INSULIN INDUCED CONGENITAL MALFORMATIONS IN RAT

Rakesh Kumar Diwan*, G.L. Shah **
*Department of Anatomy, C.S.M.Medical University,Lucknow.
**Institute of Medical Sciences, Banaras Hindu University, Varanasi

ABSTRACT

In the present experiment, hypoglycaemia was induced by injecting Insulin, in primigravida Charles Foster (CF strain) female rats during early pregnancy and teratogenic effects were studied in new born.

On the 9th day of gestation, 20 I.U. of Insulin was injected intra peritoneally in the rats of study group (Average wt =200 gm). At the same time normal saline was injected in the pregnant rats of control group . Fetuses were collected on 20th day of gestation by laparotomy, and were examined by naked eye. Fetal mortality (7%) was observed in treated rats while it was nil in control group. 48% new born treated rats showed gross congenital malformations. There was significant growth retardation, cataract, exophthalmos, limb anomalies and haemorrhages over head region in treated fetuses.

The present study revealed that the maternal hypoglycaemia even for a brief period, during early pregnancy, induces teratogenic effects ranging fetal mortality to gross malformations. Its implications in the human being must be seriously taken in to account.

Keywords: Insulin, hypoglycaemia, congenital malformations, teratogenecity.

INTRODUCTION:

Lack of antenatal care and disturbances in fetal environment have been described to cause congenital anomalies since long, and now it is well accepted that these are responsible for about 70% of congenital malformations (Kalter's & Warkany, 1959)[1]

Cell death at critical points of development may lead to serious structural defects. Likewise excessive cell proliferation may induce malformations. Thus agents that interfere with the cell metabolism either by mitotic interference or alteration of nucleic acid function may affect cell proliferation to a greater or a lesser degree and initiate a mechanism of malformation.

The frequency of congenital malformations is much more in diabetic mothers as compared to normal. The patients of Type I Diabetes Mellitus during pregnancy, if treated with an optimal dose of insulin, show less number of congenital malformations. The teratogenic effect of hypoglycaemia during the early period of human pregnancy is uncertain (Mills et al, 1988)[2]. Kawaguchi et al (1994)[3] reported significant growth retardation of the embryos but no teratogenic effect of maternal hypoglycaemia on the rat embryos. Administration of an optimum dose of insulin was reported to be successful in reducing malformation rates in infants born to diabetic mothers Sadler and Horton, 1983)[4]. On the other hand the practice of intensive insulin therapy in such cases may pose a potential risk of life threatening hypoglycaemia, the syndrome of hypoglycaemic unawareness due to loss of normal counter regulatory hormonal responses to hypoglycaemia, which not only endanger maternal life but is also crucial for fetal development. The present work is an attempt to find out the gross malformations, induced due to maternal hypoglycaemia secondary to insulin administration in a rat model system.

It was first noticed by Landauer (1945)[5] that injection of Insulin in the yolk sac of chick embryos, resulted in absence of tail vertebrae and some of the synsacrocaudal vertebrae. Landauer and Bliss (1946)[6] found that increasing the dose of Insulin from 5 I.U. to 15 I.U. led to an increase of rumplessness from 4.5% to 31.7%. Landauer (1947)[7] reported that a different syndrome of defect is exhibited by embryo when the injection of Insulin is delayed. It affected the normal development of the beak, extremities and eyes. The developmental defects included short upper beak, micro-melia (shortening of extremities) and malrotation of limbs as well as anophthalmia,

Correspondence
Dr. Rakesh Kumar Diwan
Asst. Prof., Deptt. of Anatomy,
CSM Medical University
Lucknow (U.P.)
Mob. : 8005335622
Email : dewanrakesh80@yhaoo.com

microophthalmia etc.

It is suggested that many of the spontaneously occurring skeletal diseases of humans like osteogenesis imperfecta may be due to insulin in early pregnancy (Duraiswami, 1950)[8]. It is also observed that the frequency of children with congenital malformations is more with diabetic mothers as compared to normal. The present study was carried out to associate and confirm the effects of insulin on the developing embryoes of rats of foetal hypoglycemia by injecting insulin to mother.

MATERIALS AND METHODS:

Fifty female rats, of 3 months age (200-250 gm) of Charles Foster strain were used for the present experiment. Female rats were kept overnight with the males of the same stock (Female: Male=3:1). Vaginal smear were examined for sperms. The day of pregnancy was taken as Gestational day-zero (GDO). The pregnant rats were weighed daily and kept individually in separate cages. Out of 50, 38 pregnant rats were treated with single dose of 20 I.U. of Insulin and 12 pregnant rats were taken as control.

