



## **The correlation between Serum Glucose levels and Glycosylated HemoglobinA1c in experimentally induced diabetes mellitus.**

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### **Abstract**

The aim of this study was to evaluate the level of Glycosylated HbA1c as diagnostic test and indicator for the severity of diabetes mellitus. Thirty six rabbits purchased from the local market, weighing 1.2-2 kg were used in the experiment. The animals were divided according to the gender into two groups and each was equally divided into diabetic and control group.

The results revealed highly significant difference in glycosylated HbA1c concentrations between diabetic groups and control groups, and the glycosylated Hb1c concentrations gradually increased after onset of diabetes, also the result revealed that values in diabetic males increased more than in than diabetic females ( $P < 0.05$ ) as well as the result revealed that the concentration of glycosylated Hb1c were increased with the increased serum glucose concentrations. The conclusion that a high HbA1c represents poor glucose control and higher levels of HbA1c are found in experimental animals with persistently elevated blood sugar, as in diabetes mellitus.

### **Introduction:-**

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia among other organs (WHO, 1999). Diabetes mellitus is a syndrome initially characterized by a loss of glucose homeostasis. The disease is progressive and associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness (Bartosikova et al., 2003). It is defined clinically as either: A fasting plasma glucose level greater than 7.8 mmol/L (140 mg/dl) or 2 hour post-prandial plasma glucose greater than 11mmol/L (200 mg/dl) (MacSween and Whaley, 1992). There are three main types of diabetes mellitus: type -1 {also known as insulin-dependent diabetes mellitus (IDDM)}, type -2 {formerly called non-insulin-dependent diabetes (NIDDM)} and gestational diabetes (Edgren, 2003).

Glycoslyated hemoglobin is formed by the non- enzymatic reaction between glucose and the amino groups of hemoglobin (Holmquist and Schroeder, 1966).The most reactive residue is the terminal amino group of the  $\beta$ -chain of hemoglobin. It has been shown that subjects with sickle cell haemoglobinopathy have decreased level of glycated hemoglobin (Abraham et al., 1980); also studies of individuals with other types of hemolytic anemia have indicated that the levels of glycation are significantly reduced during hemolytic crises (Penzer et al., 1982 and Baule et al., 1983). The etiology of anemia in type 1- diabetes is divers. Diabetic patients who through neglect or ignorance, do not follow the appropriate dietary regimes, are at-risk of



developing nutritional deficiency anemia, especially iron and folate deficiency. More over, diabetics with poor glycaemic control are susceptible to recurrent attacks of ketoacidosis which may be accompanied by anorexia, severe vomiting with frequent hospitalization and excessive caloric loss (Dikow, 2002). The occurrence of diabetic nephropathy with ultimate renal failure is important cause of anemia in these patients (Bosman, 2002). The association of type 1 diabetes and coeliac disease has been widely reported, the latter being associated with iron, folic acid and vitamin B12 deficiency, Hashimoto's thyroiditis, resulting in acquired hypothyroidism, is strongly associated with type 1 diabetes and is commonly accompanied by anemia (Lorini, 1996). Thalassaemia minor is relatively common in the Mediterranean area and should be considered in the differential diagnosis of anemia (Schwartz, 2000).

### **Materials and Methods:-**

Thirty six rabbits purchased from the local market, weighing 1.5-2 kg were used in the experiment. Animals were housed in cages with dimension (130×100×70) under 12/12 h light/dark cycle at 25±2 °C & 60% relative humidity with standard granulated food, & water available ad libitum. The animals were divided according to the gender into two equal groups:

1. Male group: includes (18) rabbits, which are subdivided into two groups:
  - A. Diabetic group denoted by (Dm) includes (9) rabbits.
  - B. Control groups denoted by (Cm) includes (9) rabbits.
2. Female group: includes (18) rabbits, which are subdivided into two groups:
  - A. Diabetic group denoted by (Df) includes (9) rabbits.
  - B. Control group denoted by (Cf) includes (9) rabbits.

