Foetal alcohol syndrome: an osteometric evaluation in the wistar rat animal model

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Abstract

Background: Foetal alcohol syndrome had been reported as a common feature in the off – springs of alcoholic mothers; and craniofacial and limb bones anomalies are usually some of the cardinal features of foetal alcohol syndrome (FAS).
Methods: These features were osteometrically assessed in the cranial and limb bones of the harvested foetuses of pregnant Wistar rats' models.
Results: Mal-developments and stunted growth with no evidence of post treatment reparation or recovery were observed in the ethanol – treated rats.
Conclusion: This observation is possibly indicative of another dimension at improving our understanding of the mechanism of the toxicity of ethanol.

Keywords: Foetal alcohol syndrome, bone anomaly, osteometry, wistar rat

Introduction

Ethanol is the main ingredient in the three classes of alcoholic beverages: distilled spirit, wines and beer. The use of alcoholic beverages by pregnant women has long been shown to have far reaching consequences on the phenotype and behaviour of the offspring such as in the foetal alcohol syndrome (FAS). [1] This syndrome is characterized by craniofacial, limb and cardiovascular anomalies as well as the pre- and post-natal growth retardation. and have been reported in the off - springs of alcoholic mothers. [1] Sequel to this observation, various skeletal abnormalities had been reported in experimental animal studies. [2 - 4] According to Krous, [5] such studies in relation to humans should be interpreted with caution, as information obtained from animals may not absolutely apply to Man.

This study reports the osteometric assessment of the craniofacial and limb bones in the fetuses of ethanol treated pregnant wistar rats as a model of foetal alcohol syndrome.

Materials and Methods

Ninety virgin healthy adult female wistar rats weighing between 200g and 250g were used in the study. They were bred in the animal holdings of our department, fed on rat pellets with clean drinking water provided liberally, and the room

kept clean. The animals were subsequently grouped into 2: A, control and B, experimental groups consisting of 45 rats each. They were caged in twos and a healthy adult male rat introduced into each cage for the purpose of mating. Confirmation of pregnancy and commencement of gestational dating followed the methods of Asling. [6]

Treatments

The experimental B rats were intubated with a dosage of 0.79g/kg of 30%v/v ethanol on days 9,10 and 11 of gestation, which coincide with osteogenic period in the rats, according to Murphy[7]. The dosage of ethanol is calculated from its equivalent g/ml weight. The animals were sacrificed on day 20 of gestation by chloroform inhalation; this was to prevent possible cannibalization of the fetuses at delivery[8]; and the fetuses were retrieved from either horns of the uterus, weighed, and fixed in 10% formol-saline. The foetal bones were dissected free of skin, soft tissue and viscera and stained in alizarin red S stains according to the methods of Dix. [9] To monitor the extent of calcification; the bones for osteometric assessment were disarticulated and air-dried, and with the aid of the vernier calipers, the methods of Edwards and Edwards [10] were adopted to quantitatively assess the craniofacial and limb anomalies from the following parameters:

1. Cranial length (G-H, Figure 1): the distance between the incisive foramen and the notch between the coronoid process and angular process.
2. Cranial width: The greatest distance between the lateral surfaces of the parietal bone.
3. Palate length (P-L, Figure 2): From the most posterior point on the palatine suture to the most anterior point on the pre-maxilla suture.
4. Mandibular length (G-H, Figure 1): The distance between the incisive foramen and the notch between the coronoid and angular processes.
5. Plane of anterior cranial fossa (A-C, Figure 1): From the anterior margin of the nasal bone to the mid-point on the greater wing of sphenoid bone.
6. Plane of basis crani (C-F, Figure 1): From the mid-point on the greater wing of sphenoid to the anterior margin of the foramen magnum.
7. Plane of alveolar point (C-E, Figure 1): From the alveolar point of incisor to the mid-point on the greater wing of sphenoid.
8. Plane of foramen magnum (F-M, Figure 2): Between the lateral extent of the foramen.
9. Palate width (P-W, Figure 2): Between the lateral extent of the pre-maxillary bones at the point of junction with the maxilla.
10. Length of shaft of limb bones (L-L, Figure 3): From the proximal to the distal articulating ends of each bone.
11. Thickness of shaft of limb bones (T-T, Figure 3): Measured at the mid-point of L-L.

These measurements were taken on the cranial and limb bones except the fibula, because of its distal union with the tibia in this animal. The minimal and maximal values obtained were recorded as range, and their mean values calculated. The statistical analysis of the comparison of these measurements in both the control and experimental groups was evaluated using the Student's t – test, at the P – value of 0.05.

