

## Glycosylation Gap in Gallstone Patients

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### ABSTRACT

**Background:** Gall stones are one of the commonest biliary diseases. Interest in the formation and clinical management of the disease dates back to ancient times. Gall stones continue to be one of the major health problems in the world today, although the exact number of patients is unknown, because there are no signs and symptoms related to the disease when there are no complications. The majority of the patients are diagnosed incidentally. However, some patients come with acute complication of gall stones. Gall stones are classified into; cholesterol gall stones, pigment gall stones, and mixed gall stones. However, all stones even pure cholesterol gall stones usually contain bilirubin (conjugated and unconjugated bilirubin) and cholesterol.

**Aim:** To determine the changes in glycosylation gap and glycation profile and the relation of this change to the changes in age and BMI on the measured parameters.

**Patients and methods:** Serum samples were collected from 40 individuals included in this work. They are divided into 2 groups. The first group included 20 apparently healthy individuals (10 males and 10 females). The second group included 20 individuals (5 males and 15 females) with Gall stone diagnosed in out-patient Clinic at Al-Jamhoory General Hospital and Neinawa private Hospital. Gall stone patients with metabolic diseases, infections and other inflammatory diseases were excluded.

**Results:** The study demonstrated a significant elevation in serum glucose, mean blood glucose, serum fructosamin, measured and predicated HbAc1 in GS patients in comparison with controls. Regarding the relation between age and measured parameters, the study showed a non-significant correlation between age and measured parameters in the control group, while in GS group there were a significant correlation ( $r = 0.05$ ) between ages and predicted HbAc1 and BMI ( $p \leq 0.03$ ). Regarding the relation between BMI and measured parameters, the study showed a significant correlation between measured parameters and BMI in GS individuals, while in control group only serum glucose and MBG in control group ( $r = 0.01$ ) ( $p \leq 0.01$ ) with MBI.

**Conclusion:** The study shows that there is a significant change in glycosylation gap and glycation profile in gall stone patients.

**Keywords:** Glycosylation, gall stones, HbAc1, Fructosamin.

### فجوة الكليكوسيلشن عند مرضى حصى المرارة

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### الخلاصة

**المقدمة:** تعتبر حصاة المرارة من أهم الأمراض التي تصيب الجهاز الصفراوي ويعود الإهتمام بكيفية تكوينها وعلاجها الى فترة ليست بالقصيرة، ولا تزال حصاة المرارة واحدة من المشاكل الصحية الكبرى في العالم اليوم، على الرغم من أن العدد الدقيق للمرضى غير معروف، لأنه لا توجد علامات وأعراض متعلقة بهذا المرض عندما لا تكون هناك أي مضاعفات. ويتم تشخيص الغالبية العظمى من المرضى بالصدفة. ومع ذلك يعاني بعض المرضى مضاعفات حادة من حصاة المرارة. تصنف حصاة المرارة

إلى: حصى متكونة من الكولسترول، حصى متكونة من الأصباغ ، وحصى متكونة من خليط من الإثنين. ومع ذلك، كل أنواع الحصى حتى التي تتكون من الكولسترول عادة ما تحتوي البيليروبين (المتحد والغير متحد مع البيليروبين).  
**الهدف من الدراسة:** هو تحديد التغيرات في فجوة الكليكوسيلشن والكلايكيشن وعلاقة هذه التغيرات مع العمر ومعامل كتلة الجسم.  
**المواد والطرق:** تم جمع عينات من مصل الدم من 40 شخصا من الأفراد الذين شملتهم الدراسة والذين تم تقسيمهم الى مجموعتين. وضمت المجموعة الأولى 20 فردا أصحاء (10 ذكور و 10 إناث). وضمت المجموعة الثانية 20 شخصا (5 ذكور و 15 إناث) مصابين بحصى المرارة التي تم تشخيصها في العيادة الخارجية في مستشفى الجمهوري التعليمي ومستشفى نينوى الأهلي. تم استبعاد مرضى حصى المرارة الذين يعانون من أمراض الأيض، أمراض معدية وغيرها من الأمراض الإلتهابية.  
**النتائج:** أظهرت الدراسة إرتفاع كبير في نسبة الكلوكوز في مصل الدم، معدل السكر في الدم، وفركتوز امين مصل الدم ونسب المقاسة عند المرضى مقارنة مع مجموعة السيطرة. وفيما يتعلق بالعلاقة بين العمر والمتغيرات التي تم قياسها، أظهرت الدراسة إرتباط غير معنوي بين العمر والمتغيرات التي تم قياسها ضمن مجموعة السيطرة بينما في مجموعة مرضى حصى المرارة أظهرت الدراسة إرتباط كبير بين العمر والمقاس ومعامل كتلة الجسم. أما بالنسبة للعلاقة بين معامل كتلة الجسم والمتغيرات التي تم قياسها، أظهرت الدراسة تغيير كبير بين المتغيرات المقاسة ومعامل كتلة الجسم بين مرضى حصى المرارة، بينما في مجموعة السيطرة فقط معدل الكلوكوز في مصل الدم ومعدل السكر في الدم أظهر تغيرا معنويا مع معامل كتلة الجسم ( $P=0.01$ ).  
**الإستنتاج:** تبين الدراسة ان هناك تغييرا كبيرا في فجوة الكليكوسيلشن والكلايكيشن بين مرضى حصى المرارة.  
**الكلمات المفتاحية:** الكليكوسيلشن، حصى المرارة، هيموغلوبين أي ون سي، فركتوز أمين.

