MATERNAL DEXAMETHASONE USE AND RISK OF CONGENITAL ANOMALIES AND PRE-TERM BIRTHS IN RATS

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Abstract: Currently, the use of dexamethasone in treatment of Covid-19 pandemic have become more intenseincluding patients of reproductive age and pregnant mothers.Dexamethasone is also commonly usedduring pregnancy as a fetal lung enhancer, correcting congenital malformation such an adrenal hyperplasiaas well as treatment of various maternal diseasesduring fetal development.Continueduse of this drug will continue to pose a risk to inducing injurious effects to the developing fetal organs.

The objective of this studywas todeterminingthe maternal dexamethasone use and risk of birth defects and pre-term births in rats.Pregnant ratswere given varied doses of oral dexamethasone during the first, second and third trimester. Daily maternal weight and food intake were recorded. The rats were sacrificedon day 20th day of gestation.A total of 158 fetuses were found to have congenital malformations whose occurrence was more pronounced in the first and second trimesters in all the dexamethasone treated groups. However, most of this malformation were majorly observed in the High dose dexamethasone groups (HDG) followed by the medium (MDG) and the low dose dexamethasone (LDG) treated groups respectively

Conclusion Prenatal administration of dexamethasone impaired fetal embryogenesis which is dependent on gestation period and amount of drug ingested. Dexamethasone when given had negative effects on the fetal organs such as a kidney, bones, placental weight, brain, organs.

Keywords: glucorticoids, dexamethasone, congenital anomalies

INTRODUCTION

Fetal therapy by use of glucorticoids have in last four decade been in used for treatment of various disease during prenatal period and newborns[1,7–9]. Dexamethasone have shown to be more efficient than betamethasone during preterm birth in maturing of fetal lung surfactant production and maturation of the fetal lung, especially to mothers who are prone to preterm deliveries pregnancy[1]. A single course of dexamethasone is given to mothers at gestation period between 24 weeks and 34 weeks with ruptured membrane and 23 weeks to 34 weeks who are at risk of preterm deliveries and between one to 7 days before delivery of multiple pregnancies[1011].

Dexamethasoneis a very potent anti-inflammatory and immunosuppressive drug and thisdrug is necessary for treatment of maternal conditions during in pregnancy. Generalindications for corticosteroidtreatment during pregnancyinclude; asthma, inflammatory bowel diseases, rheumatoid arthritis and the treatment of the current covid -19 pandemic indicatingincreased use of dexamethasone in pregnancy. Corticosteroids have beensuspected to be teratogenic, although the existing evidence about teratogenicity of corticosteroids is uncertain.

Glucorticoids have copious detrimental effects to the developing organs of the developing fetuses encompassing muscles, liver, kidneys, brain, lung, ,placenta, spleen and heart[1]. For instance, when administered during early pregnancies leads to placenta insufficiency by inhibiting placental VEGF expression [1].In brain, leads to decline of the blood brain barrier permeability, reduction of fetal cerebral blood flow , hypoxia of brain, reduction in hippocampal sizeto learning and attention disorders[2,3]. In the kidneys, it causes diminution of nephron and glomerular of the kidney and reduction of the glomerular number resulting glomerulosclerosisand hypertension[45,6].Clinically ,prenatal dexamethasone reduces neonatal mortality, short term respiratory morbidity, severe neurological deficit and, necrotizing enterocolitis [4,8,1012]. Dexamethasoneits ability to cross the placenta have helped in treatment of virilizing congenital adrenal hyperplasia(CAH) which is an autosomal recessive disorder of steroidogenesis, caused by lack of 21-hydroxylase[13,14].The prenatal treatment of CAH is dispensed on5th week of intrauterine period, when genitalia are developing to stabilize androgen precursor[1,13,15]. Clinical studies on human

have also shown, dexamethasone is clinically effective in treatment of third-degree heart block[4,16],also a drug of choice in treatment of Congenital cystic adenomatoid malformations [17,18].Dexamethasone has also been shown to be effective in decreasing risk of periventricular leukomalacia in low birthweight (≤1.75 kg) infants[19]. Contrarywise, to its numerous clinical important, dexamethasone have also numerous adverse effects. this have been reported on studies done on animal and human studies to the fetus[20] .Dexamethasone use for instance contribute to multifarious metabolic effects like glucose intolerance, hyperglycemia which could have teratogenic effect to the organsof the developing embryo or fetus[21,22]. Studies done on animal and human have proved that prenatal dexamethasone impedes the metabolism of the developing fetus resulting to low birth weight, intrauterine growth retardation, thin fetuses, increased hypothalamopituitary adrenal axis activity, reduced brain growth with delayed myelination and hypertension[2,3,23–26].