Animals were left (1) month for adaptation. The animals were given Clopidol 0.06 mg/kg with feed as a prophylaxis drug against coccidiosis during adaptation period.

#### **Weighing of Animals:-**

Animals were weighed immediately after buying them and weighed again before the beginning of the experiment. Afterwards they were weighed weekly.

#### **Blood Collection:-**

The blood was collected according to the following equation: Total blood volume (TBV) = 6% of lean body weight. Maximum blood collection = 20% of total blood volume every two weeks.

Animal weight in kg × 0.06 × 0.02 × 1000 = ( ) ml. (McGuill and Roman, 1989).

The blood collected at the following periods: Zero day (before the injection of alloxan), three days, ten days, twenty days, thirty days and forty days after injection of alloxan.

#### **Site of Blood Collection:-**

The blood was collected from marginal ear vein with empty stomach. Some of the collected blood was used for estimation glycosylated Hb1c and



the rest was centrifugated to obtain the serum. The serum used immediately for checking Fasting serum glucose (FSG the blood collected in test tubes contains sodium fluoride (Naf) (Christopher and Neill, 2000).

### **Induction of Diabetes Mellitus:-**

Diabetes mellitus was induced in over night fasting rabbits by a single injection of alloxan (alloxan monohydrate) at dose 100 mg /kg into marginal ear vein .Each 100 mg of alloxan was diluted in 1 ml of 0.9% normal slain. (Lukens, 1948).

Immediately, after alloxan injection, 10 ml of 20% glucose I.V & 5 ml of 20% glucose I.P was given to the rabbits in order to overcome sudden decrease in blood glucose level (hypoglycemia).The rabbits were prevented from feeding for 12 h and the drenching water replaced by 5% glucose for 24h.The procedures of administrations and blood collection made under sedation of animals by using kitamin 44 mg /kg and xylazine 5 mg/kg. The control groups were I.V injected with 1 ml of 0.9 % of normal slain (EFPIA & ECVAM, 2000).

### **Laboratory Examination:-**

#### **Estimation of Fasting Serum Glucose:-**

After 3 days of alloxan injection the animals were fasting overnight and bled for checking the hyperglycemia .Fasting serum blood glucose (FSG) was measured by using special kit prepared by (SPINREACT, S.A.Ctra.Santa Coloma, 7E-17176SANT ESTEVE DE BAS (GI) SPIN), then the (FSG) concentrations were checked every 10 days .

#### **Estimation of Glycated Hemoglobin:-**

Glycoslyated hemoglobin were estimated every 10 days intervals from the beginning of the experiment by using Column chromatography method (Mallia etal., 1981).

### **Results**

#### **1. Fasting Serum Glucose Concentrations (FSGCs):**

On day zero, the results revealed no significant differences among all groups (Dm, Df, Cm and Cf). On day ten after alloxan injection there were significant differences between diabetic and control groups ( $P<0.05$ ). On day twenty the results revealed significant differences between diabetic (the FSG concentrations in diabetic group were increased beyond the normal levels) and control groups ( $P<0.05$ ). On day thirty, the differences between diabetic males and diabetic females were statically significant ( $P<0.05$ ) and there were statistical differences between diabetic groups and control groups ( $P<0.05$ ). On



day forty there were statistically significant differences between diabetic and control groups ( $P < 0.05$ ).

A significant difference between the genders was revealed ( $P < 0.05$ ). The results also revealed that the difference between days of the experiments is highly significant at ( $P < 0.05$ ). The fasting serum glucose concentrations are presented in chart (1).

## 2. The Hb<sub>A1c</sub> Rate:-

On day zero, the Hb rate had an average value of  $167.33 \pm 12.31$  in males and  $175.38 \pm 17.61$  in females. On day ten after alloxan injection the Hb values had increased to  $340.27 \pm 28.89$  in males and  $207.83 \pm 42.00$  in females. On day twenty the values in males were  $346.10 \pm 11.83$  while  $238.55 \pm 15.60$  in females. On day thirty the Hb values in males were  $382.33 \pm 12.56$  while  $323.70 \pm 19.66$  in females. The Hb values had been reached its peak levels in males  $705.73 \pm 21.3$  and  $404.48 \pm 29.90$  in females.

