Original paper

Morphological Study of the mice's Islets of Langerhans and β-cells mass assessment during Pregnancy and lactation conditions

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Abstract

Background: Islets of langerhans adapts to changing insulin demands in the body. One of the most amazing reversible islets of langerhansadaptations occurs during pregnancy and postpartum conditions. During pregnancy,β-cells mass expand by increasing their number and size . and they are rapidly reversed at end of pregnancy by β- cell apoptosis and retained to normal level .
Objectives: This study attempt to demonstrate the changes of the islets of langerhans that happen during pregnancy and lactation including changes in the general morphological and histological features, changes in the certain islets' parameters e.g. number ,diameter and mean areas, and changes in the β – cells mass.
Material and Methods: We were used thirty female mice which divided into (3) groups ten for each group. Group(A): served as a control. Group(B) was pregnant group (at day 15 of gestation). Group(C) was lactating group (at day 4 of Postpartum). Tissues were processed for both paraffin block and semithin plastic sections . Tissue sections were stained with H&E stain, Gomoritri chrome stain and NDS. Two digital image analysing soft wares were used in this study: Image J program and Image Scope program
Results: It was demonstrated that the formation of new islets, enlargement of islets by union of adjacent small islets and by increased their cellularity were common features of pregnant group. also it was showed that in postpartum group, apoptosis of beta -cells started to restore the beta- cell number to normal level. The morphometric analysis of the islets parameters in this study showed a highly significant differences among the studied groups with P value ≤ 0.001. The mean number , mean diameter and mean area of islets were significantly higher in pregnant group, intermediate in lactating group and lowest in control group. In this study, the result also showed that the β-cell mass rose near the doubled during pregnancy as compared with control group, then decreased by 25% at 4th day of postpartum. This study conclude that islets of langerhans subjected to natural compensatory changes during pregnancy then they retain to normal state when pregnancy ended
Keywords: Islets of landerhans, pregnancy, postpartum,β-cell

Introduction

Endocrine portion of pancreas is a dynamic tissue with a capability to change its mass in response to variation in the metabolic status of the body such as that occur during pregnancy and obesity[6]. During pregnancy, there is a state of enhanced insulin resistance produce

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by the effect of placental hormones[7]. To overcome this state, the pancreatic islets of langerhans undergo structural and functional changes to increase insulin release with normal glucose level. β-cells mass expand by increasing their number and size. These changes are induced by placental lactogen (hPL)[12] and they are also rapidly reversed at end of pregnancy due to progesterone- controlled activation of β-cells apoptosis[2,9,10].

We aimed in this study to demonstrate the changes of the islets of langerhans that happen during pregnancy and lactation on the following aspects:

- Changes in the general morphological and histological-changes.
- Changes in the certain islets' parameters e.g. number, diameter and mean areas .
- Changes in the β – cells mass

Material and Methods

We were used thirty female mice identical in age. They were divided into (3) groups ten for each group.
Group(A): served asa control.
Group(B) was pregnant gp (at day 15 of gestation).
Group(C) was lactating gp at day 4 of Postpartum).
Tissues were processed for paraffin block[4] and semithin plastic section and thetissue sections were stained with following stains:

1) Haematoxylin and Eosin (H&E) Staining [3]:
The staining was done to demonstrate the morphologic appearance and the integrity of the specimens before Gomori trichrome staining. Paraffin sections were cut at 7um, deparaffinisedinxylene, embedded in descending grades of alcohol, washed in water, then stained with H&E and mounted.

2) Gomori trichrome Staining [27]:
According to Sheehan & Hrapchak 1987, sections were immersed in Harris haematoxylin for 5 minutes, and washed with tap water. Then, immersed in Gomori trichrome stain for 10 minutes, followed by differentiation in 0.2% acetic acid. Sections were then dehydrated in ascendin galcoholconcentrations, cleared in xylene, mounted and covered.

