Prevalence of prenatal drugs exposure malformations in fetal mice

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Abstract: Preventive Medicine Health Promotion involves empowerment of the community in improvement its health through education, and provision of preventive health services. Aiming Educating community at risk and medical staff about determinants of diseases and their prevalence, and preventing them. The drug abuse is of increasing concern, and prevalence of simultaneous ingestion of alcohol by cocaine users was to be high, therefore, pregnant mice were used to study the teratogenic effects of alcohol and a combination of alcohol and cocaine. In the alcohol group, pregnant female mice were given alcohol at 2.5 gm/kg twice daily 5 hours apart by orogastric intubation. In the combined group, pregnant females received alcohol at 2.5 gm/Kg body weight twice daily and cocaine hydrochloride at 20 mg/Kg body weight daily by intraperitoneal injection. The control group was given saline. The treatment was started from 6th day to 15th day of gestation. The dams were sacrificed on 20th day of gestation (one day before fill-term. The uterine contents were evaluated and the number of the dead and live fetuses was determined as well as the number of resorptions. The living fetuses were examined for skeletal anomalies. The interventional Randomized Control Trial (RCT) teratological study revealed a significant increase in the number of dead fetuses in the combined group compared to the control group. Fetal growth parameters showed a significant decrease in the combined group. A significant increase of the skeletal anomalies was detected in the alcohol group, but became more marked in the combined group. The present study indicated that alcohol alone mainly affects the skeletal anomalies, and the cocaine in the combined group increased and potentiated the teratogenic effect of alcohol on the skeletal anomalies in mice. Prenatal alcohol and other drugs administration potentiate the teratogenic effects in the vertebrates, and those abusers should be identified for prevention.

Keywords: Health promotion, prenatal malformations, drug abuse, randomized control trial.

Introduction

Medicine was not succeeding in curing the new generation of chronic degenerative diseases, its attempts to do so were involving escalating cost, what might not be cured should be prevented, and human behavior was implicated in the etiology and management of preventable diseases. People should persuade to adopt behaviors in the interest of disease determinants prevention. As preventive medicine: health promotion is process of enabling people to increase control over, to improve their health and to reach a state of complete physical, mental, and

social wellbeing; which is positive concept emphasizing physical capacities. Behavioral and attitudinal factors are one of main identified influences on people health. People cannot achieve their fullest health potential unless they are able to take control determinants of health and disease which apply equally to man and women (1). Women who drink while pregnant are at a high risk of giving birth to children with neuro-developmental disorders. Fetal alcohol syndrome (FAS) is facial features. It was clinically described in USA in human more than 30 years ago (1973), while historically alcohol's teratogenic effects were identified in the early 20th century in a mix with the prohibition cause of the period. Consuming alcohol during pregnancy is the cause of FAS, consisting of a variable degree of birth defects and mental retardation, initially identified by a reduced head size and distinctive facial features (2). A study with human found that newborns of mothers that consumed light amount of alcohol during pregnancy had a higher frequency of facial malformations and alterations in umbilical cord artery contractility.

A recent brain imaging study demonstrated that low, moderate alcohol exposure during fetal development was associated with reduction in grey matter volume in several brain regions at approximately 20 years of age (3). Cocaine is a tropine alkaloid with local anesthetic and CNS stimulant properties. Cocaine is legitimately used as a topical local anesthetic is mainly abused by insufflations (snorting) but it can also be injected, smoked or ingested. It causes acute vasoconstriction which may cause fetal hemorrhage and hypoxia (4). Cocaine use is one of the most common causes of drug-induced medical problem in USA). Cocaine use is contraindicated in pregnancy.

Data on cocaine use is heavily confoundded, however, cocaine use during pregnancy gas been associated with increased risk of spontaneous abortion, placental abruption, premature labor, intrauterine growth retardation and sudden infant death syndrome (5). In spite of that combination of alcohol and cocaine were widely abused in the recent decades, the studies of the teratogenicity of these combinations were surprisingly limited. Therefore, comprehensive studies on teratogenic effects of alcohol and a combination of alcohol and cocaine on the skeletal anomalies of fetal mice was designed.