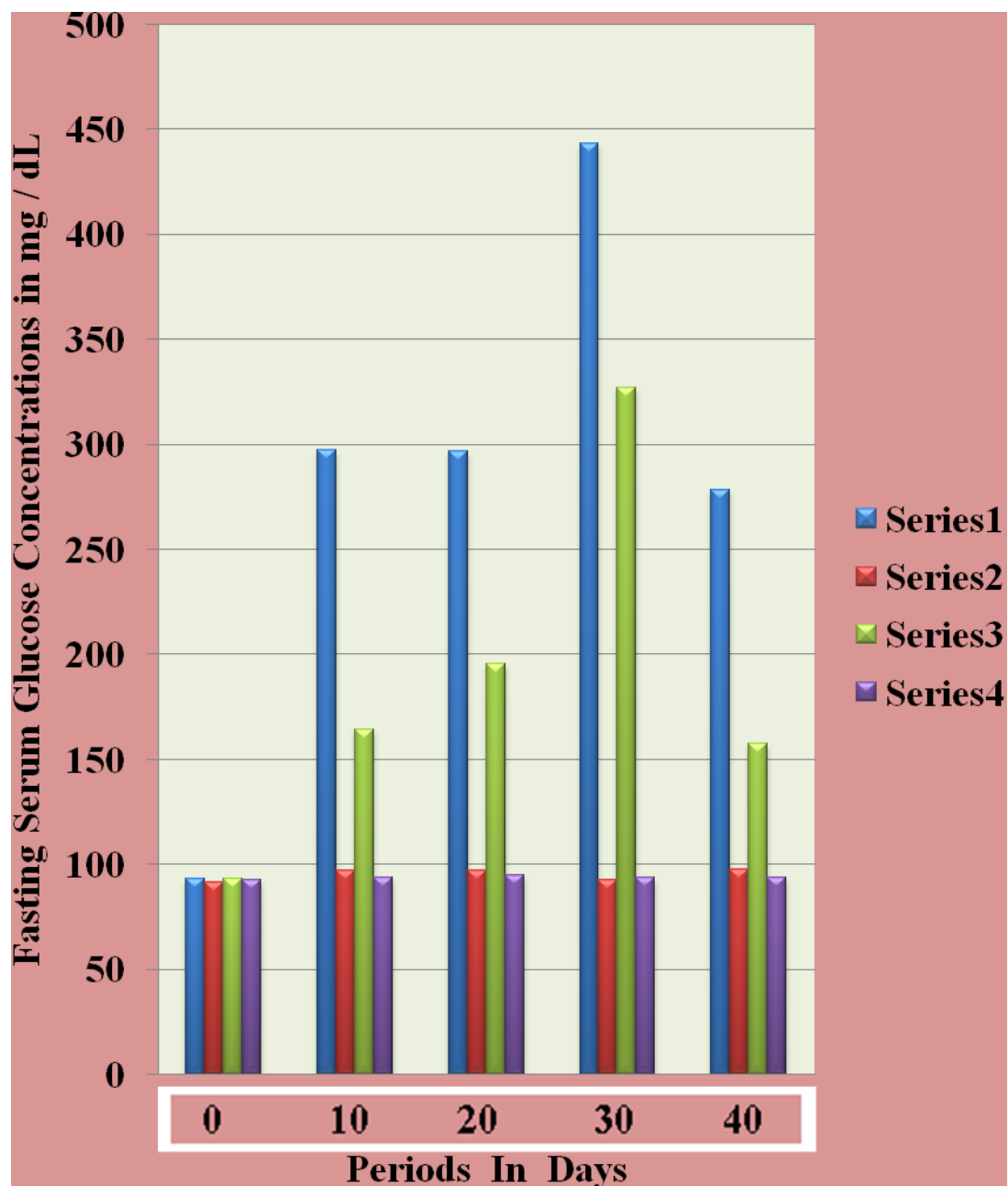
Whereas in control groups the Hb rates continued in the same values or nearby till the end of the experiment, which were  $182.18 \pm 13.41$  in males and  $167.12 \pm 2.26$  in females.

