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ORIGINAL ARTICLE

Intra Uterine Fetal Morphine Exposure Delays Normal Development of the Brain Bubbles and Vision System in Wistar Rat Embryos

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ABSTRACT
Previous studies have shown that morphine consumption during pregnancy may delay embryo development or cause abnormal nervous system function. The present study focused on impact of maternal morphine consumption on the brain bubbles and vision development in Wistar rats. The number 24 female wistar rats (170-200g) were used throughout. The experimental groups received 0.05 mg/ml of morphine by tap water while, the control group only received water. On 10th and 17th day of pregnancy, the pregnant animals were anesthetized by chloroform and the embryos were removed surgically. The embryos were fixed in formalin 10% for one week. Then, tissue processing, sectioning and staining hematoxylin and eosin (H&E), were applied for the embryos. The sections were examined for in terms of area measurement the brain bubbles (Rhombencephal and Prosencephal) and vision (Retina), brain cortex layer thickness development by light microscope and MOTIC, SPSS software. Severe reduction of the area Rhombencephal and Prosencephal but increased thickness brain cortex was observed in the experimental group regarding to control groups. Furthermore, thickness reduction of retina and area reduction of lens were observed in the experimental group regarding to control groups.The study showed oral morphine consumption has caused to primary brain bubbles decrement and visual system .This defect may lead to tardy on function and development central nervous system(CNS).
Keywords*: Brain Bubbles, Morphine, Rat, Development, Visual System*

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INTRODUCTION

Opiates production and consumption, is growing on and also the routine stress and problems increase the tendency for opiates consumption. All human beings are in risk for opiates dependency and addiction. Mothers also are in dependency risk to opiates. In pregnant mothers, addiction side effects will affect them and the next generation as well [1-3].Opioid abuse and the subsequent side effects are the problems of today's world and are growing as time goes by. Among the most fragile drug abusers are the pregnant mothers. Based on the previous research, the side effects of opioid drug consumption, such as morphine, will harm not only the mother but also the placenta and embryo and the abnormalities will be transmitted to the next generation [4, 5] . Research done on rat placenta and embryo proves that morphine will have effects on the placenta directly and in addition, it will cause disturbance in the development of embryonic cavities, such as the amniotic cavities, by passing through the placenta barrier [5-8]. Most of the embryo development disorders and abnormalities, which are caused by pregnant mothers' morphine consumption, are connected to the central nervous system. Delay in the natural growth of any parts of the central nervous system will bring about a disorder in the growth of other parts of the nervous system. Oral morphine consumption by pregnant mothers who are dependent on the consumption period, will cause several abnormalities and it will also cause deficiency on the duration of

growth of different parts of the embryo based on the pregnancy day [9, 10] .Another abnormal effect of morphine on rat central nervous system is disorder in choroid plexus and neural cavities development, the result of which is changes in the cerebrospinal fluid which leads to disorders in nutrients supply and the natural differentiation of neuroblast cells. Natural development of different parts of the nervous system and their mutual communicative relations is of high importance [11, 12]. The natural functioning of the nervous system as the receiver, processor, and transmitter of the neural messages in the embryo has great effects on the embryo's health. Having said that, delay in the natural growth of the central nervous system will lead to dysfunctions in the system and disorders in embryo's growth [13]. Teratogenic foreign substance at the time of pregnancy causes deficient embryonic growth in the brain vesicles and several systems including the visual system [15, 16].The aim of this study is to examine In this research, the effect of maternal oral morphine consumption on the brain bubbles and vision system development in rat embryo.

MATERIAL AND METHODS

Female Wistar rats with an average weight of 170 to 200 grams were used in this research. The rats were kept in pair cages, under the ambient temperature (24±1ºC) and with natural photoperiods (12 hours light and 12 hours dark). During the experiment, the rats were given adequate food and water. The ethical standards established with laboratory animals were compiled (under the supervision and approval of the ethics committee of Baqiyatallah University of Medical Sciences).

Drug

In this study, Morphine sulfate provided by the Iran Atomic Agency (Tehran, IRAN) was used orally.

Animals

24 rats were divided into three groups (I, II, and III) as follow Group I (control group=12), Group II (the 10th day pregnancy group=6), and group III (the 17th day pregnancy group=6). A total of 12 female rats in dual groups copulated with adult male rats. After confirmation of pregnancy (observation of a vaginal plug and the existence of sperm in the vagina), the following morning they were separated from male rats and kept in the same dual-groups. Thereafter, the experimental group (first group from day 0 until 10 of pregnancy and second group from day 0 until 17 of pregnancy) received a daily dose of 0.05 mg/ml (5 mg morphine in 1000 ml tap water for six rats). The amount of consumed morphine in 10 ml water for every 100 g of the rat's weight was computed and attempts were made to give the rats the amount they needed. Control groups were treated with normal tap water. After treatment, all groups were anesthetized by chloroform. Embryos with the placenta were separated from the mother rats and transmitted to a 10% formalin solution for two weeks. Next, Embryos with the placenta were processed, molded, sectioned in 5 µm thicknesses, and fixed on glass slides for additional evaluation. The slides that were prepared with 5 µm sections were stained using the hematoxylin and eosin (H&E). Samples were then examined by light microscope and MOTIC software. The MOTIC software was used for tissue measurement, which consists of a microscope which is connected to a computer and a monitor through a software program. This software provides the possibility off scanning the slides and is capable of performing different measurements.