Materials and methods

Mature mice (25-30 gm body weight) twenty males and sixty females were used in the present study. Mice were mated one male to three females. The day in which the sperm was identified in the vaginal smear was considered day zero. The pregnant female mice were randomized into three groups as follows:
Alcoholic group (13 pregnant female mice): received alcohol as 2.5 gm/Kg twice daily. Combined group (18 pregnant female mice): received alcohol as 2.5 gm/kg twice daily and cocaine hydrochloride as 20 mg/Kg daily. Control group (13 pregnant female mice). Pregnant female mice were given alcohol as 2.5 gm/Kg body weight twice daily 5 hours apart by orogastric intubation. The alcohol was diluted as 50% in water to avoid gastric irritation according to. Cocaine hydrochloride in a crystalline form was dissolved in 0.9% normal saline and given as 20 mg/Kg body weight daily

by intraperitoneal injection according to (6). On 20th day of gestation (one day before term), the dams were sacrificed by decapitation by scissors. The abdominal wall of the dam was opened, the living and dead fetuses were distinguished immediately by the appearance of a moving reflex after touching the fetus with a tweezers. The number of dead and living fetuses was determined as well as the number of resorptions. Individual fetal weight, length and sex were recorded and a gross examination for external fetal malformations was made macroscopically and in case of uncertainties by means of a magnifier. The live fetuses were eviscerated, put in 95% alcohol for one week, and then cleared by 2% KOH for 6 hours to be stained by Mall's solution for 24 hours for skeletal examination according to (7).

Results

The effect of alcohol and combined alcohol and cocaine on pregnant mice was shown in Table (1). A slight increase in the percentage of the female deaths was observed during pregnancy in the combined group, compared to the control and alcohol groups (Table 1). The female who died in the control group was due to large axillary swelling. The female who died in the alcohol group was due to poor gavages technique. Three females died in the combined group after rapid intraperitoneal injection of cocaine. The average number of implants/ pregnant female mouse slightly increaseed in the combined group, while the incidence of resorptions in the alcohol group slightly increased (Fig. 1).

The total number of dead fetuses and dead fetuses/pregnant female mouse (Fig. 2) significantly increased in the combined group compared to the control group (Table 1, Fig. 3). Also in the combined group, fetal growth parameters showed a significant decrease in the body weight, while the fetal length significantly decreased in both alcohol and combined groups compared to the control group. Table (2) showed the type of skeletal anomalies found in the present study. The total number of fetuses with skeletal anomalies significantly increased in the combined group comparing to the control group (Fig. 4).

Parameters	Control group	Alcohol group	Combined group
no. of females	13	13	18
no. dead females	1	1	3
no. liters examined	12	12	15
Total no. of implantations	149	157	183
no. of implants/female	12.0 ± 0.7**a**	12.3 ± 1.0	12.2 ± 0.7
Male/female live fetuses	69/67	65/63	73/68
% of live fetuses	88.6 ± 2.2**b**	81.2 ± 5.1	75.6 ± 2.8
Total no. of resorptions	7	17	17
No. resorptions/female	0.6 ± 0.33**a**	1.4 ± 0.37	1.1 ± 0.33
No. of females having resorptions	4	8	10
% of resorptions /affected female	14.5 ± 7.18**b**	20.5 ± 5.08	11.7 ± 7.18
Total No. of dead fetuses.	6	12	25
No. of dead fetuses/female	0.5 ± 0.33**a**	1.1 ± 0.33	1.6 ± 0.30 **d**
No. females having dead fetuses	5	7	13
Fetal body weight (gm)	1.2 ± 0.02**c**	1.2 ± 0.03	1.05 ± 0.01 **d**
Fetal length (cm)	2.3 ± 0.03**c**	2.2 ± 0.02 **d**	2.1 ± 0.02 **d**

Table 1: Effect of alcohol and combined alcohol and cocaine on pregnant female mice

However, alcohol treatment also significantly increased the skeletal anom-

alies compared to control group (Figs. 5, 6, 7). The skull examination revealed that the incomplete ossification of the frontal bones, the wide separation of the parietal bones and incomplete fusion of the occipital bone significantly increased after combined treatment of alcohol and cocaine (Figs. 5, 7, 8, 10, 11).

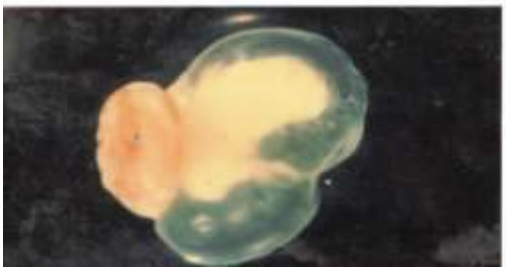

Figure 1: A photograph showing a resorbed fetus inside the amniotic sac (A) connected with the placenta (P) of the pregnant female mouse of the alcohol group. X 10

Figure 2: A photograph showing a distorted non-viable fetus of a pregnant female mouse of the combined group X 10

