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Growth and Development Effects Following In Utero Exposure to Varied Doses ofEnarapril in Albino Rats (*Rattus Norvegicus*)

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Abstract: In utero exposure to Enarapril; a widely prescribed antihypertensive due to its success as a monotherapy, cost effectiveness, relative paucity of side effects, bone, cardio and nephron protective effects in comparison to standard therapy for equal level of blood pressure control has been associated with foetal deleterious effects. These include renal failure, intrauterine growth retardation, hypocalvaria, persistent patent ductus arteriosus and cerebral complications. It blocks Foetal renin- angiotensin- system that comes to play during the second and third trimesters of pregnancy documenting it safe during first trimester. In contrast, other studies reports major deleterious effects during the first trimester including cardiovascular, nervous, and renal and limb defects effects yielding conflicting report on the most critical periods. This has seen Enarapril labelled as class D drug by American FDA, a rationale based on data extrapolated only in single-case reports or in small case series, with no confirmed effects of specific drugs, doses and durations, considerations of severity of hypertension or gene mutations of the RAS System
This experimental studyusedthirty gravid rats. Three were controls and twenty-seven grouped into three different trimesters of nine animals each. These subgroups divided into three groups of three animals each, receivedconventional Enarapril dosages converted to animal equivalents of 20mg, 10mg and 5mg given daily for twenty one days, fourteen days and seven days for trimesters one, two and three respectively. All animals were sacrificed on day twenty-gestation, three fetuses of median weights from each rat was taken and a sample of ninety fetuses were evaluatedfor meanweights, kidney weights, and heart weights and head circumference.
This study elucidated that there seems to be little Enarapril specificity in the association between maternal use and an increased risk for foetal deleterious effects the main association being foetal systemic hypotension and foetal circulation disturbances following placental transfers that can equally occur with other classifications of antihypertensives.Notably, these effects were dose but not time dependent.

I. INTRODUCTION

Enarapril, an antihypertensive drug in the class of angiotensin converting enzyme inhibitors is widely prescribed in management of essential and gestational hypertensiondue to its success as a monotherapy, cost effectiveness, minimal side effects[1],cardio-nephron protective and bone healing effects[2,34]in comparison to standard therapy for equal level of blood pressure control in women of reproductive age. However, its use during the second and third trimesters, has been associated with foetal renal failure, intrauterine growth retardation, hypocalvaria, persistent patent ductus arteriosus and cerebral complications[567] by blocking Foetal renin- angiotensin- system that comes to play during the same perinatal period. In contrast, retrospective studies by cooper et al[8],reports major deleterious effects during the first trimester including cardiovascular, nervous, and renal effects conflicting previous literature that Enarapril is safe during first trimester[9–11]. Nephrogenesis in humans commences by gestation day 22[12] and teratogens during this period supports the theory that first trimester exposure is not safe. Enaraprilcontraindication in pregnancy is un justified considering that the standardizing studies for this rationale was extrapolated only in single-case reports and in small case series, with no confirmed effects of specific drugs, doses, durations and not considering severity of hypertension or gene mutations of the RAS systems[13,14],depriving women of reproductive age all the benefits conferred by Enarapril.

II. Material and methods

Study Area: ***Safari,*** a small animal facility for Research and innovation in Jomo Kenyatta university of Agriculture and technology. The study lasted from August 2018 to January 2019.

Chemicals:

Conventional Enarapril maleate tablets of 5, 10 and 20 mg formulations from *Novartis* were converted to animal equivalent doses by multiplying with 6.2[15]. Appropriate concentrations of 1 ml/gm body weight wasprepared using distilled deionized water. Throughout the dosing period, rats' body weights determined daily Enarapril doses volumes.

Animalhusbandryand pregnancydetermination:

Mature Albino rats weighing 250-300 grammes purchasedfrom *safari;* a small animal facility for Research and innovation in Jomo Kenyatta university of Agriculture and technology were acclimatized for seven days. Later they were caged with males and separated on evidence of mating (copulatory plug or vaginal sperm)[16].This was designated gestational day (GD) 0. Gravid animals were housed under a 12-h light: dark cycle in polycarbonate cages with heat-treated wood shavings supplied as bedding, fed on standard feed Rodent pellets obtained from UNGA Mills and water ad libitum.

Experimental Design:

This was a randomized experimental study using thirty gravid albino rats sampled using resource equation method([17]). Twenty-seven experimental animals grouped into three different trimesters of nine animals each were Sub grouped into three groups of three animals each.

To evaluate the critical dose and period, each rat received conventional Enarapril dosages converted to animal equivalents[15] of 20mg as high Enarapril group,10mg as medium Enarapril Groups and 5mg as low Enarapril group given orally by gavage and daily for twenty one days, fourteen days and seven days for Trimesters one, two and three respectively. Control group of three animals received oral distilled deionized water. Three foetuses of median weights from each animal recruited in the study totalled to a sample size of ninety foetal rats.

All rats were examined throughout the experimental period for clinical signs of toxicity and in accordance withnational guidelines for animal care and use[18].