3) Nuclear Differentiation Stain [19] (NDS): composed of two solutions:
Solution A: Basic fuchsine 0.4 gm. in 100 ml of (2.5%) methanol. Solution B: Prepared by mixing equal volumes of: Azure II, Methylene blue, Na_2 Co_3 inethanolalcohol.
Histometrical evaluations was done by taking digital images to slides and analysing with Image J program[25] to obtain 100 measurements of the islets parameters (number, diameter and mean area) for each group. Data were expressed as mean ± standard error of mean.
Assessment of the β- cells mass was done using Image Scope [26] program. This program has been pre-configured for brown color quantification in the three intensity range (yellow, orange, red). Pixels which are stained and fall into the color specification are considered positive, and pixels which are stained and not fall into color specification are considered negative. So that the negativity of the pixels can calculate as a fraction of the negative to total number (positive + negative) is) which represents the β-cell mass. Calculation of the negativity of the pixels was done by taking digital images to slides and analysing with Image Scope program to obtain 100 measurements for each group. Data were expressed as mean ± standard error of mean.
F-test was applied in ANOVA[30] table to compare the results among studied groups and for finding out the

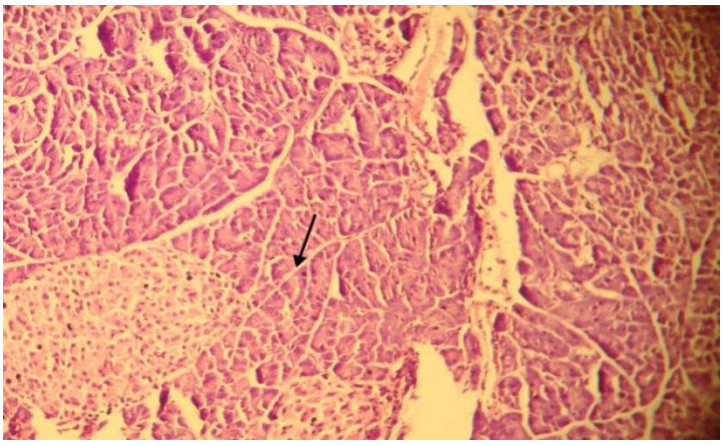
source of difference, we applied Independent –sample **t**- test to compare the results between each two groups. The values were considered statistically significant when p- value < 0.05.

Results

A) General histological study

Light microscopic examination of the pancreatic sections of control group revealed thatthe exocrine component formed the majority of the pancreas which consisted of closely packed secretory serous acini arranged into small lobules. Among them interlobular connective tissue septa seen. Within the pancreatic exocrine tissue islets of langerhans were embedded, they appeared as pale stained rounded or oval area (Figure 1). And they formed of group of cells arranged in an irregular branching and anastomosing cords (Figure 2).

During pregnancy , There were appearance of multiple, small ,newly formed islets and enlargement of the islets either by union of adjacent small islets or by increase its size seen(Figure 3,4&5).



Figure(1): pancreatic section in control group(A),showing multiple lobules. separated by (⟶)interlobular septa and contain both exocrine and endocrine components. Control group, H&E, 40X

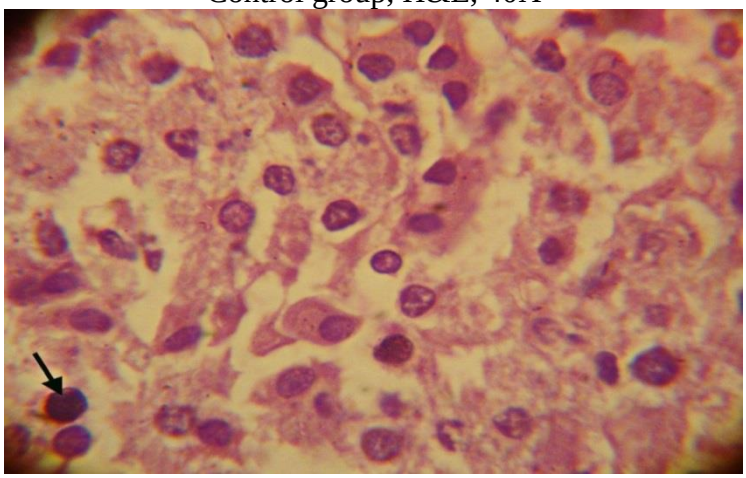


Figure2.pancreatic section in control group(A),showing irregular branched cords of islets cells. Control group ,H&E, 400X

