
<th>BMI Group</th>
<th>S.G.S Patients N=50 (57%)</th>
<th>Control N=37 (43%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>13 (26%)</td>
<td>23 (62.16%)</td>
<td></td>
</tr>
<tr>
<td>(18.5-24.9)Kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over weight</td>
<td>17 (34%)</td>
<td>13 (35.14%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(25-29.9)Kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese &gt;30 Kg/m²</td>
<td>20 (40%)</td>
<td>1 (2.70%)</td>
<td></td>
</tr>
</tbody>
</table>

Total mean ±SEM(Kg/M²) 27.58±0.51 25.22±0.38

N=number of patients or controls.

Table (1-3): Age sub groups of SGS-patients and controls.

<table>
<thead>
<tr>
<th>Age/year</th>
<th>S.G.S-Patients (N=50) (57%)</th>
<th>Control (N=37) (43%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>4 (8%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td>25-34</td>
<td>9 (18%)</td>
<td>11 (30%)</td>
</tr>
<tr>
<td>35-44</td>
<td>11 (22%)</td>
<td>12 (32%)</td>
</tr>
<tr>
<td>45-54</td>
<td>17 (34%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td>55-64</td>
<td>4 (8%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>≥65</td>
<td>5 (10%)</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>

Mean ±SEM 44.14 ±1.92 39.51 ±1.89

N=number of patients or controls.
The present study shows that (42%) of males with symptomatic gallstone disease was smoker and (58%) were nonsmoker, while all of the healthy controls were nonsmoker as stated in table (1-1). In concordance with the findings of previous studies, a positive association of smoking with gallstone disease is seen in present study. Naseem Aslam Channa, et al. (19)," who reported a moderate increase in the incidence of gallstones among cigarette smokers as compared with nonsmokers. Biological mechanisms are unclear even if cigarette smoking may increase a predisposition to gallstones. Lower concentrations of plasma high density lipoprotein cholesterol associated with cigarette smoking may be relevant to the positive association between smoking and gallstones”. However, a study from Germany did not show any positive correlation between history of smoking and presence of gallstones (20). The present study shows that only (2%) of males with symptomatic gallstone disease were drinker and (98%) were nondrinker, while all of the healthy controls were nondrinker as stated in table (1-1). TimoSahi, et al. (21),"who did not observe any relation between alcohol consumption and risk of gallbladder disease”.As stated in table (1-1),the present study also shows (76%) of the patients were office worker,(6%) retired, (8%) non worker, (4%) student, & (6%)military. While (92%) of healthy controls were office worker, (3%) retired, & (5%) student. Epidemiologic evidence suggests that increased physical activity is associated inversely with the risk of gallstone formation (22). Physical activity may play an important role in the prevention of symptomatic gallstone disease in men; it was observed that symptomatic gallstone disease in men could be prevented by increasing exercise to 30 minutes of endurance type training for five times per week. Regular exercise, in addition to facilitating weight control, alone or in combination with dieting, improves several metabolic abnormalities related to both obesity and cholesterol gallstones (23). The present study finds that (22%) of patients had cardiovascular diseases while all of the healthy controls had not any cardiovascular diseases. In agreement with Field AE, et al.(24)“ who found a link between GD and cardiovascular diseases and that was demonstrated in a large prospective study where overweight and obesity were the common risk factors”. The main pathologic effect involved insulin resistance associated with central adiposity: both factors are clearly associated with GD and cardiovascular diseases. Excess cholesterol is one of the main characteristics shared between cardiovascular diseases and GD. In the first case, cholesterol is deposited in the arterial wall, in the second case it precipitates in the gallbladder(25). The present study finds that (18%) of patients and only (3%) of controls had renal stones as shown in table (1-1). In agreement with Sakhaee K, et al. (26)&Tsai CJ, et al.(27) "they said the mechanisms underlying the association between gallstone disease and kidney stones are unknown. Insulin resistance is associated with an increased risk of gallstones and kidney stones”. Indeed, obesity and the metabolic syndrome(28,29), lower intakes of many factors such as fruit, vegetables, magnesium, coffee and alcohol (30,31,32) have been established as risk factors for kidney stone and gallstone.

