STEREOLOGICAL TERATOGENIC EFFECTS OF PRENATAL EXPOSURE TO VARIED DOSES OF DEXAMETHASONE ON FETAL PANCREAS IN ALBINO RATS

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Abstract: The normal morphogenesis of the fetal pancreas has been shown to be disturbed by the prenatal exposure to dexamethasone. There is however, paucity of data on the histo-stereological effects of dexamethasone when prenatally exposed at different gestation periods. In addition, data on whether the prenatal exposure to dexamethasone is dose and time dependent is yet to be elucidated.The main objective of this study is to evaluate the histo-stereological teratogenic outcomes of the prenatal exposure to varied doses of dexamethasone on the development of the fetal pancreas in albino rats. A total of 40 albino rats weighing between 250 to 300 gms were used as the albino rats experimental model. These 40 dams were assigned into two study groups of 36 dams as the experimental category and 4 dams as the control group. The stereological sections were analyzed using Stepnizer stereological software version 23 for windows.The dexamethasone treated groups was marked with significant decrease ($p<0.05$) in volume densities of islets, acini, blood vessels as well as volume density the ducts in the all the dexamethasone treated groups (LDG, MDG, HDG) when compared with control. These teratogenic effects of dexamethasone on the developing fetal pancreas were also noted to be time and dose dependent with the most injurious effects experienced in the first and second trimester (TM1 and TM2) respectively in all the dexamethasone treated groups.

Keywords: glucorticoids, dexamethasone, prenatal, albino rats

INTRODUCTION

Dexamethasone, a synthetic drug in the class of corticosteroids is commonly used as an anti-inflammatory agent due to its inhibitory effects to substances that cause inflammation in the body [1]). It is used in treatment of different inflammatory conditions such as allergic disorders and skin conditions. It has a molar mass of 392.464g/mol, formula C22H29FO5 and a bio availability of 80-90% with excretion of 65% in the urine [2]. Moreover, because of its molecular weight, when used during pregnancy it readily crosses the maternal placental barrier accumulating in the developing fetal tissues subsequently perturbing their histomorphogenesis [3]. It has a function as an adrenergic agent, an antiemetic, an antineoplastic agent, an environmental contaminant, a xenobiotic, an immunosuppressive agent and an anti-inflammatory drug [4]. It is highly soluble, at an 80-90% rate and has a half-life of approximately 3 hours [5]. Dexamethasone is metabolized by the liver and excreted in the urine mainly [5]. Dexamethasone can be used in the treatment of congenital adrenal hyperplasia, to prevent virilization of a female fetus (Karlssan et al., 2018). Unbound dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic

glucocorticoid receptors [7]. This complex binds to DNA elements (glucocorticoid response elements) which results in a modification of transcription and, hence, protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue [8,9,10]

Glucorticoids administration to rodents during the early stages of pregnancy, leads to general reduction in the size of all organs composing the digestive tract with volume reducing by17% of that of an age-matched wild-type pancreas [11,12,13,14]. Glucocorticoid levels play a role in the apoptotic process of various endodermal derived cell types [11,15]. More specifically, it has been shown that dexamethasone treatment leads to an increased expression of the anti-apoptotic factors Bcl-2, Bcl-xL or cFLIP (an inhibitor of caspase-8) in human and rat hepatocytes, or C-IAP2 (cellular inhibitor of apoptosis 2) in human lung epithelial cells [16,17,18]

When prednisolone is administered to Wistar Bonn/Kobori rats, it has been shown to cause chronic pancreatitis, decreased pancreatic weight, low endogenous corticosterone levels and increased acinar cell apoptosis [19,20]. Glucocorticoids also show direct effects on beta cell expansion, rather than increased beta cell mass [21].

The impairments of the normal pancreas morphogenesis have been linked to adult pancreatic disorders like pancreatitis, diabetes mellitus and pancreatic cancer among others when tumor suppressor genes such as DPC4, P16, and P53, BRCA2 are interfered with by dexamethasone during development [14,21]. Glucocorticoids tend to stimulate cancerous cells that express multiple cell-type markers, for example, cytokeratin, insulin, and glucagon [22] at the same time studies have shown that glucocorticoids like dexamethasone also trigger the immune mediated diabetes mellitus that account for about 5–10% of all diabetes patients, and is a result from a cellular-mediated autoimmune destruction of the β-cells of the pancreas [14,23]. Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, auto antibodies to GAD (GAD 65), and auto antibodies to the tyrosine phosphates IA-2 and IA-2β [14]. Type I diabetes arises from pancreas, and specifically β cells, are destroyed by auto-immune attack [13].