On GD20 pregnant rats were sacrificed with an overdose of ether anaesthesia. The uterine horns were exteriorized after opening the abdomen by midline incision. The sacs were inspected for sites of resorptions and viable fetuses. The fetuses were removed from the uterus and were blotted on blotting paper. After taking record of weight and C.R. length, the fetuses were observed for gross malformations.

RESULTS:-

No abnormalites could be detected in fetuses of control group collected on day 20 of gestation while, 48% of viable fetuses from treated group, showed congenital malformations. On gross examination of dissected, treated rats, there was fetus resorption in 7% cases as compared to that in control where there was no mortality (Table I). There was marked growth retardation in the insulin treated fetuses as compared to control fetuses (fig-1). The average weight of control fetuses was 4.36 gm while the average weight of treated group was 2.90 gm (Table II). At the same time there was marked reduction in CR length of treated fetuses as compared to control (Table III).

	Day of treatment	Total no. of experimental rats	Total no. of embryo	No. of embryo dead	No. of embryo living	No. of abnormal living embryo
Control	9th	12	108	0	108	0
Treated	9th	38	228	16 (7%)	212 (93%)	87 (48%)

Table-1
Teratogenic effect of Insulin (20 IU/rat) in developing rat embryos on 9th day of gestation (average weight of females rat = 200 gm).

	Control (n=108)	Treated (n=212)
Range	3.30-5.90 gm	2.20-4.40 gm
Mean	4.36 gm	2.90 gm
S.D.	0.70 gm	0.61 gm
t test	19.02	
P value	<0.001	

Table II
Effect of Insulin (20 IU/rat) on weight of rat embryos:

	Control	Treated
Range	2.60-4.80 (cm)	2.73-5.10 (cm)
Mean	3.880	3.577
S.D.	0.363	0.414
t test	3.855	
P value	P<0.001	

Table III
Effect of Insulin on C.R. length of rat embryos:

Defects	Control (Number)	Treated	
		Number	Percentage
Total no. of abnormal embryos	0	102	15.87%
Limb Anomalies	0	39	44.82%
Haemorrhage over body wall	0	28	32.18%
Thin anterior abdominal wall	0	14	16.09%
Exophthalmos	0	4	4.59%
Excencephaly	0	2	2.29%

Table IV
External malformation of rat embryos treated on day 9th of gestation with Insulin 20 I.U/rat (Percentage out of living abnormal embryos).

On gross examination, various external malformations were recorded in treated rats (Table IV). Limb anomalies were recorded as notable feature, torsional and angular deformities were observed specially in forelimbs .There was marked digital hypoplasia causing brachydactyly without any variation in the number of digits. In some treated fetuses, one of the forelimbs was developed incompletely (fig-2). A large number (32.18%) of fetuses showed haemorrhagic patches over face and head, exophthalmos along with wide palpebral fissure. Anterior abdominal wall was very thin in 16% fetuses and excencephaly was observed in 2.29 % fetuses (fig-3).

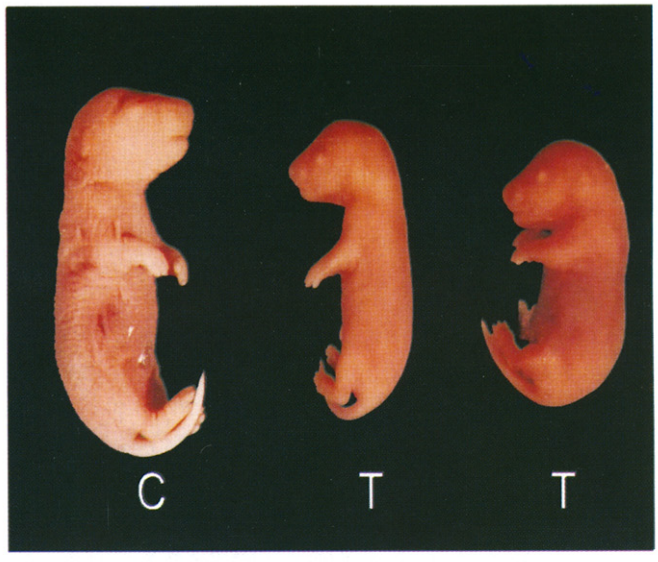

Fig-1: Photograph showing reduced size of treated embryos in comparison to control group (C=control. T=Treated group)