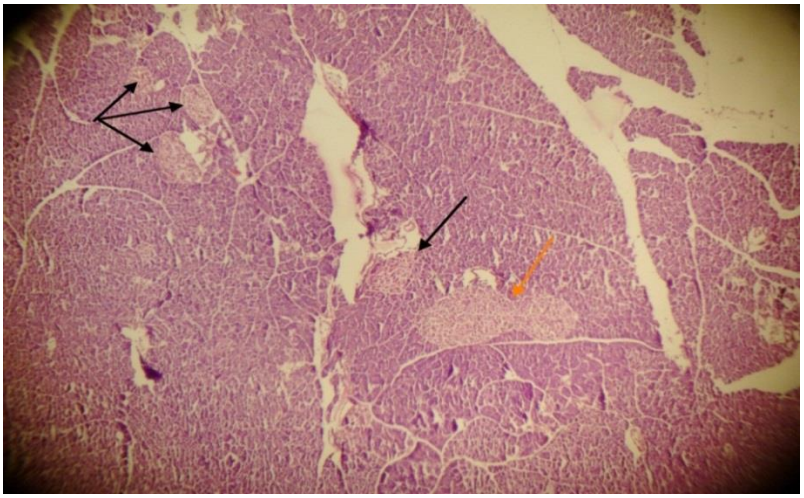


Figure3. pancreatic section in pregnant group (B), showing multiple newly formed islets (⟶),coalescence of adjacent islets(⟶). Pregnant group, H&E,40X

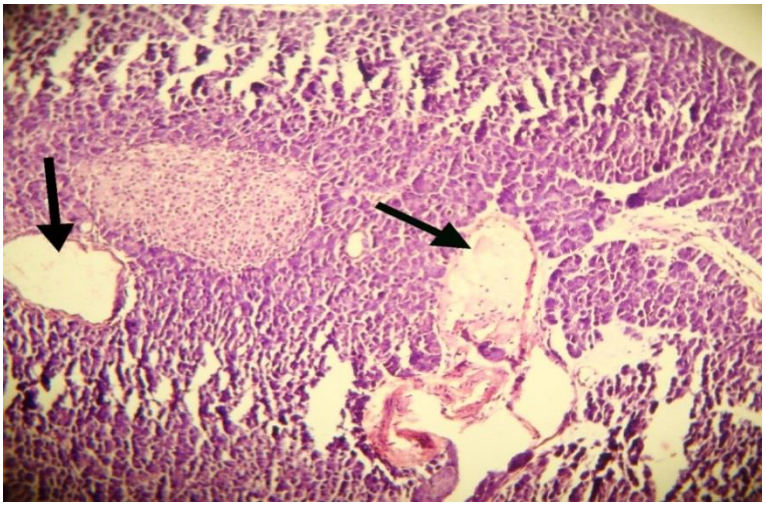


Figure4.pancreatic section in pregnant group(B),showing enlargement of the islets caused by its size.Pregnant group, H&E,40X