**Table (1-4): Distribution of Androgens (free testosterone (FT), dihydrotestosterone (DHT), and sex hormone binding globulin (SHBG) levels in SGS-patients and controls.**

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>S.G.S Patients N=50</th>
<th>Controls N=37</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free testosterone (FT) (pg/ml)</td>
<td>6.066± 0.679</td>
<td>9.1729± 1.085</td>
<td>P=0.013</td>
</tr>
<tr>
<td>Serum dihydrotestosterone (DHT) (pg/ml)</td>
<td>399.26± 27.51</td>
<td>626.53± 47.78</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Serum sex hormone binding globulin (SHBG) (nmol/L)</td>
<td>58.205±5.667</td>
<td>31.036±4.014</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

-Data are expressed as mean ±SEM-N=number of patients or controls

**Table (1-5): Distribution of estradiol and progesterone levels in SGS-patients and controls.**

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>S.G.S Patients N=50</th>
<th>Controls N=37</th>
<th>T-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum estradiol (E2) (pg/ml)</td>
<td>20.581±1.23</td>
<td>12.096±0.99</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Serum progesterone (ng/ml)</td>
<td>0.656±0.078</td>
<td>0.501±0.045</td>
<td>P=0.121</td>
</tr>
</tbody>
</table>

-Data are expressed as mean ±SEM-N=number of patients or controls

**IV. Discussion**

The present study suggests that (42%) of males with symptomatic gallstone disease was smoker and (58%) were nonsmoker, while all of the healthy controls were nonsmoker as stated in table (1-1). In concordance with the findings of previous studies, a positive association of smoking with gallstone disease is seen in present study. Naseem Aslam Channa, et al. (19)," who reported a moderate increase in the incidence of gallstones among cigarette smokers as compared with nonsmokers. Biological mechanisms are unclear even if cigarette smoking may increase a predisposition to gallstones. Lower concentrations of plasma high density lipoprotein cholesterol associated with cigarette smoking may be relevant to the positive association between smoking and gallstones”.