MATERIAL AND METHODS

Animals

Pregnant ratsweighing between 150g to 200 grams were resourced from SAFARI animal in Jomo Kenyatta University of Agriculture and Technology (JKUAT). They were harbored in standard rat cages and subjected to 12-hour dark cycles under humid tropical conditions 24°C at the same resource. The cages were indicated with a cage label showing experimental name of the animal, initial date of experiment, prescribed amount of the drug, Age, total sum of experimental rats, type of the rat. The rats were allowed limitless access to standard feed Rodent pellets resourced from UNGA Mills as founded by American institute of nutrition (1977 (Unga feeds Kenya). and water ad libitum throughout the experimental period. The rats were carried out in carried out with the guiding principles of laboratory animals 'principles. Two females were instituted to one male albino rat put into a cage overnight. The next day, the males were taken back to their individual cages.Vaginal smears were taken from the 12 mated females the next morning and pregnancy was determined by the presence of spermatozoa in the smears followed by vaginal wash twenty four hours later to determine changes in estrous which will denote the first day of gestation (GD1)[27,28]. The animals were operated solitary by the skilled investigator associate for the determination of procurement each day weights, consumptions and dexamethasone dispensation. All animals were killed on day 20th using carbon dioxide gas asphyxiation[29]. After pregnancies were established, animals were randomly assigned to either the control or the experimental group. The 36 rats in the experimental category were divided into three marked study groups of 12 rats each allotted low dexamethasone group (LDG), medium dexamethasone group (MDG) and high dexamethasone group (HDG). Each of the marked subgroups was further subdivided into first trimesters (TM1), second trimester (TM2) and third trimester (TM3) trimesters comprising of 4 rats each. All animals received rodent pellets and water ad libitum.Dams were given peritoneal dexamethasone via intragastric gavage (Gauge 1.8 2R2 needle) at through the pregnancy period in first trimester, second trimester and third trimester[30] .

Feeding and Prenatal Dexamethasone dispensation

All experimental group received oral dexamethasone dissolved in normal saline via gastric gavage (Gauge 1.8 2R2 needle) and rodent pellets and water ad libitum between 8:00 am to 9: am.The control group received only the rodent pellets and water ad libitum between 8:00am to 9: am.The dexamethasone groups received (LDG 0.5mg/kg/d, MDG 5mg/kg/d, HDG 7 mg/kg/d) during the gestation period in first trimester, second trimester and third trimester. The dosage used in this study have been found to be comparable with human dose used during pregnancy (0.5-10mg/kg).The ratsin trimester 1 received dexamethasone treatment from day one of gestation all through to day 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 ratsin trimester III start receiving the dexamethasone treatment from day 14 all through to- day 20 the last day of gestation.

Calculation of the dexamethasone doses:

Calculation of Albino rats Equivalent Dose weighing average 150g. (Nair and Jacob, 2016).

Dexamethasone dosage in human

Dexamethasone is given in usual doses of 0.5 to 10 mg daily, depending on the disease being treated. In more severe disease conditions doses above 10 mg per day may be required. (Arifin and Zahiruddin, 2017b)

Antenatal dexamethasone 12 mg for five days (Grgić, Fatusić and Bogdanović, 2003)
AED (mg/kg) =human dose (mg/kg) ×Km ratio.
AED (mg/kg) in low dose=0.5mg/kg× (37/7) =2.6mg/kg.
2.6 mg/kg given to a rat weigh average weight of 150g=
= (2.6×150)/1000
=0.4 mg/kg

AED (mg/kg) in medium dose =4 mg/kg× (37/7) =21.2 mg/kg.
10 mg/kg given to a rat weigh average weight of 150g=
= (10×150)/1000
=3mg/kg

AED (mg/kg) in high dose=12mg/kg× (37/7) =63.6 mg/kg.
21mg/kg given to a rat weigh average weight of 150g=
= (21×150)/1000
=9.5 mg/kg.

Ethical approval

The ethical approval was obtained from JKUAT Animal Ethical Committee (AEC) before commencement of the study [29]

RESULTS

Statistical analysis

The study sought to analyzedexamethasone fetal outcomes. The data was analyzed using SPSS and Excel statistical software and was expressed as mean ± standard error (SEM). The study compared how the three dose levels (Low, medium and high dexamethasone groups) and control in the three trimesters (TM1, TM2 and TM3), affected the different parameters. To determine the significance, a one-way analysis of variance with Tukey post hoc test was used and 5% significance level (α = 0.05) was assumed. The results were considered to be significant whenever the probability value (sig. value) is less than 0.05 ($p<0.05$). The results were presented below per each trimester.