MATERIAL AND METHODS

Animal subject

A total of 40 nulliparous albino rats weighing between 200g to 300 grams sourced from Jomo Kenyatta University of Agriculture and Technology (JKUAT) were used. The albino rats were used in the study due to the following facts: They have low incidence of spontaneously occurring congenital defects, relatively short gestational span, a large litter size, low cost of maintaining the albino rats and, considerable amount of the reproductive data on the rat is already available [24]. They were housed in polycarbonated rat cages 3 rats per cage and exposed to 12-hour light, 12-hour dark cycles under humid tropical conditions 24°C at the same resource. The cages were marked with a cage tag showing experimental name of the albino rats, initial date of experiment, prescribed amount of the drug, Age, total sum of experimental rats, type of the rat.

Drug administration

All experimental groups received oral dexamethasone dissolved in normal saline via gastric gavage (Gauge 16) between 8:00am to 9: am. The dexamethasone groups received (HDG 4mg/kg/d, MDG 2mg/kg/d, LDG 0.5 mg/kg) during the gestation period in first trimester, second trimester and third trimester.

The 12 albino rats in trimester 1 received dexamethasone treatment from day one of gestation all through today 20; those in trimester two study category received dexamethasone treatment starting day 7 all throughout to the last day of gestation day 20, while the 12 albino rats in trimester III start receiving the dexamethasone treatment from day 14 all through to- day 20 the last day of gestation.

Preparation of tissues for stereology

The fetal pancreas for stereological analysis was removed and cleaned of fatty tissue, placed in cold saline solution and weighed, and immersed in 10% neutral buffered formalin for 24 hours at room temperature (230c) to allow for proper fixation. After tissue processing the tissues was allowed to settle haphazardly in palaplast (the embedding

media) to allow for physical randomization of tissue orientation in the blocks. Pancreas sections were processed and were stained with hematoxylin and eosin [25].

The primary volume of the pancreas was measured using the immersion method. Also, tissue shrinkage was estimated and the final pancreas volume was corrected without the need for serial sectioning. A limited number (10) of the isotropic uniform random slabs of each pancreas was embedded in the same block.5µm sections were obtained and stained with a hematoxylin and eosin. The point counting and point-sampled intercept methods were used to estimate the volume density of the islet and the mean cell volume, respectively.

In this study a total of 120 pancreases obtained from the study sample of 120 fetuses harvested from 40 dams were routinely processed for light microscopy and for stereological analysis using hematoxylin and eosin stain. Slides for stereological analysis were selected in systemic uniform random sampling where the starting point was randomly determined that systematic sampling for Nth followed a systematic order [25].

The stereological sections were analyzing using Stepnizer stereological software version 23 for windows where both the parenchyma and stromal tissues were further subjected to its analytical components including Cavalieri, optical dissector, point sample intercept and point counting method to determine entire pancreatic mass, volume density of the islets, volume density of the blood vessels, volume density of the connective tissue stroma and volume density of the ducts.

Statistical analysis

The stereological parametric data was expressed as mean volumes and standard error of the mean. The data was put in excel spread sheets, exported in Statistical Package for Social Sciences (SPSS) version 23.0 and statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc for multiple comparison tests. The results were considered to be significant at $P<0.05$.

Ethical approval

All procedures were performed with approval of Albino rats Ethics Committee of Jomo Kenyatta University of Science and Technology. The albino rats were only used once in the experiment. They were all sacrificed using humane end points at the end of the study [38].

RESULTS

Trimester one (TM1) findings on the Primary Pancreatic Volume, Final Pancreatic Volume and Shrinkage

When dexamethasone was administered in trimester one (TM1), it was observed thatprimary pancreatic volume, final pancreatic volume and shrinkageas well a dose response relationship in the all the three parameters decreased with the increasing doses of dexamethasone treatment (table 1).

The comparative intragroup and intergroup comparisons also shown that all values were statistically significant difference by both P-values ($p<0.001$) as well as by F-values between LDG, MDG and HDG against control.