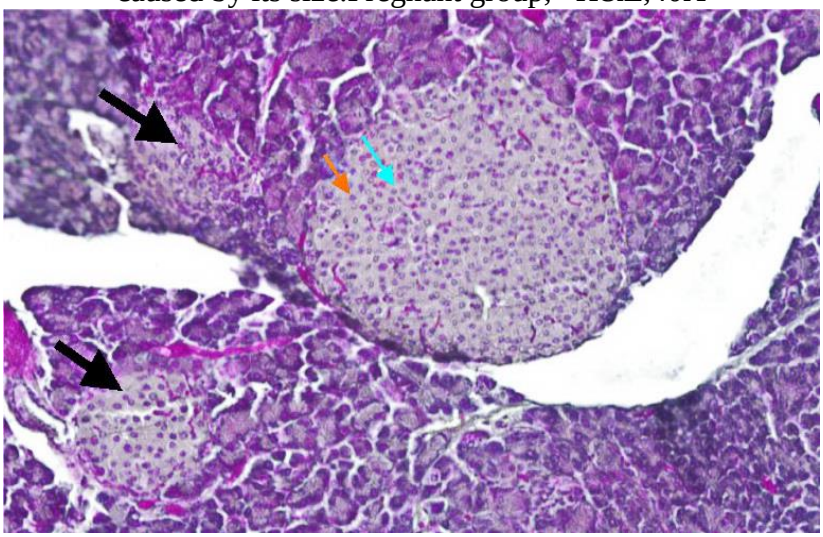


Figure5. pancreatic section in pregnant group(B),showingincrease cellularity of islets with dominancy of β- cells (⟶),α cell (⟶) and newly formed islets (⟶). Pregnant group, Gomoritrichrome, 40X

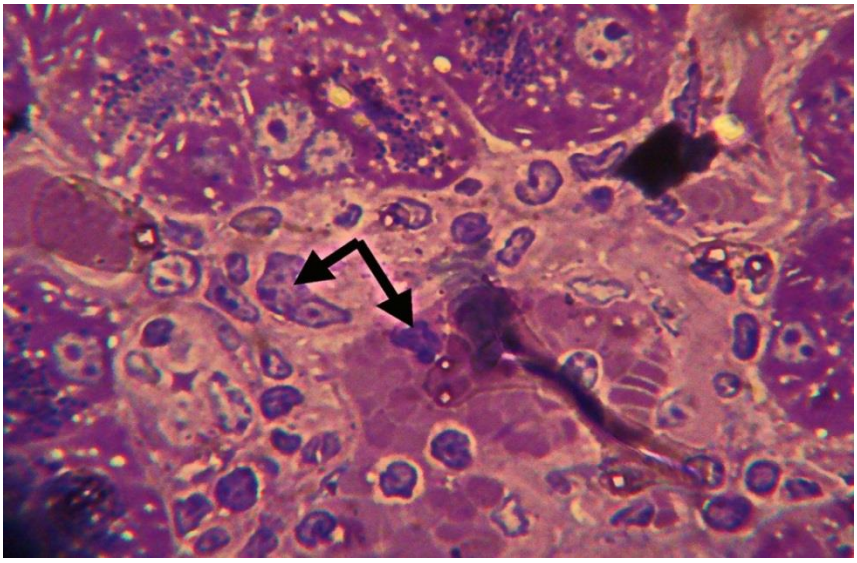
In lactating group, islets cells were characterized by losing their regularity ,their nuclei started to diminish in size and also started to change their shape from well-rounded to different irregular shapes. Another feature was the appearance of high number of the apoptotic condensed and fragmented nuclei (Figure 6&7).

B) Morphometrical Study

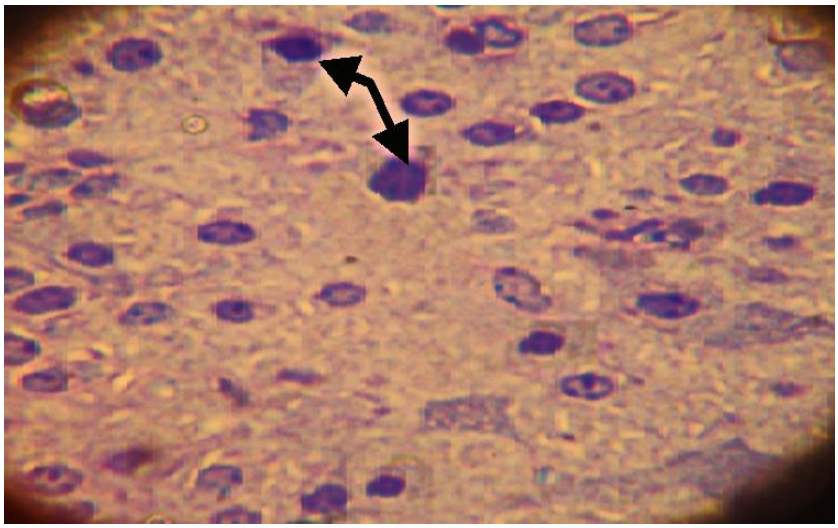
1) Number of Islets of Langerhans.