The difference between the control groups and hyperglycemic groups were highly significant at ( $P < 0.0001$ ).

The result also revealed that there was a significant difference between the gender  $324.22 \pm 147.25$  mg/dl in males and  $216.02 \pm 105.47$  mg/dl in females at ( $P < 0.0001$ ).

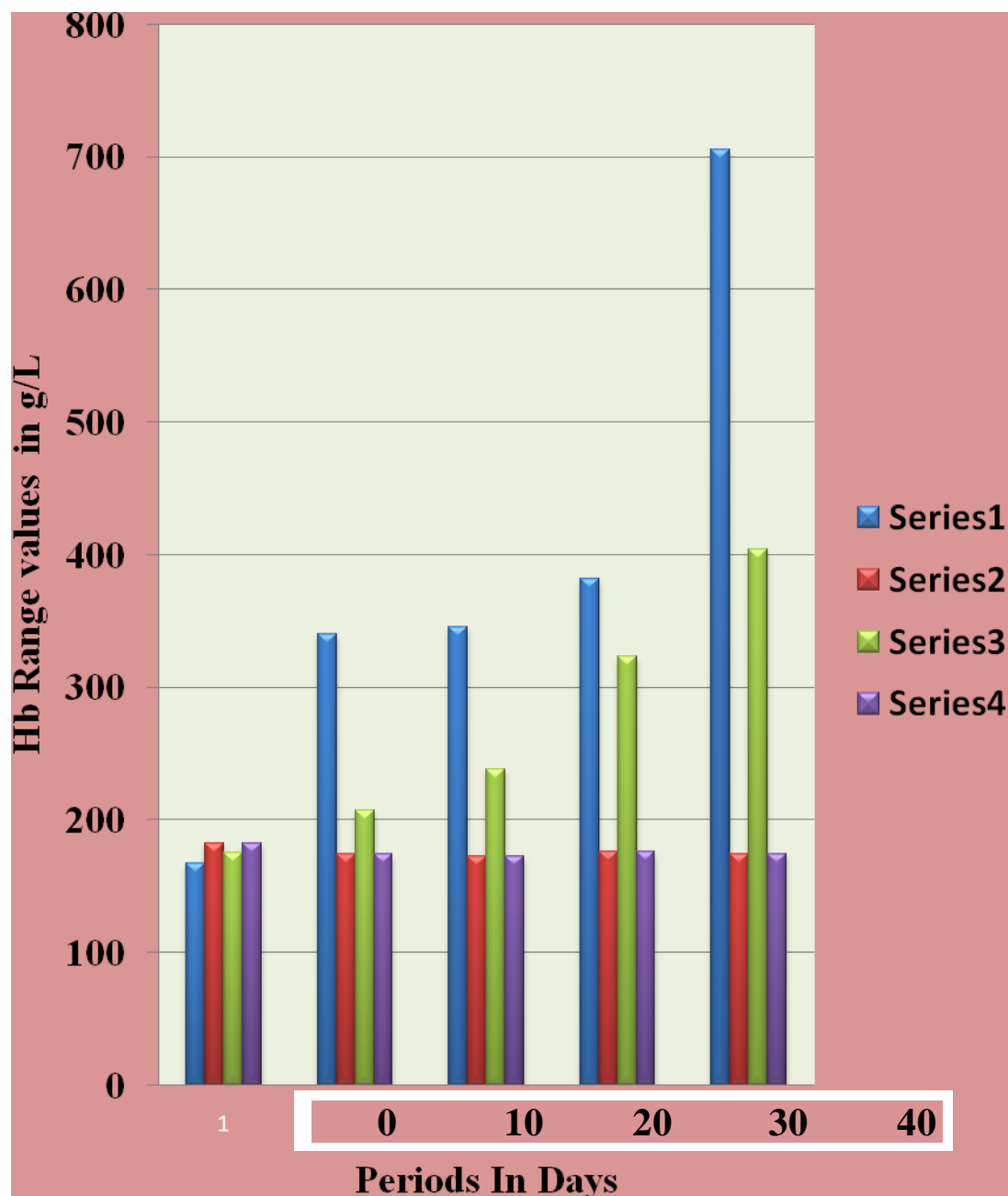
The result also revealed that the difference between days of the experiments is statistically difference at ( $P < 0.000$ ).