Results

The ethanol treated foetuses recorded lower mean weight, 3.64 $\pm$ 0.74 compared to the control, 5.07 $\pm$ 0.52g; likewise the skeletal (wet) weight was 1.92 $\pm$ 0.03 and 3.21 $\pm$ 0.57g respectively in the experimental and control group. The osteometric values for the control and experimental rats are shown in the following tables 1 – 3. Almost all the parameters were significantly higher in the control than the ethanol treated rats.

Figure 1: Lateral view of skull of wistar rat foetus, including the points at which measurements were taken
A-B: cranial length, A-C: palane of anterior cranial fossa, C-E: plane of alveolar point, C-F: plane of basis crani, G-H: mandibular length
Fig. 2: Ventral view of the skull of wistar rat foetus indicating the points at which measurements were taken
P-L: palate length, P-W: palate width, F-M: plane of foramen magnus
Fig. 3: Limb bones of wistar rat foetus indicating the points at which measurements of length (L-L) and thickness (T-T) were taken
(i): humerus,(I)i: ulnar and radius,(iii): femur, iv: tibia

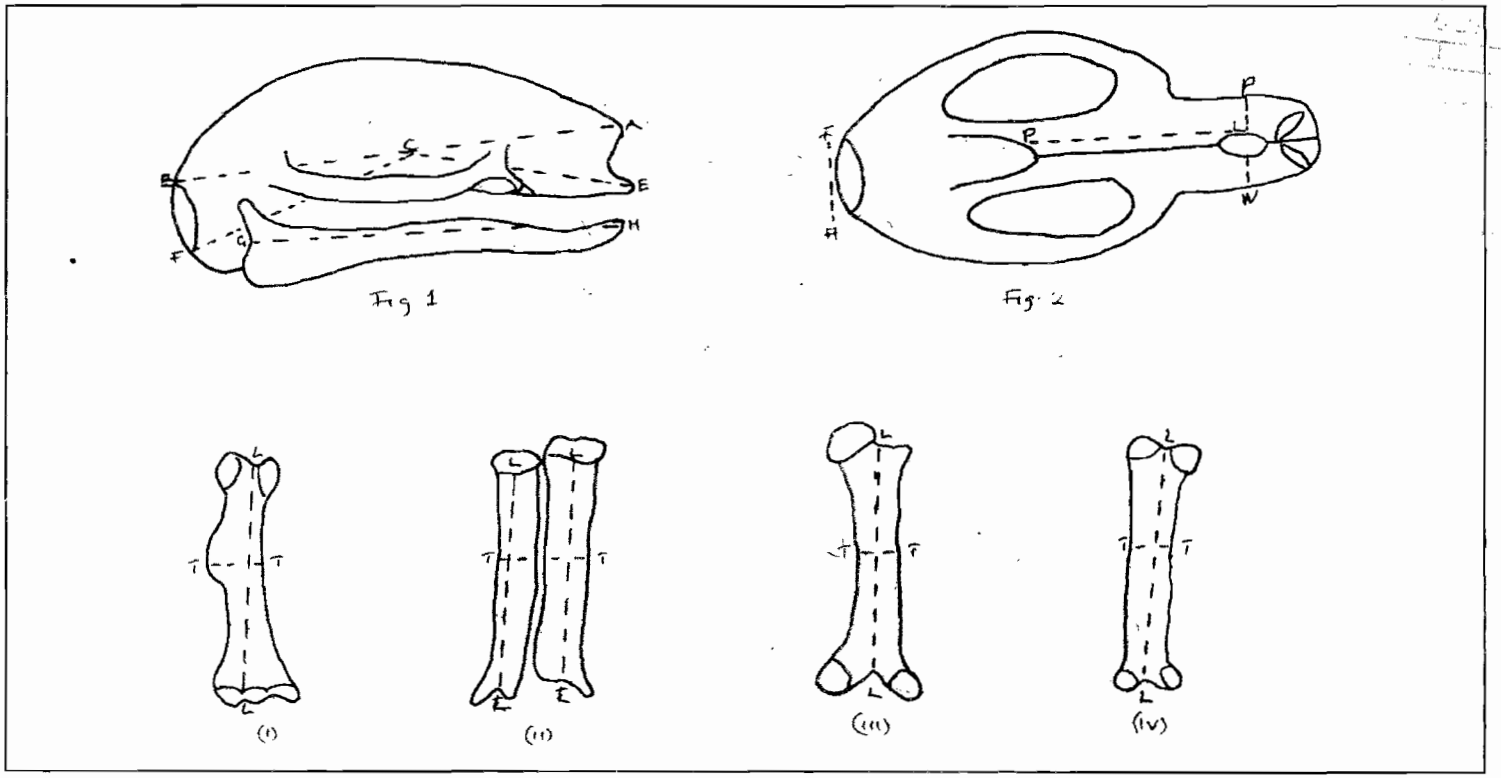

Table 1: Dimensions of the skull (mm) in the control 'C' (n = 145) and experimental 'T' rat foetuses (n = 136)