Mean number of the islets for each group was calculated as shown in the (table 1). From this table, mean number of islets in pregnant group increased then decreased in lactating group.

Statistical analysis with(Independent – sample **t**-test) showed a highly significant differences between pregnant and control groups and also between pregnant and lactating groups while it showed a significant difference between control and lactating groups as shown in(Table 2)



Figure(6):pancreatic section in lactating group(**C**),showing islets cells lost the regularity of their nuclei ( ). Lactating group,NDS, 1000X



Figure(7):pancreatic section in lactating group(**C**),showing appoptotic islets cells with condensation and fragmentation of the nuclei, (Lactating group,NDS, 1000X

2)Diameter of Islets of Langerhans:
Mean diameter of the islets for each group was calculated as shown in the (table 3).From this table, mean diameter of islets in pregnant group was the highest among the others.
Statistical analysis with(Independent – sample **t**-test) showed a highly significant differences between pregnant and control groups and also between pregnant and lactating groups while it showed a significant difference between control and lactating groups as shown in (Table 4)

3) **Mean area of isletsof langerhans:**
Mean area of the islets for each group was calculated as shown in the (table 5).From this table,mean areaof islets in pregnant gp was markedly increased then it started to decline inlactating group.

Table 1. Mean number of islets of langerhans of each group.

Groups	**Mean number of islets per field**±SR
Group A	1.2727 ± 0.823
Group B	2.2897 ± 0.948
Group C	1.5647 ± 0.756

Table 2. Comparison of mean ± SE of the number of islets per section in all groups according t- test

Groups	Mean ± Std. Error	P value
Group A	1.2727 ± 0.823	≤0.001**
Group B	2.2897 ± 0.948	
Group A	1.2727 ± 0.823	= 0.005*
Group C	1.5647 ± 0.756	
Group B	2.2897 ± 0.948	≤0.001**
Group C	1.5647 ± 0.756	

**Highly statistical significant difference.
*Statistical significant difference.

Table 3. Mean diameter of islets of langerhans of each group

Groups	**Mean diameters of islets(um)**
Group A	106.510 ± 3.983
Group B	175.215 ± 6.959
Group C	149.925 ± 5.161

Table 4. Comparison of mean ± SE of the diameter of islet s per section in all groups according t- test.

Groups	Mean ± Std. Error	P value
Group A	106.510 ± 3.983	≤0.001**
Group B	175.215 ± 6.959	
Group A	106.510 ± 3.983	0.005*
Group C	149.925 ± 5.161	
Group B	175.215 ± 6.959	≤0.001**
Group C	149.925 ± 5.161	

** Highly statistical significant difference.
* Statistical significant difference.

Table 5. Mean area of islets of langerhans of each group

Groups	***Mean area of islets(um)2***
Group A	**431.98 ± 12.515**
Group B	**910.86 ± 16.826**
Group C	**516.078 ± 20.179**

Statistical analysis with(Independent – sample **t**-test) showed a highly significant differences between pregnant and control groups and also between pregnant and lactating groups while it showed a significant difference between control and lactating groups as shown in (Table 6)

A) Assessment of the β- Cells Mass:

Mean area of the islets for each group was calculated as shown in the (table 7).From this table, mean of β- cell mass in islets of pregnant group rose near the doubled, then it decreased by 25% at day 4 of postpartum in lactating group.

Table7. Mean of the negativity of the pixels for each islets per section

Groups	Mean of β-cells mass in the islets per section
Group A	58.029 ± 3.024
Group B	98.097 ± 1.514
Group C	73.4433 ±3.763

Statistical analysis with (Independent – sample **t**-test) showed a highly significant differences between pregnant and control groups and also between pregnant and lactating groups while it showed a significant difference between control and lactating groups as shown in (Table 8) and(Figure 11).

Discussion

Islets changes during pregnancy was the focus of many studies from the mid 1960's and the functional adaptation of the endocrine pancreas during pregnancy and lactation was a subject of wide controversy. Adaptation of pancreatic islets cells during these two events were our main objective in this study.