Fetal congenital malformations

From Table 1 below, a total of 158 fetuses were found to have musculoskeletal congenital malformations whose occurrence was more pronounced in the first and second trimesters in all the dexamethasone treated groups. However, most of this malformation were majorly observed in the High dose dexamethasone groups (HDG) followed by the medium (MDG) and the low dose dexamethasone (LDG) treated groups respectively. In addition, the teratogenic induction of these malformations was also seen to be more pronounced when dexamethasone was administered the first and the second trimester (TM1, TM2)

Table 1; Shows the types and the distribution of congenital anomalies observed in the LDG, MDG & HDG compared with the control when treatment was done at TM1, TM2 AND TM3

Type of congenital	Number of fetuses	Distribution of fetuses with anomaly across the study group				Percentage out of the total number of fetuses
		HDG	MDG	LDG	Control	
Cleft palate and lip	15	8	4	2	1	25%
syndactyl	10	4	3	2	1	17%
Limb Amelia	10	4	3	2	1	17%
Limb's hypoplasia	3	1	1	0	0	5%

Maldevelopment of the anterior abdominal wall	9	5	3	2	0	15%
microcephaly	2	1	0	0	1	3%
Congenital club foot	9	4	3	1	1	15%
Total	58	27	17	9	5	100%

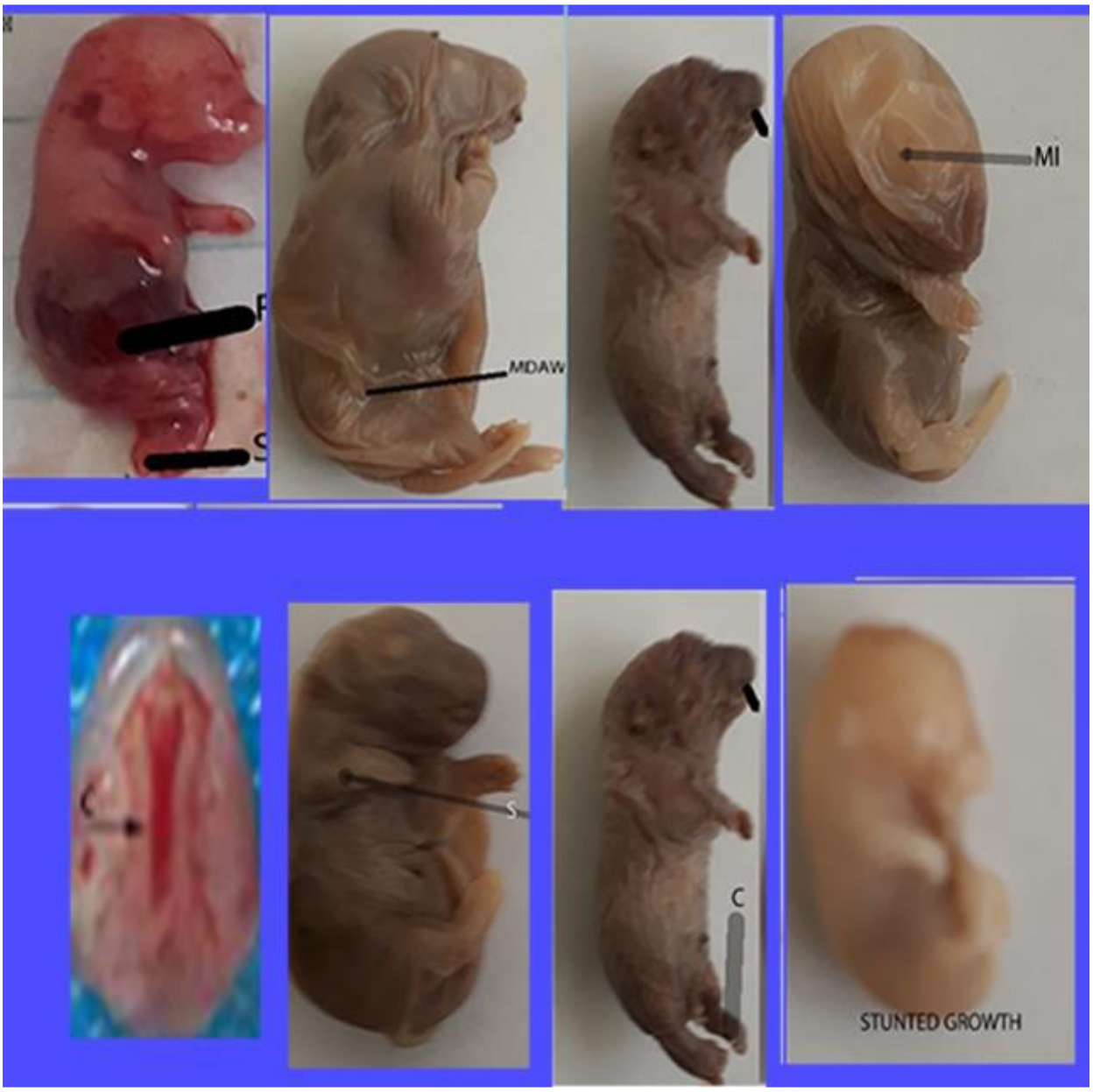

Figure 4:1: A photograph showing ventral view and lateral view of fetuses with congenital anomalies observed from the LDG, MDG, and HDG.

{KEY: A: cleft palate; F: failure of closure of anterior abdominal wall; S: syndactyl; MDAW: mal-developed anterior abdominal wall; C: congenital club foot; Stunted growth; M-microcephaly}.

Table 2: Showing the liner correlation by Pearson and P values of the maternal pregnancy outcomes for the dexamethasone treated groups (LDG, MDG and HDG) against control in the TM1, TM2 and TM3

		Mean weight gain	Mean little size	Mean resorbed fetuses	Mean placental weight	Mean dead fetuses
Mean weight gain	r	1				
	P					
Mean terminal maternal weight	r	.177				
	P	.303				
Mean little size	r	.603**	1			
	P	.000				
Mean resorbed fetuses	r	-.722**	-.403*	1		
	P	.000	.015			
Mean placental weight	r	.715**	.457**	-.773**	1	
	P	.000	.005	.000		
Mean dead fetuses	r	-.639**	-.470**	.731**	-.843**	1
	P	.000	.004	.000	.000	

NB: r is the Pearson's correlation coefficient, P is the p-value, * and ** indicate significance i.e. $p<0.05$

The intra and intergroup liner Correlational analysis of the maternal and fetal pregnancy outcomes