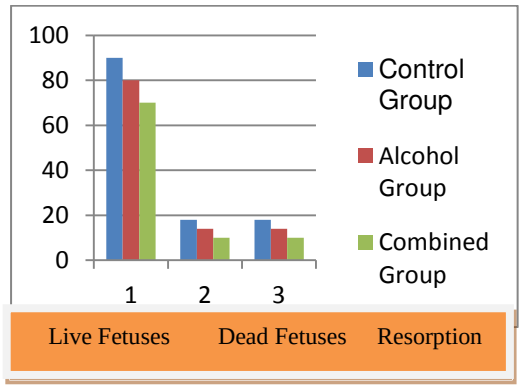

Figure 3: Effect of alcohol and combined alcohol and cocaine on mice reproduction

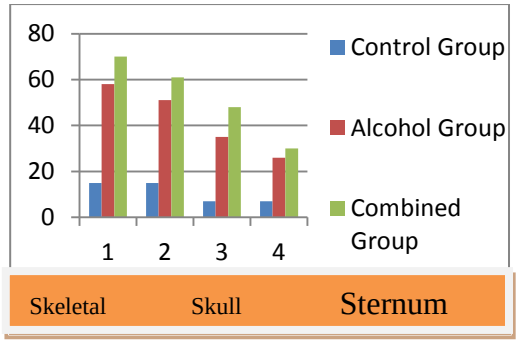

Figure 4: Effect of alcohol and combined alcohol and cocaine on the skeletal anomalies of the fetal mice.

Alcohol treatment also increased the number of incomplete ossification of the frontal bones, wide separation of the parietal bones and delayed ossification or incomplete fusion of the occipital bone (Figs. 5, 6, 8, 9). The cervical vertebrae showed delayed ossification in both alcohol and combined groups (Figs. 5, 6, 7). The fetuses of both alcohol and combined groups showed increasing in the sterna anomalies comparing with the control group (Table 2, Fig. 4). The most common sterna anomalies in the alcohol group were the zigzag-shaped sternum (Fig. 12). In the combined group, three types of the sterna anomalies were encountered, namely the bipartite sternebrae, incomplete ossification of the 5th sternebrae and also zigzag-shaped sternum (Figure 13).

Paw examination showed a significant increase in delayed ossification in both alcohol and combined groups compared to the control group (Table 2, Figure 4) but the combined group was more significant than alcohol group where marked delayed ossification of paws was identified (Figs. 14, 15).

Figure 5: A photograph of a fetal mouse from a pregnant female mouse of a control group showing a normal frontal (F), parietal (P), interparietal (I), occipital (O) bones and ossified cervical vertebra (arrow). X 5

Parameters	Control group	Alcohol group	Combined group
No. of litters examined	10	12	14
No. of fetuses examined for skeletal anomalies.	50	54	71
No. of fetuses with skeletal anomalies	8	32 d	49 d
fetuses with skeletal anomalies/female	0.8 ± 0.32 **a**	2.7 ± 0.51 **b**	3.5 ± 0.40 **b,c**
Skull			
***** No. of fetuses with skull anomalies.	8	41 **d**	44 **d**
***** No. of skull anomalies/female	0.8 ± 0.32**a**	2.3 ± 0.46	3.1 ± 0.46**b**
***** No. of litters having fetuses with skull anomalies	5	9	14
***** Incomplete ossification of frontal bone	4	28 **d**	40 **d**
***** Incomplete ossification of frontal bone/female	0.4 ± 0.16a	2.3 ± 0.46**b**	3.0 ± 0.46**b**
*Wide separation of parietal bones	0	2	20 **d**
* Wide separation of parietal bones/female	0.0	0.3 ± 0.41	1.2 ± 0.23**b**
* Incomplete fusion of occipital bone	7	18	32 **d**
* Incomplete fusion of occipital bone/female	0.4 ± 0.16**a**	1.5 ± 0.33	2.2 ± 0.43**b**
Sternum			
* No. of fetuses with sternum anomalies	4	29 **d**	34 **d**
*No. of fetuses with sternum anomalies/female	0.4 ± 0.16**a**	1.6 ± 0.35	2.9 ± 0.46**c**
* No. of litters having fetuses with anomalies	4	9	14
* delayed ossification of 5th sternebrae	0	8	15 **d**
* Delayed ossification/female	0.0	0.6 + 0.18	1.3 ± 0.28**b**
* Bipartite sternebrae	4	19 **d**	23 **d**
* Bipartite sternebrae/female	0.5 ± 0.18**a**	1.5 + 0.35	1.7 ± 0.36**b**
* Zigzag-shaped sternum	0	6	3
Paws			
* Delayed ossification	3	16 **d**	21 **d**
* Delayed ossification/female	0.4 ± 0.18**a**	1.3 + 0.35	1.7 ± 0.43**b**
* No. of litters having fetuses with anomalies	3	9	12

Table 2: Effect of alcohol and combined alcohol and cocaine on skeletal anomalies of the fetal mice
(a) Values represent mean per female ± standard error
(b) Significantly different from control by ANOVA, P < 0.05.
(c) Significantly different from control by ANOVA, P < 0.01.
(d) Significantly different from control based on Chi-square test calculated from the –ve log likelihood.