Table 1: The TM1 intra and inter group comparative on the Primary Pancreatic Volume, Final Pancreatic Volume and Shrinkage between the dexamethasone treated groups LDG, MDG, and HDG against the control

Variable	Control	LDG	MDG	HDG	F value	P-value
Primary Pancreatic Volume	2.71±0.087a	2.07±0.011b	1.76±0.034c	1.30±0.005d	159.118	0.004*
Final Pancreatic Volume	2.66±0.091a	2.00±0.013b	1.71±0.031c	1.26±0.004d	145.002	<0.003*

Pancreatic Shrinkage	0.021±0.002a	0.033±0.002a	0.028±0.004a	0.025±0.004a	2.577	0.066

*Notes: The means, followed by the same letter in a row are not statistically different at (P<0.05) using one-way ANOVA.with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).*

The results in Table 1 indicates that the fetal pancreas volume estimation through water immersion (primary volume) method in control group (2.71±0.087) in trimester one was significantly higher than the (LDG 2.07±0.011), MDG (1.76±0.034), HDG (1.30±0.005), p=<0.001.

The fetal pancreas volume estimation through cavalier method water immersion (final volume) method in control group (2.66±0.091) in trimester one was significantly higher than the (LDG 2.00±0.013), MDG (1.71±0.031), HDG (1.26±0.004), p=<0.001.

However, shrinkage in control group (0.021±0.002) in trimester one was found to be insignificantly higher than the, LDG, (0.033±0.002), MDG (0.028±0.004), HDG (0.025±0.004), this was indicated by a significant p-value (p=<0.308).

Trimester two (TM2) findings on the Primary Pancreatic Volume, Final Pancreatic Volume andPancreatic Shrinkage When dexamethasone was administered in trimester two (TM2), it was observed that primary pancreatic volume, final pancreatic volume and shrinkage as well a dose response relationship in the all the three parameters decreased with the increasing doses of dexamethasone treatment (table 2). The comparative intragroup and intergroup comparisons also shown that all values were statistically significant difference by both P-values (P<0.001) as well as by F-values between LDG, MDG and HDG against control .

Table 2:The TM2 intra and inter group comparative on the Primary Pancreatic Volume, Final Pancreatic Volume andPancreatic Shrinkage between the dexamethasone treated groups LDG, MDG, and HDG against the control

Variable	Control	LDG(0.5mgkg/bt)	MDG(2mgkg/bt)	HDG(4mgkg/bt)	F value	P-value
Primary Pancreatic Volume	2.91±0.087a	2.19±0.020b	1.91±0.023c	1.57±0.026d	101.714	<0.001*
final Pancreatic Volume	2.6±0.091a	2.15±0.020b	1.87±0.027c	1.54±0.026d	89.026	<0.001*
Pancreatic Shrinkage	0.021±0.002a	0.015±0.0006a	0.022±0.005a	0.021±0.003a	1.235	0.308

*Key: the means, followed by the same letter in a row are not statistically different at (P<0.05) using one-way ANOVA.with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).*

Table 2 indicates that the fetal pancreas volume estimation through water immersion (primary volume) method in control group (2.91±0.087) in trimester two was significantly higher than the LDG (2.15±0.020), MDG (1.87±0.027), HDG (1.54±0.026; p=<0.001.

The fetal pancreas volume estimation through cavalier method water immersion final volume) method in control group (2.66±0.091) in trimester two was significantly higher than the LDG (2.15±0.020); MDG (1.87±0.027); LDG (1.54±0.026); p=<0.001.

However, shrinkage in control group (0.021±0.002) in trimester two was found to be insignificantly higher than the, LDG (0.015±0.0006); MDG (0.022±0.005); HDG (0.021±0.003), this was indicated by a significant p-value (p=<0.308).

Trimester three (TM3) findings on the Primary Pancreatic Volume, Final Pancreatic Volume and Pancreatic Shrinkage

When dexamethasone was administered in trimester three (TM3), it was observed that primary pancreatic volume, final pancreatic volume and shrinkage as well a dose response relationship in the all the three parameters decreased with the increasing doses of dexamethasone treatment (table 3). The comparative intragroup and intergroup comparisons also shown that all values were statistically significant difference by both P-values ($p<0.001$) as well as by F-values between LDG, MDG and HDG against control.