When the linear correlation analysis was done within and across the dexamethasone treated groups on the maternal and fetal pregnancy outcomes to establish the correlation significance levels, the strength and direction of the linear relationship on the maternal and fetal pregnancy outcome parameters namely: initial maternal weight, terminal maternal weight, weight gain, litter size, Resorbed fetuses, Placenta weight, and Dead fetuses shown a strong linear relationship between all this variables when dexamethasone was administered at trimester one and two (TM1 & TM2) variables across and within groups. A value between 0.3 and 0.5 (**table 2**) indicates a moderate linear relationship while a value below 0.3 indicated a weak relationship, an absolute correlation value of 0.5 and above indicated weak relationship. On the other hand, a Pearson correlation value of **0** as indicated in the table 4.5 below indicates absence of a linear relationship between the stated variables. The direction of the relationship is indicated by the sign of the Pearson Correlation value(r). Finally, the significance of the relationship was achieved through p-values of ($P<0.05$). where all relationships in table 4.5 below that were less than 0.05 at 95% confidence level did indicates that the linear relationship between variables of maternal and fetal outcomes were statistically significant and vice versa. indicates a moderate linear relationship while a value below 0.3 indicated a weak relationship, an absolute correlation value of 0.5 and above indicated weak relationship. On the other hand, a Pearson correlation value of 0 as indicated in the table 4.5 below indicates absence of a linear relationship between the stated variables. The direction of the relationship is indicated by the sign of the Pearson Correlation value(r). Finally, the significance of the relationship was achieved through p-values of ($P<0.05$). where all relationships in table 4.5 below that were less than 0.05 at 95% confidence level did indicates that the linear relationship between variables of maternal and fetal outcomes were statistically significant and vice versa.

DISCUSSION

Findings from the cohort studies suggested that exposure to corticosteroids in the first trimester of pregnancy may be associated with a marginally, but not statistically significantly, increased risk of major malformations.

In this study, the effects of antenatal exposure to wide range of glucorticoids levelwere observed to have negative manifestation to the developing fetus. Prenatal administration of dexamethasone caused congenital anomaliessuch

cleft palate and lip, syndactyl, limb Amelia, limb's hypoplasia, maldevelopment of the anterior abdominal wall, microcephaly, and congenital club foot. These prejudicial effects were consequently observed to be dependent on the gestation period and amount of the dexamethasone administered. The major impairments were observed in the first and second trimester and in low and high dexamethasone doses. From this study, when dexamethasone is given throughout the gestation period at high doses leads to major detrimental effects to both fetus and the mother wellbeing, this is controversial to what had been described before[31].