## INTRODUCTION

**G**all stones are one of the commonest biliary diseases. Interest in the formation and clinical management of the disease dates back to ancient times. Gall stones continue to be one of the major health problems in the world today, although the exact number of patients is unknown, because there are no signs and symptoms related to the disease when there are no complications. The majority of the patients are diagnosed incidentally<sup>1</sup>. However, some patients come with acute complication of gall stones<sup>2</sup>.

Gall stones are classified into; cholesterol gall stones, pigment gall stones, and mixed gall stones. However, all stones even pure cholesterol gall stones usually contain bilirubin (conjugated and un conjugated bilirubin) and cholesterol<sup>2</sup>.

The prevalence of gall stones continues to rise with age, and it is higher in women than men. This may be due to the increase of cholesterol content in the bile by the effect of estrogen<sup>2</sup>. Many hypotheses explain the reasons that can lead to gall stones, most of them may have a relation to lipid metabolism<sup>3,4</sup>.

Glycosylation is a non-enzymatic process in which proteins react with reducing sugar molecules causing impair of their function and change of their characteristics<sup>5</sup>. Glycosylation usually occurs in diabetes, aging and many other diseases where accumulation of glycosylation end products involve

in pathogenesis of these diseases and their complications<sup>6</sup>.

Glycated HbA1c result from non-enzymatic concentration dependent covalent bonding of glucose to Hb within the erythrocytes, thereby HbA1c is a good indicator for glycemic control over long period 2-3 month and also its good indicator for intracellular glycation process<sup>7-10</sup>.

The extracellular glycation process can also be evaluated using serum fructosamin, a glycated end product of serum protein<sup>7</sup>. Fructosamin is a measure of average glycemic control over a shorter period 2-3 week and its plasma concentration is much more stable than that of glucose itself and much more easier to measure than true mean blood glucose (MBG), which requires continuous blood glucose monitoring<sup>8-11</sup>.

Glycosylation gap (GG) defined as the value that results from subtraction of direct measured HbA1c value minus the predicted HbA1c value obtained from equation using another indicator of glycemic control, fructosamin (FA)<sup>7</sup>.

GG is use as clinical research tool for evaluating physiological sources of variation that affects glycemic control and it can also be used as a tool to define the direction of glycosylation process<sup>6-13</sup>.

The aim of this study is to determine the changes in glycosylation gap and glycation profile and the relation of this change to the changes in age and BMI on the measured parameters.

## PATIENTS AND METHODS

This study was conducted during the period from October 2011 to January 2013 in the department of clinical pharmacy - college of pharmacy in cooperation with the department of surgery-college of medicine; under approval of scientific and ethics committee in Ninawa health office. Serum samples were collected from 40 individuals whose included in this work they are divided into 2 groups.

The first group included 20 apparently healthy individuals (10 males and 10 females). The second group included 20 individuals (5 males and 15 females) with Gall stone whose diagnosed in out-patient Clinic at Al-Jamhoory General Hospital and Ninawa private Hospital. Gall stone patients with metabolic diseases, infections and other inflammatory diseases were excluded.

Serum fasting glucose was assayed by glucose oxidase/peroxides colorimetric method<sup>14</sup>, FA by NBT-spectrophotometric method<sup>15</sup> and HbAc1% measured by Chromatographic-spectrophotometric method<sup>16</sup>, While mean blood glucose (MBG) predicted HbAc1 and Glycosylation gap were calculated using equations<sup>13,17</sup>.

$$\text{MBG} = 1.76 \times (\text{HbAc1}) - 3.67 \text{ mmol/L}$$

$$\text{P-HbAc1} = 0.017 \times \text{FA} + 1.61$$

$$\text{GG} = \text{M-HbAc1} - \text{P-HbAc1}$$

Data are presented as mean  $\pm$ SD, 2-sample t-test was used to compare between measured parameters in test group and control group. The relationship between age or BMI and the measured parameters were determined by Pearson correlation.