In pregnancy, insulin demands of the mother dramatically increase due to the enhanced insulin resistance of the maternal tissue and due to increase food intake especially during the latter third of pregnancy[18]

Spellacy and Goetzreported that there was a progressive increase in both fasting and stimulated insulin secretion throughout the period of pregnancy[31].

Table 6. Comparison of mean area of islets ± SE of islets per section in all groups according t- test.

Groups	Mean ± Std. Error	P value
Group A	431.98 ± 12.515	≤0.001**
Group B	910.86 ± 16.826	
Group A	431.98 ± 12.515	0.005*
Group C	516.078 ± 20.179	
Group B	910.86 ± 16.826	≤0.001**
Group C	516.078 ± 20.179	

** Highly statistical significant difference.
* Statistical significant difference.

Table 8. Comparison of mean ± SE of theβ- cells mass per islets per section in all groups according t- test.

Groups	Mean ± Std. Error	P value
Group A	58.029 ± 3.0243	0.001**
Group B	98.097 ± 1.514	
Group A	58.029 ± 3.0243	0.005*
Group C	73.443 ± 3.763	
Group B	98.097 ± 1.514	0.001**
Group C	73.443 ± 3.763	

** Highly statistical significant difference.
* Statistical significant difference.

The main features noticed in islets of pregnant group in the present study, were formation of numerous, small islets and evidence of enlargement of islets by coalescence of adjacent islets and by increased its cellularity.

Green and Taylormentioned that enhanced β- cell proliferation and hypertrophy were two of the main cellular processes involved in increased islet volume during pregnancy[14]. Genevay et alreported that islets not only were larger during pregnancy, but possibly islets neogenesis[13]. Study done by Maryline et al; Bertelli et alreported that islets neogenesis is the mechanism that triggers the generation of new cells from precursor cells which could potentially originate from: Ductal cells by ductal neogenesis, already differentiated pancreatic cell (i.e., exocrine, acinar or ductal) or extra-pancreatic cells through a mechanism so called trans differentiation. and an islet precursor cell by intra- islet neogenesis[18,5]

Sylvie et al reported that some cells of the liver Hering duct had ability to proliferate and regenerate upon hepatic lesion called oval cells harbour some epithelial markers as CK19. These cells have been found in pancreas as a response to specific stimuli and it was found in all models of transdifferentiation from liver into pancreas. Oval cells were found in pancreas and more precisely in the ductal tree or adjacent to it[32].

There is a uniform agreement in different studies that the pregnancy results in an increase in the total pancreatic mass of islets. This growth in the islets is due to both β- cell hyperplasia and hypertrophy. The increase in β- cell proliferation is first observed around the day 10 and peak around the day 14 of pregnancy[29]

In nondiabetic rats, lactation accelerates the restoration of pancreatic β- cell function after the period of increased secretory activity associated with pregnancy[15]. Maternal pancreatic islets undergo a transient burst of progesterone- induced apoptosis as early as the 4th day post-partum, in spite of high levels of circulating hPL[16,23].

The present study reported that number, diameter and mean area of the islets in lactating group were decreased. The islets cells lost their regularity of nuclei and their nuclei changed from well-rounded to different irregular shapes with the appearance of high number of characteristic apoptotic condensed and fragmented nuclei. This finding was in agreement with Carla et al who demonstrated that β-cell mass has increased by both replication and hypertrophy during the first two- third of gestation. After parturition, maternal β- cell mass returned to non-pregnant level by β-cell apoptosis which was maximum at 4th day after delivery[9].

Scaglia et al reported that the normal low rates of apoptosis in β-cells were upregulated postpartum in the mother and in the neonate, and they were also increased after glucose deprivation[24] and Sara et al mentioned that the process of cell death could be influenced by the cells environment[22]. Both withdrawal of trophic factors and change in the hormonal environment have been suggested to trigger apoptosis in hormone dependent tissues in postpartum rat pancreas. In the postpartum rat pancreas, a significant and rapid involution in the β- cell mass has been described as a response to the rapid change in the hormonal milieu that happens after delivery.