Figure 6: A fetal mouse from of alcohol group showing wide separation of parietal bones (P), delayed ossification of occipital bone (thin arrow) and delayed ossification of the cervical vertebra (thick arrow). X 5

Figure 7: A fetal mouse of combined group showing wide separation of the parietal bone (P), delayed ossification of the frontal (F) and occipital (O) bones. There is also delayed ossification of cervical vertebra and the phalanges (arrows). X 5

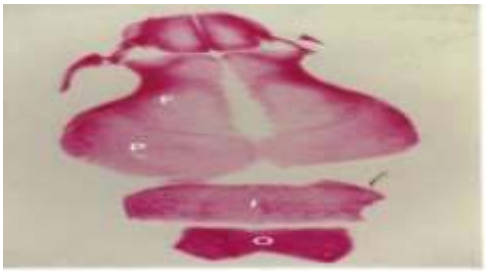

Figure 8: A fetal mouse control group showing a normal frontal (F), parietal (P), interparietal (I), occipital (O) bones X 10

Figure 9: A fetal mouse of alcohol group showing delayed ossification of the frontal bone (F) and incomplete fusion of occipital bone (arrow). X 10

Figure 10: A fetal mouse combined group showing delayed ossification of the frontal bone (F), wide separation of the parietal bones (P) and delayed ossification of the occipital bone (arrow). X 7

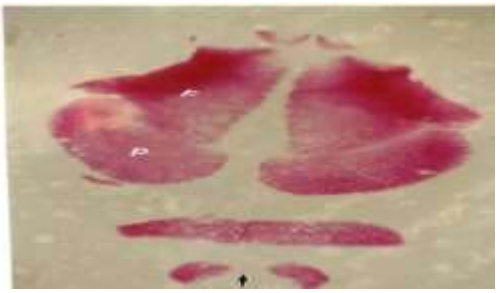

Figure 11: A fetal mouse of combined group showing delayed ossification of the frontal bone (F), wide separation of the parietal bones (P) and delayed ossification of the occipital bone (arrow). X 10

Figure 12: A fetal mouse of alcohol group showing zigzag-shaped sternum (arrow). X 5

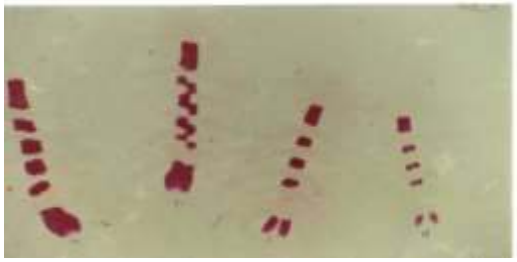

Figure 13: Showing (a) control sternum, (b) zigzag-shaped sternum, (c) delayed ossification of 5th sternebrae & (d) bipartite sternebrae. The last 3 anomalies from three fetal mice from pregnant female mice from combined group. X 6

Figure 14: A forelimb (F) and a hind-limb (H) of a fetal mouse of the control group showing normal ossification of the paws (arrows). X 6

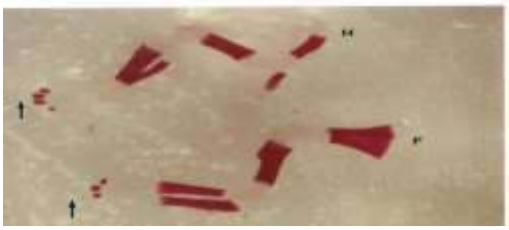

Figure 15: of a forelimb (F) and a hind-limb (H) of a fetal mouse of the combined group showing marked delayed ossification of the paws (arrows).

Discussion

The prevalence of ingestion of alcohol alone or simultaneous ingestion of alcohol and cocaine users is high. Therefore, comprehensive study on the teratogenic effect of alcohol and a combination of alcohol and cocaine on the skeletal anomalies of fetal mice were designed. The present study demonstrated that the number of resorptions increased slightly in the alcohol group while co-administration of alcohol and cocaine did not change the number of resorptions. In the combined group, the number of dead fetuses increased and fetal growth parameter significantly affected. A significant increasing in the skeletal anomalies was detected in the alcohol group, but these skeletal anomalies became more marked in the combined group.