## RESULTS

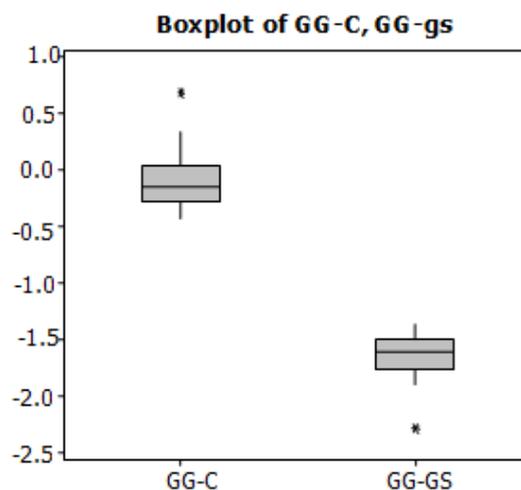
The study demonstrated a significant elevation in serum glucose, mean blood glucose, serum fructosamin, measured and predicated HbAc1 in GS patients in comparison with controls **Table 1**.

The glycosylation gap shows a significant increase in GS patients when compared to controls.

**Table 1:**

| Parameter             | Control           | Gallstone patients |
|-----------------------|-------------------|--------------------|
| Serum fasting glucose | 4.40 $\pm$ 0.91   | 5.7 $\pm$ 0.2*     |
| Serum fructosamin     | 237 $\pm$ 11.01   | 423 $\pm$ 33.8*    |
| Measured HbAc1        | 5.58 $\pm$ 0.33   | 7.11 $\pm$ 0.48*   |
| Predicted HbAc1       | 5.59 $\pm$ 0.3    | 8.71 $\pm$ 0.42*   |
| Mean blood glucose    | 6.32 $\pm$ 0.51   | 8.70 $\pm$ 0.51*   |
| Glycosylation Gap     | -0.02 $\pm$ 0.332 | -1.74 $\pm$ 0.244* |

\*= P<0.05



**Figure 1.** Glycosylation gap in gallstone and controls.

Regarding the relation between age and measured parameters, the study showed non-significant correlation between age and measured parameters in the control group, while in GS group there were a significant correlation ( $r = 0.05$ ) between ages and predicted HbAc1 and BMI ( $p \leq 0.03$ ).

Regarding the relation between BMI and measured parameters, the study showed a significant correlation between measured parameters and BMI in GS individuals, while in control group only serum glucose and MBG in control group ( $r = 0.01$ ) ( $p \leq 0.01$ ) with MBI.

## DISCUSSION

Glycosylation gap is defined as the difference between the measured HbAc1 and HbAc1 predicted from glucose serum protein based on the HbAc1 from regression equation

GG= HbAc1 measured-HbAc1 predicted.  
Glycation has both physiological and pathophysiological significance<sup>18</sup>.

Gallstone disease is one of the most prevalent gastrointestinal Diseases with a substantial burden to health. Etiology and pathogenesis of cholesterol gallstones are still not well defined, and strategies for prevention and efficient non-surgical therapies are missing<sup>19</sup>.

Based on the obtained results, it can be clearly stated that the glycosylation gap has elevated level in GS patients when compared to control group; furthermore, it demonstrated a significant correlation ( $r=0.05$ ) between age, predicted HbAc1 and BMI ( $p\leq 0.03$ ) among GS group<sup>20</sup>.

Thus we can hypothesize that significance correlation between predicted HbAc1 and BMI in GS may contribute to the increase in GG. This is in accordance with AL-kataan who described a significance correlation between MBG, serum fructoseamine, and GG with BMI in obese individuals<sup>1</sup>. as well as it may be associated with relation to an increase in insulin resistance that in turn associated with intravascular hyperglycemia and hypertriglyceridemia that associated with obesity and agrees with the results described by Ruderman *et al.*, Resnick *et al* and Boden *et al.*<sup>21-23</sup>

The study showed a significant difference in mean blood glucose between GG patients and control group which is in accordance with AL-Kataan *et al* who demonstrated that there is a significance increase in fasting glucose ( $P < 0.01$ ) in GS patient prior and posterior of Cholecystectomy<sup>24</sup>.

The study also revealed that the relation between age, BMI and measured parameters shows non- significance correlation between age and measured parameter in control group.

## CONCLUSION

The study shows that there is a significant change in glycosylation gap and glycation profile in gall stone patients.

## RECOMMENDATION

The researchers recommend further work on glycation end products, ROS formation and nitro-reductases activity to define the relation between GSD and glycemic control in this group of patients before and after treatment.

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