In the present work, the mean of number of islets per section in pregnant group was statistically significant increased as compared with control group and lactating

group (2.2897 ± 0.948 *v 1.2727± 0.823, p*≤0.01) and (2.2897 ± 0.948 v1.5647±0.756.≤0.001) respectively. The mean of diameter of islets per section in pregnant group was statistically significant increased as compared with control group and lactating group (175.215± 6.959 *v 106.510±3.983,p≤0.001)* respectively and the mean square area of islets per section in pregnant group was statistically significant increased as compared with control group and lactating group (175.215±6.959 *v 149.925±5.161,p*≤0.001)respectively.

The appearance of these morphological changes reflects the effect of placental hormones on the pancreatic islets as an attempt to compensate the state of insulin resistance that occur in pregnancy by increase islets mass to increase insulin secretion with euglycemia.

The result of present study matched with numbers of previous morphometric studies. These studies indicated that there was an approximate two fold increase in islets mass[33,34,21]. Some of these studies used software package (e.g. Image Pro Plus V4.5, software Optimas v6.21), other used morphometric method of Chalkley in their morphometric measurements of islets parameters

Costrini and Kalkhoff; Dunger et al ; Green and Taylornoticed that DNA content per islet also indicated an increase in islets mass, and both morphometric and DNA to protein ratio methods indicated β-cells hyperplasia and hypertrophy[10,11,14]. Rhodesreported thatβ- cell mass was determined by the product of the number and size of the pancreatic β-cells. Compensatory changes in β- cell mass were controlled by increases in cell size and adjustment to the rate of β-cell proliferation and death. Current evidence suggested that dysregulation of these mechanism was an essential feature in the pathogenesis of diabetes mellitus[20]. A variety of quantitative methods have been used to measure islets mass during pregnancy, These include quantitative histomor-phometry of the endocrine pancreas at light and electron microscope levels, islets diameter, DNA and protein content of islet isolated and tritiated thymidine or BrdU incorporation into islets cell DNA. Histomor-phometry provides the best estimate for the amount of pancreatic islets mass [28].

Studies done by Aler et al; Butler et alhave determined beta cell mass by point counting stereology on Gomori trichrome-stained and immunohistochemical stained sections respectively. In the present study, assessment of β- cells mass was done by using Aperio positive pixel count algorithms program[1,8].

The result of the present work showed the mean of the β- cell mass in pregnant group was statistically significant increase as compared with control group (98.097 ± 1.515 *v* 58.029 ± 3.0243, *p*≤0.01) and the mean of β- cell mass for lactating group was statistically significant less than pregnant group (73.443 ± 3.763 *v* 98.097 ± 1.515 , *p*≤0.01) .

From the result of the present study, β- cell mass increased nearly 100% in pregnancy (at 15th day gestation) as compared with control group and it started to decreased to reach 73% at 4th day after delivery in lactating group (decrease 25%). Several reports existed in accordance with the observation concerning with β- cell mass which showed that β- cell mass in rodents increase 1-3 fold during gestation and reach the peak about two- thirds of the way through the gestational period, then decrease reaching pre- pregnancy level shortly before parturition within ten days. The pregnancy paradigm was a unique example of rapid and reversible β-

cell mass expansion, with distinct bursts of both β- cell proliferation and β-cell apoptosis in a physiological setting[10,8,21].

In the light of the findings of the present study, it was concluded that islets of langerhans subjected to natural compensatory changes during pregnancy and lactation. The observed changes were:

1- Increment in number and size of islets were the main features that observed during pregnancy .
2- Increase in the number of the islets in pregnant group was evidenced by coalescence of adjacent islets and by increased their cellularity.
3- Increased islets cellularity during pregnancy due to hypertrophy and hyperplasia of β-cells as evidenced by increase the beta- cells mass in pregnant group as compared with control group.
4- Apoptosis of beta -cells during postpartum period started to restore the beta- cell number to normal level as evidenced firstly by increment in the appearance of the characteristic apoptotic condensed and fragmented nuclei, secondly by decrease in beta- cells mass (about 25%) as compared with pregnant group.

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