Indeed, obesity and the metabolic syndrome(28,29), lower intakes of many factors such as fruit, vegetables, magnesium, coffee and alcohol (30,31,32) have been established as risk factors for kidney stone and gallstone.
formulation (28,29). Factors affecting the intestinal handling of bile acids and oxalate may account for the associations between gallstones and kidney stones(27, 28). As stated in table (1-1), the present study also shows that (8%) of patient had diabetes mellitus while all of the healthy controls had not diabetes mellitus. In concordance with the findings of Neil Bajwa et al. (13) who said that diabetes mellitus seem to facilitate the development of gallstone formation secondary to increased triglyceride levels associated obesity as well as promoting gallbladder hypomotility and stasis”. The present study also shows that (4%) of patient had thalassemia while all of the healthy controls had not thalassemia. Swee Lay Thein, et al. (35) who said that hyperbilirubinemia and a propensity to gallstone formation is a common complication of thalassemia and is attributed to the rapid turn-over of the red blood cells, and bilirubin being a break-down product of hemoglobin”. The present study shows that (2%) of patient with pancreatic cancer while all of the healthy controls had not pancreatic cancer. Sun Yan, et al. (36) who reported a correlation between pancreatic cancer and gallstone, which might be due to infection in biliary tract, chronic pancreatitis, hormone, diet and obesity etc. Pancreatic cancer is more likely to occur in aged patients with gallstone”. As stated in table (1-1), this study also shows that (2%) of patient had duodenal ulcer (DU) while all of the healthy controls had not duodenal ulcer (DU). Davide Festi, et al. (37) “who reported peptic ulcer as a risk factor for gallstone disease in males”. Cabrol J, et al. (38) “who conclude that gallstone disease is not always accompanied by an increased duodenogastric reflux”. The results obtained from this study may agree with other study which considers obesity as an important risk factor for cholelithiasis in man (39). Most of the symptomatic gallstone patients were overweight or obese; table (1-2) reveals that (40%) of patients were obese, (34%) were overweight and only (26%) were normal weight, while control group had mostly normal weight with (62.16%), the percentage was (35.14%) for overweight and only (2.7%) for obesity in controls. Gallstone patients had significantly higher BMI (27.58±0.51 kg/m²) than control (25.22±0.38 kg/m²). Obesity is recognized as a major gallstone risk factor; it has been associated with gallbladder dysmotility (40) and increased biliary secretion of cholesterol from the liver, producing cholesterol-supersaturated bile, which lead to gallstone formation (41). Table (1-3) shows that the age is divided into 6 subgroups: (<25), (25-34), (35-44), (45-54), (55-64) and (65-). The most age group affected were between (45-54y), it form 34%, it nearly similar to previous studies have done in Iraqi in which the peak age group of gallstones affected were the fifties years. In U.S.A most ages affected between (50-65y). This result could be due to a linear relationship between increasing age and prevalence of cholelithiasis. In elderly men increased incidence of gallstone disease due to alteration in the ratio of androgen and estrogen (9). From a biochemical standpoint, age itself may increase cholesterol saturation of bile with enhanced hepatic secretion of cholesterol to secondary to increased levels of HMG co-A reductase, decreased synthesis of bile acids may occur secondarily to decreased cholesterol 7 α-hydroxylase enzyme activity, as age advances (42). Table (1-4) shows that the mean of serum free testosterone (FT) was (6.06±0.679) for patients and (9.1729±1.085) for controls, the level of serum free testosterone in the patients was significantly lower (P<0.05) compared to healthy volunteers, the table also shows that the mean of serum dihydrotestosterone (DHT) (399.26±27.51) of SGS-patients was significantly lower (P<0.05), than that of controls (626.53±47.78). The data support other studies (43, 44) which suggested that the level of testosteronine male patients with gallstones were significantly decreased. J. Ahlberg, et al. (45) "who reported that decreased 5α-reductase activity in the gallstone patients”. The conversion of testosterone to the more potent androgen dihydrotestosterone(DHT) is catalyzed by 5α-reductase (46). Inhibition of 5α-reductase activity is lead to the suppression of serum dihydrotestosterone(DHT) levels(47). Thus the present study may suggest that the levels of dihydrotestosterone(DHT) decrease in male patients with gallstones as a result of decreased 5α-reductase activity. As a result low androgen levels may promote stone formation, and there is imbalance in the level of male hormones in the patients with cholesterol gallstones, so androgens play a protective and preventive role in the occurrence of cholesterol gallstones (48). It was suggested that the synthesis or metabolism of pituitary-gonad axis hormones in male patients with cholelithiasis was significantly abnormal (44). The table (1-4) also shows that the mean of serum sex hormone binding globulin (SHBG)(58.20要不要±5.667) of SGS-patients was significantly higher (P<0.05), than that of controls (31.036±4.014). The possible explanations for the elevation of serum sex hormone binding globulin (SHBG) in males patients with symptomatic gallstone disease may be due to:the decline in the free testosterone is amplified by a concomitant increase in sex hormone-binding globulin (SHBG) levels (49). As this study discussed above there is decline in the free testosterone levels in males patients and this should be accompanied by increasing levels of sex hormone-binding globulin (SHBG) in male's patients as compared to the controls. Advancing age and cigarette smoking suggested to increase sex hormone-binding globulin (SHBG) levels (50). At the study showed above that increasing age associated with increase in number of symptomatic gallstone-patients and (42%) of males with symptomatic gallstone disease were smoker. According to these results levels of sex hormone-binding globulin (SHBG) in males' patients should be increased. Higher levels of estradiol (E2) were associated with higher levels of sex hormone-binding globulin (SHBG) in men (51). As the study will show below these levels of estradiol (E2) in patients with symptomatic gallstone disease were higher as compared to the healthy controls. Thus the levels of sex hormone-binding globulin (SHBG) in
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patients should be increased. Table (1-5) shows that the mean of serum estadiol (E2) was (20.58±1.23) for patients and (12.096±0.99) for controls, the level of serum estadiol (E2) in the patients was significantly higher (P<0.05) compared to healthy volunteers. In agreement with the June Li, et al. study (43) "who found that estadiol (E2) levels of males with gallstone were higher than healthy people, indicating that patients with high estrogen levels are susceptible to gallstone formation". The liver has estrogen receptors, and the presence of endogenous estrogens causes cholesterol saturation in the bile, inhibition of chenodeoxycholic acid secretion, and an increase in cholic acid level (52). Additionally, high levels of estrogen could impair gallbladder motility function and consequently induce gallbladder hypomotility (53). Table (1-5) also shows that there is no significant change (P>0.05) in the level of progesterone in the serum of symptomatic gallstone patients (0.65±0.078) in comparison with healthy control group (0.50±0.045). The present study is in agreement with a study performed by Russo F, et al. (54) "who showed that there is no significant differences between males with gallstone and controls". The present study disagree with a study performed by Rui Jing, et al. (44) "who showed that progesterone of the male patients with gallstones were higher as compared with the healthy people". The present study is disagree with the Cohen G, et al. study (55) " who showed that there is no significant difference in the plasma concentrations and the urinary excretion rate of sex hormones (testosterone, dihydrotestosterone, and estradiol), as well as in the plasma sex hormone binding globulin in male patients with asymptomatic gallstone disease as compared with the healthy group".

V. Conclusion

In conclusion, elevation of SHBG & estradiol (E2) levels, lower free testosterone & DHT levels in the patient’s serum compared to controls, which conclude that male patients with symptomatic gallstone disease were associated with some hormonal abnormality that may be the cause or the result of gallstone formation. Physical inactivity, smoking, cardiovascular diseases, DM and renal stones were all associated with cholelithiasis in men. The present study could be vital in achieving a better understanding of the pathogenesis of gallstones formation.

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References

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