Humane ends: Ongestationday 20, all animals were euthanized using concentrated carbon dioxide and sacrificed by hysterectomy. Mean Fetal weights, Foetal head circumference, and weights of heart and kidneyswere determined.

Statistical analysis:

Data was analyzed using SPSS and Excel statisticalsoftware and expressed as mean ± standard error. (SEM)

The study compared how the three dose levels (Low, medium and high) and control in the three trimesters (T1, T2 and T3) affected each of these parameters.

To determine the significance, a one-way analysis of variance with Tukey post hoc test was used and 5% probability

Significance level ($\alpha = 0.05$) was assumed.

The results were considered to be significant whenever the value was less than 0.05 ($p<0.05$).

Ethical Approval:

The experimental protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Animal ethical Committee (JKUAT AEC) and was executed as per theNational Guidelines for Care and Use of Laboratory Animals in Biomedical Research[18]

III. Results

The decrease in, foetal weights, head circumference, heart and kidney weights occurred across all groups, was statistically different among the groups and they decreased as the dosages increased. However with high Enarapril dosing there were significant differences compared with control group across all trimester, ($p<0.05$).For example, high dose trimester one affected foetal weight in comparison to high dose trimester three that affected the mean kidney weights.

Post hoc analysis revealed differences across the trimesters but thisdifference was not statistically significant for these parameters in any particular trimester from control. ($p>0.05$).For example foetal weights $F(2,15) = 0.179$, $p=0.8$,head circumference$(2,15)=0.693$,$P=0.5$,mean kidney weights$(2,15)=0.113$,$P=0.8$ and heart weights $F(2,15)=0.5$,$P=0.615$) in all trimesters of all groups.

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 was less than 0.05 (p<0.05). The results were presented in Table 1.

Table 1; Comparison between Mean ± SD of different fetal parameters in control with LEG, MEG and HEG in the Trimester 1, 2, and 3 (TM1, TM2, and TM3) (Intergroup and Intragroup comparison)

GROUPS	CRITICAL PERIOD OF ENARAPRILT REATMENT	MEAN FOETALWEIGHTS ± SE	MEAN HEAD CIRCUMFERENCES ± SE	MEAN KIDNEYWEIGHTS ± SE	MEAN HEARTWEIGHTS ± SE
Control (C)		6.746± 0.03	4.386± 0.01	0.347±0.11	0.300±0.02
Lowdose Enarapril group (LEG)	**Trimester 1**	5.521± 0.01*	3.920±0.55*	0.311±0.003*	0.289±0.01*
	Trimester 2	5.905± 0.04*	3.609±0.11*	0.271±0.04*	0.276±0.03*
	Trimester 3	6.339±0.31*	4.265±0.256*	0.279±0.05*	0.282±0.03*
Medium dose Enarapril group (MEG)	**Trimester 1**	5.314± 0.27*	3.727±0.270*	0.279 ±0.05*	0.254±0.03*
	Trimester 2	5.489± 0.21*	3.376±0.234*	0.270±0.05*	0.257±0.01*
	Trimester 3	5.77± 0.55*	3.925±0.222*	0.254±0.05*	0.223±003*
High dose Enarapril group (HEG)	**Trimester 1**	3.073± 0.38*	1.609±0.34*	0.255±0.003*	0.91±0.01*
	Trimester 2	3.542± 0.46*	1.468±0.26*	0.112±0.026*	0.113±0.03*
	Trimester 3	3.757± 0.6*	1.955±0.38*	0.110±0.00*	0.125±0.06*

***Shows the figures that have a statistically significant difference compared with the controls p<0.05*
S.E=Standard Error