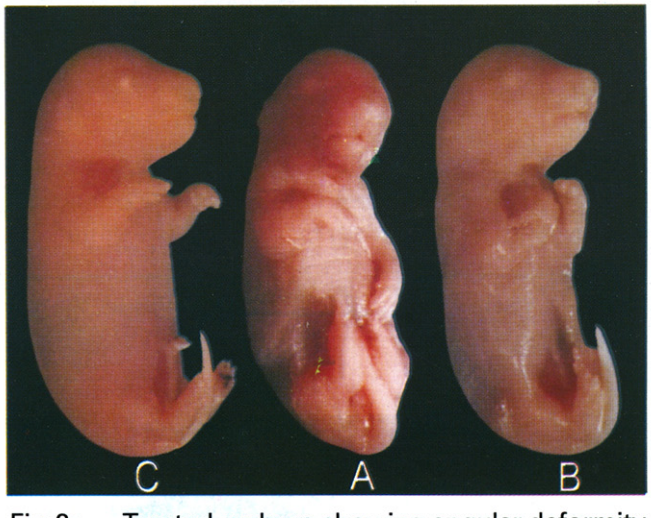

Fig-2: Treated embryo showing angular deformity of limb (A) with poorly developed upper limb (B). (C-control)

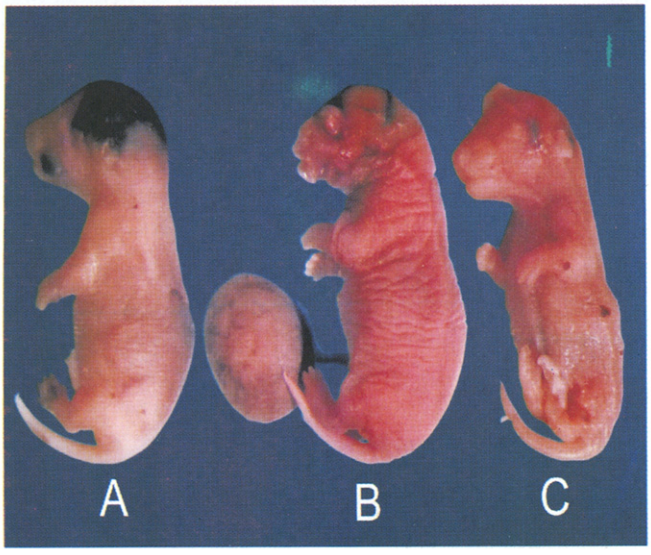

Fig-3: Treated embryo showing hemorrhages over cheek, upper jaw and parietal region of head (A); exophthalmos with wide palpebral fissure (B); thin anterior abdominal wall (C).

DISCUSSION :

In the present study, the findings of the fetuses of Insulin treated group were compared with those of control, to relegate the reported controversy regarding teratogenic effects of insulin. Sadler and Horton (1983)[4] and Buchanan et al (1986)[9] reported insulin to be non-teratogenic in different experimental animals including rat.

The limb defects observed in the present investigation could be due to direct effect of insulin or insulin induced hypoglycaemia, or an indirect one as secondary to primary effect on the central nervous system. Normal development in rats during the early phase of organogenesis of embryogenesis depends on uninterrupted glycolysis and its interruption leads to dysmorphogenesis (Freinkel et al, 1983[10] and Buchanan et al 1985). Duraiswami (1950)[12] drew attention to the disease osteogenesis imperfecta. Saddler (1995) reported cataract, microphthalmia due to maternal infection during embryonic period of gestation. Gene mutation is also reported to cause micro ophtalmia (Mueller young 1998).[13] In the present study however 4.59 % fetuses have exophthalmos.

Foetal stunting due to anoxia induced by uterine artery clamping was noted by Chinara (1980)[14]. Fetal stunting and microcephaly due to ischemia have also been observed by Wigglesworth (1964)[15]. The present investigation also showed stunting as evidenced by reduction in weight and C.R. length. Although the high percentage of malformation may be attributed to the small sample size, it is evident that maternal hypoglycaemia during early organogensis causes teratogenic effects in the rats. Therefore, during insulin therapy of pregnant mothers a careful monitoring of blood glucose is very very crucial.

If the embryo is not going to develop congenital malformations of untreated diabetic mother because of hyperglycaemia, it will develop defects due to uncontrolled hypoglycaemia if treated with insulin.

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