The fasting serum glucose Concentrations presented in chart (2).



**Chart (1): represent Fasting Serum Glucose Concentrations in mg/L.**

- Series 1 represent diabetic males (Dm).
- Series 2 represent control males (Cm).
- Series 3 represent diabetic females (Df).
- Series 4 represent control females (Cf).



**Chart (2): represent Glycated hemoglobin values in g/L.**

- Series 1 represent diabetic males (Dm).
- Series 2 represent control males (Cm).
- Series 3 represent diabetic females (Df).
- Series 4 represent control females (Cf).



## Discussion:-

The usefulness of glycated hemoglobin in monitoring the long – term metabolic control in diabetic patients is now widely recognized (Koenig et al., 1976). Although the exact chemical nature of human or rabbit HbA1c is unknown, both contain a sodium-borohydride-reducible linkage on the beta chain which is a presumed Schiff base between a sugar moiety and the protein (Blevins et al.; 2008). The diabetic animals show the increase approximately 4 weeks after the onset of the signs of diabetes. This rise is brought about by an increase in a circulating factor that determines directly or indirectly the synthesis of mouse HbA1c as a post-synthetic modification of HbA1c (Wang and Chalmers, 2008). If glycosylations of basement membrane proteins and hemoglobin proceed via a common mechanism, then the monitoring of HbA1c could provide a useful model for studying the early events of basement membrane thickening (Vracko, 1970). It should be noted that the measurement of glycated hemoglobin correlates well with mean serum glucose determinations over the periods (Dix et al., 1979). It can be stated that determination of glycated hemoglobin is a useful parameter for diabetic control in addition to the urinary and blood glucose levels (Menard et al.; 1980). Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. A diabetic person with good glucose control has an HbA1c level that is close to or within the reference range. The International Diabetes Federation and American College of Endocrinology recommend HbA1c values below 6.5%, while American Diabetes Association recommends that the HbA1c be below 7.0% for most patients (Rohlfing *et al.*, 2002). A high HbA1c represents poor glucose control. Persistent elevations in blood sugar (and therefore HbA1c) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene, and gastropathy (slowed emptying of the stomach) (Larsen et al., 1990). A 1% change in an A1c result reflects a change of about 30 mg/dL (1.67 mmol/L) in average blood glucose. For instance, an A1c of 6% corresponds to an average glucose of 135 mg/dL (7.5 mmol/L), while an A1c of 9% corresponds to an average glucose of 240 mg/dL (13.5 mmol/L). The closer a diabetic can keep their A1c to 6%, the better their diabetes is in control. As the A1c increases, so does the risk of complications (ADA, 2007).

The correlation between mean plasma glucose (MPG) levels and A1c levels is estimation only, dependent on methodology used for the calculation as well as other factors, such as the red blood cells life span (CDA, 2007).



## References:-

**Abraham, E.C.; Stalling, M.; Cameron, B.F. and Huisman, T.H.J. (1980).** Minor hemoglobin in sickle cell heterozygotes and homozygotes with and without diabetes. *Biochem. Biophys. Acta.* 625:109-117.

**American Diabetic Association [ADA]. (2004).** Elevation in markers of liver Injury & Risk of Type 2 Diabetes. *Diabetes*, 53(10): 2623-2632.