From this finding it shows that thecleft palate and cleft lip were the most commonly reported anomaly this is in agreement with a systematic study that reported more cleft lip as compared in other anomalies [33–35].According to these study maldevelopment of the anterior abdominal wallwas the second highest congenital anomalies this is contributed by the factor that intrauterine glucorticoids impair development of the intestines especially small intestine resulting to immobility of the gut[36].Dexamethasone effects caused reduced brain growth with delayed myelination and hypertension[2,3,23–26].additional effects on the restrict growth of the brain include ;decline of the blood brain barrier permeability, reduction of fetal cerebral blood flow ,increased hypothalamopituitary adrenal axis activity, , hypoxia of brain, reduction in hippocampal sizeto learning and attention disorders[2,3].Feng et al found that prenatal dexamethasone exposure on day 16 to 18of embryogenesis led to reduced fetal body weight and intrauterine growth retardation thus explaining the direct effect growth restriction[37]

The findings of this study on the mean fetal weight gain throughout the entire gestation period were seen to depict an inverse dose response relationship in that with the increasing dexamethasone doses there was significant reduction ($P<0.03$) in mean maternal weight gain (table 2). This reduction in mean fetal weight gain and fetal pregnancy outcomes distinctly reveals that the maternal and fetal pregnancy outcome parameters are an important indicator to the kind of perturbations caused in the development of the fetal organs like the pancreas. This study established that there is a very strong correlation (0.3-0.5) between the maternal and fetal pregnancy outcomes with the overall gross, histo-morphological and stereological teratogenic outcomes to the developing fetal pancreas. This is in agreement with a study done on rats and humans which reported that glucorticoids leads to decline in progesterone hormone upsurge, prostaglandin synthetase activity and prostaglandin F2α generation in early pregnancy, leading to the abortions, resorption of fetuses and dead fetus (Yahi et al.,2017).

For instance, in this study it was established that any increase in the dexamethasone, had a subsequent significant ($P<0.05$) reduction in all fetal growth parameters including the mean little size, mean resorbed gland, mean placenta weight, mean crown-rump and the mean percentage embroylethality. This was observed to have a dose response relationship in that these parameters decreased with the increasing dexamethasone treated groups (LDG, MDG and HDG) (table 4.2 and 4.3 respectively). This is also in agreement with findings of a study that reported that glucorticoids inhibits hypothalamic corticotropin-releasing-hormone resulting in reduction in body weight set point[38,39,22,40,41,42].

Maternal glucorticoids to fetus are protected by a barrier in consist majorly of 11β hydroxysteroid dehydrogenase type 2 enzyme that converts exogenous and endogenous glucorticoids into their inactive 11 keto metabolite[7,13,22]. Another factor that contributes to protect the fetus from excess glucorticoids is p glycoprotein 1(p-gp1)which is a multidrug resistant protein[1] .as the pregnancy progresses towards parturition the effect of 11β hydroxysteroid dehydrogenase type 2 enzyme declines exposing the fetus to maternal glucorticoids thus explain the reason for poor development of fetus at last gestation period([1,9,45–47]). Research done on pregnant ewe administered with intramuscular dexamethasone on 40th and 41th day of gestation period showed decline in number of binucleate cell (BNCs), increased Bax p53 and impaired placenta apoptic markers, sex-specific impairment of in placental development affecting the fetal growth which may lead to intrauterine growth retardation ([7,9,46])

CONCLUSION

The administration of prenatal dexamethasone has tremendous benefit from preventing the loss of huge of pregnancies, fetal mortality and preventing significant maternal morbidity especially in prevailing covid-19 pandemic among others. However, it is also clear that prenatal dexamethasone is not harmless, and may have substantial and sustained irreversible adverse effects on fetal well-being. In conclusion this study has established that prenatal exposure to dexamethasone is teratogenic to the developing fetal pancreas and these teratogenic outcomes are dose and time dependent. The critical dose of dexamethasone teratogenicity was found to be the high and medium dexamethasone dose when exposed at first and second window period. Such effects of dexamethasone on fetus

born to mothers may predispose to congenital anomalies in postnatal period. LDG and MDG trimester three had no significant outcomes except when administered on high doses. The most vulnerable window period for dexamethasone teratogenicity was however established to be the first trimester while the most critical dose was 9.5mg/kg/bwtwhich is equivalent to human dose of 12mg/kg/bwt.

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