Fetal weight in the control group (6.74±0.03) was found to be significantly higher than that in the trimester one low dose group (5.521±0.11), trimester two low dose group (5.905±0.04) and trimester three low dose group (6.339±0.31), F (3, 8) p = < 0.0001. The weight in low dose trimester one, low dose trimester two and low dose trimester three was statistically different, but no statistical difference was between control and trimester three group. The head circumference under the control group (4.386±0.030) was found to be significantly higher followed by the low dose trimester three group (4.265±0.256), followed by the low dose trimester one group (3.920±0.001) and lowest in the trimester two low dose group (3.609±0.11). This was indicated by a significant p-value, p<0.0001 which was less than 0.05 significance level. No statistical difference was observed across all trimesters, but it was found to be lower in trimester one. Thekidney weights under the control group (0.347±0.11) was found to be significantly higher followed by the low dose trimester one group (0.311±0.037), followed by the low dose trimester three group (0.279±0.05) and lowest in the trimester two low dose group (0.271±0.04). This was indicated by a significant p-value, p<0.0001 which was less than 0.05 significance level. No statistical difference was observed between trimester one and three low dose groups but was lower in trimester two. The effect of the low dosage on mean heart weights in all trimesters was statistically significant with the lowest (0.271±0.04) in trimester two and highest (0.289±0.01) in trimester one. Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than trimester one medium dose group (5.314±0.027),trimester two medium dose group (5.489±0.021) and trimester three medium dose group(5.777±0.055) p=<0.0001. The results for the post hoc test also revealed that the fetal weight of medium groups in all the trimesters was not statistically different but was higher in trimester three. (4.115).Head circumference in the control group (4.386±0.01) was found to be significantly different (higher) than the medium dose trimester one group 3.727±0.270), the medium dose trimester two group (3.376±0.254) and the trimester thee dose group (3.925±0.22), p=<0.0001.Mean kidney weights in control group (0.347±0.11) was found to be significantly different (higher) than the medium dose trimester one group (0.279±0.05), the medium dose trimester two group (0.270±0.05) and the trimester three medium dose group (0.254±0.0045), p=<0.0001.Mean heart weights in the control group (0.300`±0.0001) was found to be significantly different (higher) than the medium dose trimester one group (0.254±0.003), the medium dose trimester two group (0.257±0.001) and the trimester three medium dose group (0.223±0.06), <0.0001. Fetal weight in the control group (6.73±0.026) was found to be significantly different (higher) than high dose trimester one (3.073±0.038), high dosetrimester two(3.542±0.46) and high dose trimester three (3.757.53±0.6), p=<0.0001. Head circumference in the control group (4.386±0.010) was found to be significantly different (higher) than the high dose trimester one group (0.1.609±0.34), trimester two high dose (1.468±0.03) and high dose trimester three group (1.955±0.06), p=<0.0001. kidney weights in the control group (0.347±0.11) was found to be significantly different (higher) than the high dose trimester one group (0.255±0.03), trimester two high dose (0.112±0.026) and high dose trimester three group (0.110±0.0001), p=<0.0001. Heart weightsin the control group (0.300±0.000) was found to be significantly different (higher) than high dose trimester one group (0.91±0.01), trimester two high dose (0.113±0.03) and high dose trimester three group (0.125±0.06), p=<0.0001.

The parameters were statistically different in all trimesters, and decreased as the dose increased. The results for the post hoc test revealed that no parameters were significantly different in all the trimesters from the control. Foetal weights F (2,15) = 0.179, p=0.8,head circumference(2,15)=0.693,P=0.5,mean kidney

weights(2,15)=0.113,P=0.8 and heart weights F(2,15)=0.5,P=0.615) in all trimesters of all groups. Again, these parameters were significantly different across all trimesters from control only in high dose group.), p=<0.0001.

IV. Discussion

Enarapril contraindication in pregnancy is un justified considering that the standardizing studies for this rationale was extrapolated only in single-case reports and in small case series, with no confirmed effects of specific drugs, doses, durations and not considering severity of hypertension or gene mutations of the RAS systems, [13,14],depriving women of reproductive age all the benefits conferred by Enarapril.

In the present study, Enarapril illustrated a decrease in all the fetal parameters including foetal weights, head circumference, kidney weights and foetal heart weights indicating a negative growth index to the fetuses. Although these were statistically different in all groups among all trimesters, statistically significant effectsoccurred in high dose groups and in different trimesters for different parameters. Mean foetal weights were lowest in trimester one of high dose group, head circumference in trimester one of high dose group, kidney weights in third trimester of high dose group and heart weights in trimester one of high dose group. Post hoc analysis did not reveal significance difference in foetal weights F(2,15) = 0.179, p=0.8,head circumference F(2,15)=0.693,P=0.5,mean kidney weights F(2,15)=0.113,P=0.8 and heart weights F(2,15)=0.5,P=0.615) in all trimesters of all groups from control. Although there was significant statistical differences of these parameters in almost all doses of all trimesters<0.05, average heart weight in low dose trimester three, mean kidney weights in high and medium doses in trimester one and two, and medium and low in third trimester had no significant difference with the control ,p=0.129,p=0.090 and p=0.190 respectively. These findings contradicts study done that second and third trimesters, has been associated with foetal renal failure, intrauterine growth retardation, hypocalvaria, persistent patent ductus arteriosus and cerebral complications[567]. This study shows that these effects occurs only in high doses with Mean growth retardation notably in trimester one of high dose group, hypocalvaria in first trimester of high dose group, renal failure in third trimester of high dose group and persistent ductus arteriosus in first trimester of high dose. The study also contradicts retrospective studies by cooper et al[8] that Enarapril is not safe during first trimester.

V. CONCLUSION AND RECOMMENDATIONS

It is conventional that in utero exposure to Enarapril is teratogenic and contraindicated in pregnancy despite its success as a monotherapy, cost effectiveness, minimal side effects[1], cardio-nephron protective and bone healing effects[2,34] in comparison to standard therapy for equal level of blood pressure control in women of reproductive age. This study shows the drug is safe in all trimesters in low and medium doses and only safe to specific parameters in high doses. A study is paramount to rule out hypertension complications associated with high dosesmanagement, gene mutations of the RAS systems and structural abnormalities through histoquantitive analysis to declare high doses of Enarapril teratogenic.

Amniotic fluid index monitoring to assess oligohydramnios is paramount in high Enarapril doses prescriptions in any trimester. Alternate antihypertensives have a role.

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