**American Diabetic Association [ADA]. (2007).** Standards of Medical Care in Diabetes. *Diabetes Care*, 30:S4-S41.

**Bartosikova, L.; Necas, J.; Suchy, V.; Kubinova, R.; Vesela, D.; Benes, L.; Bartosik, T.; Illek, J.; Salphachta, J.; Klusakova, J.; Bartosova, L.; Strnadova, V.; Frana, P. & Franova, J. (2003).** Monitoring of Anti oxidative Effect of Morine in Alloxan- induced Diabetes mellitus in the Laboratory Rat. *Acta. Vet. Brno.* (72): 191-200.

**Baule, G.M.; Onorato, D.; Talo, G.; Forteleoni, G. and Meloni, T. (1983).** Hemoglobin A1c in subjects with G6PD deficiency during and after hemolytic crises due to favism. *Aeta. Haemat.* 69:15-18.

**Blevins, T.; Schwartz, S. L.; Bode, B.; Aronoff, S.; Baker, C.; Kimball, K. T.; Harrist, R. B.; Donnelly, C., Burns, L. C. and Wooldridge, A. M. (2008).** A Study Assessing an Injection Port for Administration of Insulin. *Diabetes Spectr.* 21: 197-202.

**Bosman, D.R. (2002).** Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non- diabetic chronic renal failure. *Diabetic medicine*, 19(1):65-69.

**Candian diabetic Association {CDA}. (2007).** HbA1c, Glycohemoglobin, Diabetic Control Index. <http://www.diabetes.ca/>

**Christopher, M.M. & Neill, S. (2000).** Effect of specimen collection and storage on blood glucose and lactate concentrations in healthy, hyperthyroid and diabetic cats. *Veterinary Clinical Pathology*, 29(1):22-28.

**Dikow, R. (2002).** How should we manage anemia in patients with diabetes? *Nephrology, dialysis, transplantation*, 17(1):67-72.



**Dix, D.; Chem, P. and Kingesley, S. (1979).** Glycohemoglobin and glucose tests compared as indicators of border line diabetes. *Clin. Chem.* 25:877-879.

**Droumaguet, C.; Balkau, B.; Simon, D.; Cases, E.; Tichet, J.; Charles, M.; Eschwege, E. & DESIR study group. (2006).** Use of HbA1c in predicting progression to diabetes in French Men & Women, Data from an Epidemiological study on the insulin Resistance Syndrome (DSIR). *Diabetes Care*, 29:1619-1625.

**Edgren, A.R. (2003).** Diabetes Mellitus. *National Institute of Health*, 94: 2098.

**European Federation of Pharmaceutical Industries Association and the European Center for the Validation of Alternative Methods [EFPIA & ECVAM]. (2000).** A Good Practice Guide to the Administration of Substances & Removal of Blood, Including Routes and Volumes. *J Appl Toxicol*, 21 15-23.

**Holmquist, W.R. and Schroeder, W. (1966).** A new N-terminal blocking group involving in a Schiff base in hemoglobin A1c. *Biochemistry*, 5:2489-2503.

**Larsen, M.L.; Hørder, M. and Mogensen, E.F. (1990).** "Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus". *N. Engl. J. Med.* 323 (15): 1021-5

**Lorini, R. (1996).** Clinical aspects of coeliac disease in children with insulin – dependent diabetes mellitus. *Journal of Pediatric endocrinology & metabolism*, 9(1):101-111.

**Lukens, F.D.W. (1948).** Alloxan diabetes. *Physical Rev*, 28(3): 304-330.

s Textbook of Pathology. 13<sup>th</sup> **MacSween, R.N.M. & Whaley, K. (1992).** Muir ed. ELBS.P:798-799.

Biological Effects of Blood Loss: "**McGuill, M.W. & Roman, A.N. (1989).** . *ILAR News*, 31(4): "Implications for Sampling Volumes and Techniques 5-20.