Table 3: The TM1 intra and inter group comparative on the Primary Pancreatic Volume, Final Pancreatic Volume and Shrinkage between the dexamethasone treated groups LDG, MDG, and HDG against the control

Variable	Control	LDG	MDG	HDG	F value	P-value
Primary Pancreatic Volume	2.91±0.087a	2.48±0.025b	2.17±0.028c	1.78±0.056d	67.856	0.021*
Final Pancreatic Volume	2.66±0.091a	2.24±0.107b	2.10±0.033b	1.74±0.026c	26.941	0.011*
Pancreatic Shrinkage	0.021±0.002a	0.096±0.043a	0.036±0.004a	0.025±0.003a	2.650	0.060

*Notes: The means, followed by the same letter in a row are not statistically different at (P<0.05) using one-way ANOVA, with Tukey test on post-hoc t-tests. * indicates significance (p<0.05).*

The results in the fetal pancreas volume estimation through water immersion (final volume) method in control group (2.91±0.087a) in trimester three was significantly higher than the LDG (2.48±0.025); MDG (2.17±0.028); HDG (1.78±0.056); p=<0.001

The fetal pancreas volume estimation through cavalier method water immersion (primary volume) method in control (2.66±0.091) group in trimester three was significantly higher than the LDG (2.24±0.107); MDG ;(2.10±0.033); HDG (1.74±0.026); p=<0.001.

However, shrinkage in control group (0.021±0.002) in trimester three was found to be insignificantly higher than the, LDG (0.096±0.043); MDG (0.036±0.004); HDG (0.025±0.003) this was indicated by a significant p-value (p=<0.308).

The trimester one (TM1) findings on pancreas volumes, islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and ducts density

When dexamethasone was administered in trimester one (TM1); it was also observed that pancreas volumes , islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and ducts density as well as dose response relationship in the all the parameters decreased with the increasing doses of dexamethasone treatment the comparative intragroup and intergroup comparisons also shown that all values were statistically significant difference by both P-values ($p<0.001$) between LDG, MDG and HDG against control (**table 4**) .

Table 4: The TM1 intra and inter group comparative pancreas volumes , isletsvolumes, acinivolumes density, stroma connective tissues density, blood vessels density and and ducts density of LDG,MDG,HDG against control

Stereological Parameters	Control N=	Low Dexamethasone group (TM1) N=10	Medium Dexamethasone group (TM1) n=10	High Dexamethasone group TM1: n=10	P -value
Total Pancreatic volume(mm3)	2.66±0.03	2.00±0.01*	1.71±0.04*	1.26±0.05*	0.003
Volume density of islets (mm^3)	0.053±0.002	0.04±0.001*	0.023±0.005*	0.025±0.002*	0.001
Volume density of acini (mm^3)	2.12±0.2	1.6±0.02*	1.37±0.01*	1.008±0.01*	0.003
Volume density of Connective tissue stroma (mm3)	0.34±0.01	0.43±0.01*	0.45±0.01*	0.53±0.01*	0.031
Volume density of Blood vessels (mm3)	0.044±0.01	0.054±0.01	0.059±0.01	0.061±0.01	0.001
Volume density of ducts (mm3)	0.039±0.003	0.03±0.03*	0.02±0.002*	0.018±0.01*	0.001

Table5: The TM2 intra and inter group comparative on pancreas volumes , islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and and ducts density of LDG,MDG,HDG against control

Stereological Parameters	Groups				p-value
	Control N=10	**Low Dexamethasone group (TM2) N=10**	**Medium Dexamethasone group (TM2) n=10**	**High Dexamethasone group TM2: n=10**	**p-value**
Pancreatic volume(mm3)	2.66±0.03	2.28±0.01	1.82±0.04*	1.73±0.05*	0.031
Total islet volume (mm^3)	0.053±0.002	0.045±0.001*	0.036 ±0.005*	0.035±0.002*	0.042
Volume density acini	2.12±0.2	1.82±0.02*	1.45±0.01*	1.38±0.01*	0.003
Volume density Connective tissue stroma(mm3)	0.34±0.01	0.42±0.01*	0.43±0.01*	0.49±0.01*	0.024
Volume density Blood vessels(mm3)	0.044±0.01	0.051±0.01	0.055±0.01	0.058±0.01	0.036

Volume density ducts(mm3)	0.039±0.003	0.034±0.03*	0.027±0.002*	0.025±0.01*	0.012

*Values are Mean ±SE. N stands for number of rats in each group. CoE for point counting in each measurement are less than 0.05*P< 0.01 when compared with the control, low Dexamethasone group and medium Dexamethasone groups.*

The Pancreatic volume in control group trimester two (2.66±0.091) was significantly higher than the LDG (2.15±0.020); MDG (1.87±0.027); LDG (1.54±0.026); p=<0.001.
During the second trimester, the total islet volume in the control group (0.053±0.002) was found to be significantly different from that in the low dose group (0.045±0.001), the medium dose group (0.036±0.005) and the high dose group (0.035±0.002), p=<0.001.