In accordance with the results of the present study, some found that short-term maternal treatment with a high dosage of ethanol at stages of pregnant mice that are equivalent to 3-4 weeks of human gestation induced craniofacial dysmorphism (8). Others reported that prenatal alcohol exposure induced increased miscarriage, stillbirth prêterm delivery and sudden infant death syndrome in human (9). Also, prenatal alcohol exposure induced neural defects with craniofacial dysmorphology in the fetal alcohol spectrum disorders. These neural defects are in the form of defective structure of the human brain (10); human cortical thinning (11).

The full picture of fetal alcohol syndrome (FAS) usually occurs in babies born to alcoholic mothers or those who drink regularly or binge-drink. However, no amount of alcohol is safe. Acetaldehyde is implicated as the cause of FSA through its inhibiting effects on DNA synthesis, placental amino acid transport and fetal develoopent (12). The biological basis for FAS is related to genetic polymerphisms identified for alcohol dehydrogenase (ADH), which converts alcohol to acetaldehyde and acetaldehyde dehydrogenase, which converts acetaldehyde to acetate. Genetic differences in ADH alleles make some infants exposed to the same level of alcohol in utero more likely to have longer or higher levels of exposure to acetaldehyde. This may explain the greater frequency in Americans blacks and Native Americans (13). Most studies focused on ethanol induced defects in a developing or mature organ fail to examine lineages of precursor cell types and their defects. In addition to molecular mechanism; genetic, nutriational and environmental factors may alter the severity of ethanol induced

defects in the developing embryo. So, multiple ethanol targets produced a complex interplay between pathways. However, there may be nutritional or developmental pathways that are centralized and may provide key support for the developing embryo (14).

Cocaine is metabolized very slowly in the fetus because the fetus has low plasma cholinesterase activity. It may alter the availability and utilization of calcium and reduce blood flow from the uterus to the placenta. The complications of abruption placenta, cerebral hemorrhage, intra-uterine growth retardation, limb defects, appear to be related to vascular disruption. Coca-unexposed fetuses also have increased incidences of prematurity, microcephaly and sudden infant death (15). In consistent with the results of the present study. Some found that the prolonged intravenous administration of cocaine increased of incidence of skeletal anomalies in mice, found that alcohol and cocaine in combination had greater effects regarding decreased birth weight, increased prenatal mortality and delayed physical maturation than either drug alone, and reported that the subcutaneous injection of acute dose of cocaine produced a variety of congenital malformations in the fetal mice especially the delayed ossification of the skull, sternum and paws. Other reported that a combination of cocaine and ketamine induced teratogenic skeletal anomalies such as incomeplete ossification of the skull bones and vertebrae, reported that mid-pregnancy cocaine exposure indced fetal growth retardation especially disproportionate brain developmental retardation (16).
Cocaine is a psycho-stimulant; children were thought to be emotionally disrupted, cognitively impaired, less likely to socially interact, and more likely to die from sudden infant death syndrome (SIDS). Thus, the term "crack-baby" was introduced to describe children exposed to cocaine prenatally. However, these original studies were confounded by very small sample sizes, polypro use, nutritional status and other psychosocial problems (17). In humans, longitudinal studies have shown that there are long-term consequences of prenatal cocaine exposure; however, the behavioral dysfunction appears to be mild, detailed studies have demonstrated that prenatal cocaine exposure can have long-lasting negative effects on cognitive and attention systems, mediated via regions such as the prefrontal cortex, and other higher order cortical areas. Recent data also suggest that there is increased likelihood that children exposed to cocaine prenatally will require special needs programs, which, from both an individual and societal perspective, is expensive (18). Ethanol induced embryonic defects are preventable diseases, many of the proposed nutritional supplements are preventable measures that may reduce the severity of its birth defects. These preventable nutritional substances are included retinoic acid supplements, folic acid derivatives choline supplementation, vitamin E derivatives (19), dietary administration of N-acetyl cysteine and lithium (20); Advances in stem cell based and other regenerative therapies could bring therapeutic benefits for ethanol-induced birth defects (21).

Recommendations: Strategies aimed at creating supportive environments to certain abusers should include offering people healthy lifestyles the health care sector has to have an integrated policy for promoting positive health within a community Consider basic principles

related to setting for health promotion. Emphasize the importance of health alliance between the various delivery systems Make the common sense that each particular context and setting has its own strengths and specific potential for promoting health Effective health promotion calls for change in certain lifestyles related to the established risk factors Call for action: Raising awareness of the changing determinants of health Accumulating knowledge on best practice. Enabling shared learning promoting solidarity in action Adopting transparency and public accountability in health promotion.

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