**Mallia, A.K.; Hermanson, G. T.; Krohn, R. I.; Fujimoto, E. K. and Smith, P. K. (1981).** Preparation and use of boronic support for separation and Quantitation of Glycosylated hemoglobin. *Anal Lett.* 649-661.



**Menard, L.; Dempsey, M.E.; Blankstein, L.A.; Aleyassine, H.; Wacks, M. and Soeldner, J.S(1980).** Quantitative determination of glycosylated hemoglobin A1 by agar gel electrophoresis. *Clin Chem.*; 26:1598-602.

**Penzer, s.; koronik, G.; Lechner, K.; Bettelheim, P.; Neumann, E. and Dudczak, R. (1982).** Glycosylated hemoglobin (G-Hb); An index of red cell survival. *Blood*, 59:1348-1350.

**Pieper, A.A; Barat, D.J; Krug, D.K.; Watkins, C.C.; Gupta, A.; Blackshaw,S.; Verma, A.; Wang, Z. & Snyder, S.H.(1999).** Poly (ADP- ribose) polymerase- deficient mice are protected from streptozotocin- induced diabetes. *Proc Natl Acad Sci.* 96(6): 3059-3064.

**Rohlfing, C.; Wiedmeyer, H.M. and Little. R (2002).** "Biological variation of glycohemoglobin". *Clin. Chem.* 48 (7): 1116-8.

**Schwartz, E. (2000).** The anemias. In: Behrman, R.E.; Kliegman, R.M.; Jenson, H.B.eds.Nelson textbook of pediatrics, 16<sup>th</sup> ed. Philadelphia, WB Saunders.Pp:1461-1463.

**Su, X.; Hu, Q.; Kristan, J.M.; Costa, C.; Shen, Y.; Gero, D; Matis, L.A. & Wang, Y. (2000).** Significant Role for Fas in the Pathogenesis of Autoimmune Diabetes. *The Journal of Immunology*, 164: 2523-2532.

**Vracko, R. (1970).** Skeletal muscle capillaries in diabetics. *A quantitative analysis. Circulation*, 41(2):271-283.

**Wang, P.H. and Chalmers, T.C.(2008).** Meta-analysis of effects of intensive blood-glucose control on late complications of type I diabetes. *Lancet* 1993;341: 1306-9.

## الإرتباط بين مستويات جلوكوز المصل و Glycosylated HemoglobinA1c

### في داء السكري المستحث بشكل تجريبي

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### الخلاصة

هدف هذه الدراسة هو تقييم مستوى Glycosylated HbA1c كإختبار ومؤشر تشخيصي لشدة داء السكري. ستة وثلاثون أرنباً إشتري من السوق المحلية، أوزانها تتراوح بين ١.٢-٢ كيلو غرام، إستعمل في التجربة. قُسمت الحيوانات بالاعتماد على الجنس إلى مجموعتين (ذكور وإناث) وكل مجموعة قُسمت بأعداد متساوية إلى مجموعة مصابة بداء السكر ومجموعة سيطرة.



كشفت النتائجُ إختلافَ هامَّ جداً في مستوى ( Glycosylated HbA1c ) بين المجموعتين المصابتين بداء السكر ومجموعتي السيطرة، وظهرت النتائج ان مستوى ( glycosylated HbA1c ) اخذ بالارتفاع بشكل تدريجي بعد بداية الإصابة بداء السكر ، وكذلك اوضحت الدراسة أن النتائج في الذكور المصابة بداء السكر اكثر اهميةً احصائياً من الإناث المصابات ( تحت مستوى معنوية اقل من ٥% ) بالإضافة إلى ذلك بينت النتائج بأن تركيز ( Glycosylated HbA1c ) ازدادَ بزيادة تراكيز جلوكوزِ المصلِ اي ان العلاقة طردية. استنتجت من الدراسة الحالية بان ارتفاع مستويات Glycosylated HbA1c يدل على سوء السيطرة على مستويات سكر الدم وبالتالي فان مستوى Glycosylated HbA1c يرتفع كما ارتفعت مستويات سكر الدم.