Dimensions	CRL		CRW		PAF		PBC		PFM		PAP		MDL		PTL		PTW	
Groups	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Range: Mn	14.51	11.30	8.80	3.21	6.56	6.00	8.61	7.31	1.81	1.50	6.10	6.01	8.05	7.54	4.50	3.25	1.32	1.12
(mm) Mx	18.50	15.31	10.01	9.10	7.48	6.80	11.58	9.00	2.00	1.77	8.58	6.91	9.25	9.15	4.85	4.65	1.56	1.35
Mean	16.30	14.41	9.21	7.51	7.21	5.43	10.15	8.45	1.90	1.60	7.07	6.16	9.05	7.15	4.75	3.95	1.50	1.25
SD	3.12	2.35	3.06	2.05	2.64	2.07	2.08	1.95	3.44	3.06	3.22	3.16	3.86	2.86	1.01	1.96	0.23	0.17
p - value	<0.05		<0.05		<0.05		NS		NS		NS		<0.05		NS		NS	

CRL: cranial lenght, CRW: cranial width , PAF: plane of anterior cranial fossa, PBC: plane of basis crani, PFM: plane of foramen magnum, PAP: plane of alveolar point. MDL: mandibular lenght, PTL: palate lenght, PTW: palate width, Mn: minimum, Mx: maximum

Table 2: The length (L-L) of limb bones in the control 'C' (n = 145) and experimental 'T' rat foetuses (n = 136)

	Humerus		Ulnar		Radius		Femur		Tibia	
	C	T	C	T	C	T	C	T	C	T
Range: Mn	4.98	3.50	4.50	3.35	3.65	2.25	4.90	2.20	5.40	3.50
(mm) Mx	5.50	4.20	5.64	4.50	5.00	4.07	5.28	4.15	6.80	6.10
Mean	4.09	3.69	4.94	3.41	4.82	2.98	5.05	2.95	5.60	3.45
SD	1.22	1.04	2.27	1.13	1.24	0.98	2.17	1.33	2.13	1.32
p - value	NS		<0.05		<0.05		<0.05		<0.05	

Table 3: The thickness (T - T) of limb bones in the control 'C' (n = 145) and experimental 'T' rat foetuses (n = 136)

	Humerus		Ulnar		Radius		Femur		Tibia	
	C	T	C	T	C	T	C	T	C	T
Range: Mn	1.10	0.98	0.78	0.56	0.55	0.45	0.80	0.68	0.98	0.70
(mm) Mx	2.10	1.26	0.92	0.89	0.75	0.71	1.00	0.90	1.00	0.90
Mean	1.60	1.02	0.82	0.64	0.71	0.56	0.98	0.79	0.99	0.79
SD	0.40	0.03	0.11	0.13	0.18	0.20	0.21	0.29	0.07	0.26
p - value	NS		NS		<0.05		<0.05		NS	

Discussion

The short stature and the skeletal disorders such as under ossification observed in the ethanol treated rats are characteristics of foetal alcohol syndrome patients as reported earlier; [2, 10, 11, 12] and this could possibly be associated with the inhibitory effects of ethanol on nucleic acid and protein synthesis and the consequent poor matrix lay down at the on set of osteogenesis. In addition, Anderson [13] had identified a group of pyrophosphatase enzymes in osteoid tissues undergoing calcification. These enzymes are capable of destroying inorganic inhibitors of calcification; these inhibitors act normally to prevent calcium deposits from forming in soft tissues by poisoning apatite crystals and changing the composition of the matrix (Anderson, 1976). Ethanol possibly enhances the action of these inhibitors by an unknown mechanism and hence, potentiates its toxic effect on osteoid tissues resulting in divers skeletal malformations. [14]

Alcohol consumption results in reduced vitamin and mineral uptake such as folic acid and calcium due to malnutrition that accompanies alcoholism. Folic acid is a necessary precursor in the course of nucleic acid and protein synthesis, hence, this added to the suppressive effects of ethanol on osteoblast activities, could also inhibit bone matrix synthesis and mineralisation; [11, 12, 14] and such cases of ethanol - induced growth retardation do not recover post-natally. [15]

The findings may suggest an improving understanding of the basis of skeletal dysgenesis, growth retardation and the short statures associated with the foetal alcohol syndrome.

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