The trimester three (TM3) findings on pancreas volumes, islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and ducts density

When dexamethasone was administered in trimester three (TM3); it was also observed that pancreas volumes , islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and ducts density as well as dose response relationship in the all the parameters decreased with the increasing doses of dexamethasone treatment in (table 6) the comparative intragroup and intergroup comparisons also shown that all values were statistically significant difference by both P-values (p<0.002) between LDG, MDG and HDG against control .

Table 6: The TM3 intra and inter group comparative on pancreas volumes , islets volumes, acini volumes density, stroma connective tissues density, blood vessels density and and ducts density of LDG,MDG,HDG against control

	Groups				
Stereological Parameters	Control N=10	Low Dexamethasone group (TM3) n=10	Medium Dexamethasone group (TM3) n=10	High Dexamethasone group TM3: n=10	P- value
Pancreatic volume(mm3)	2.66±0.03	2.59±0.09	2.13±0.06*	2.03±0.06*	0.003
Total islet volume (mm³)	0.053±0.002	0.05±0.001*	0.023±0.005*	0.025±0.002*	0.011
Volume density acini (%)	2.12±0.2	1.6±0.02*	1.37±0.01*	1.008±0.01*	0.001
Volume density Blood vessels	0.044±0.01	0.051±0.01	0.055±0.01	0.058±0.01	0.041
Volume density Ducts (%)	0.039±0.003	0.038±0.01	0.031±0.01	0.03±0.003	0.002
Volume density Connective tissue stroma (%)	0.34±0.01	0.37±0.01*	0.39±0.01*	0.42±01*	0.043

DISCUSSION

Histostereological findings in this study on both the parenchymal and stromal tissues show that there was significant increase P< 0.005 in connective tissue deposition, across the entire pancreases (head, body and tail regions). Inaddition, there was a significant reduction in the total islet volume and islet masses, reduced acinar sizes and number of cells per acini with thickening of stromal tissue septations in all dexamethasone groups. The overall total pancreatic volumes were also seen to be significantly reduced in all dexamethasone treated groups with marked significant decrease ($p<0.05$) in volume densities of islets, acini, blood vessels as well as volume density the ducts in the all the dexamethasone treated groups (LDG, MDG, HDG) when compared with control (table 1-6). These teratogenic effects of dexamethasone on the developing fetal pancreas were also noted to be time and dose dependent with the most injurious effects experienced in the first and second trimester (TM1 and TM2) respectively in all the dexamethasone treated groups.

This is in conformity with studies that reported that administration of high dose glucorticoids caused atrophy and apoptosis in pancreatic endocrine cells [23,22]. Impairments of the massive volume of apoptotic cells in the dexamethasone induced rats pancreas on various endodermal derived cell types is due to a raised manifestation of the anti-apoptotic factors Bcl-2, Bcl-xL in human and rat hepatocytes [26,27]. Moreover, laboratory studies have proved that dexamethasone impairs glucose metabolism which resulted to a decrease of islets numbers insulin resistance, reduction of B cell mass, induce increased B-cell proliferation in islet of pancreatic and the hypersecretion of somatostatin, amylin and ghrelin, [28, 29, 30, 31, 32, 33].

In the current study the total islet volume densities of the pancreas were observed to reduce in the dexamethasone treated groups in a dose and time dependent manner. This effect was also reported in studies that shown that dexamethasone treated groups exhibited decrease in the total islet volume during physiological growth leading to islet cells atrophy [34, 35, 36, 37].

CONCLUSION

In conclusion, dexamethasone is teratogenic to the developing fetal pancreas as it causes significant reduction $P<0.005$ in both the parenchymal and stromal tissues of the fetal pancreas. These teratogenic outcomes were also observed to be time and dose dependent in that dexamethasone exposure in the first trimester presented the best "window of opportunity" for expression of dexamethasone teratogenesis' to the developing fetal pancreas in albino rats and particularly when administered in high doses of 4gms/Kg/Bwt per day.

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