Statistical analysis

Data were reported as mean ± SEM. Differences between group means were assessed by a one-way analysis of variance(ANOVA) and unpaired sample *t*-test using the SPSS/PC computer program(version 18.0). (a) *P* value of <0.01 and (b) value of <0.05 were considered as significant.

RESULTS

Our results showed the effect of edible morphine use by pregnant mother is quite obvious in pregnancy 10th- day which had been revealed by morphological outcome on sections of 5 µm thickness using H and E staining method. Morphine causes an increase total surface of the brain bubbles (Rhombencephal and Prosencephal) but increased thickness brain cortex were observed in the experimental group regarding to control groups had a significant decrease in size compare with control group (figure C1, M1, C2, M2). Again morphine causes an increase in total surface of retina simultaneously. In 17th- day of pregnancy, a decrease in lens total surface was seen Morphological data from Morphometric aspect revealed that in morphine group, total surface of embryo and lens have a significant increase in size compare with 17th-day control group. Again thickness reduction of retina was observed in the experimental group regarding to control groups (figure C3, M3, C4, M4).

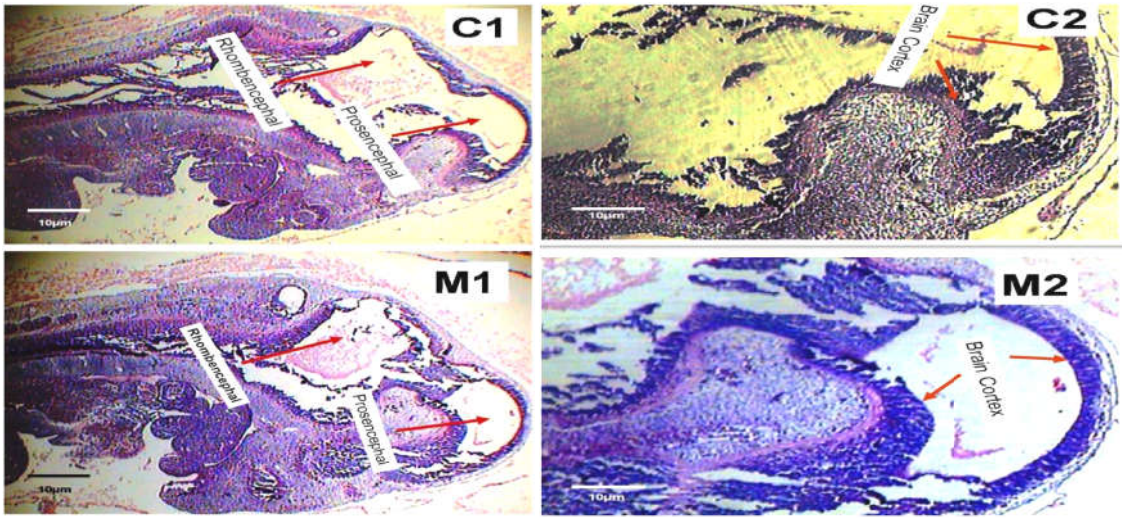

Figure 1,2: The effect of oral morphine consumption in the brain bubbles (Rhombencephal and Prosencephal) (M1,C1)and the thickness brain cortex (M2,C2)Comparison of oral morphine effect on of the experimental group (M) and control group (C) pregnancy 10th- day of the embryos. Sagital section and, staining (H &E) (×40 , x100)

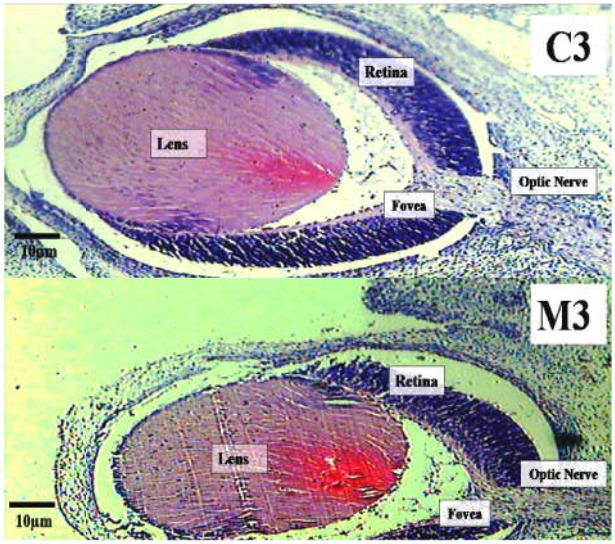

Figure 3,4: The effect of oral morphine consumption in lens (M3,C3)and thickness of retina (M4,C4) Comparison of oral morphine effect on of the experimental group (M) and control group (C) pregnancy 17th- day of the embryos. Transverse section and staining (H &E) (×40 , x100).

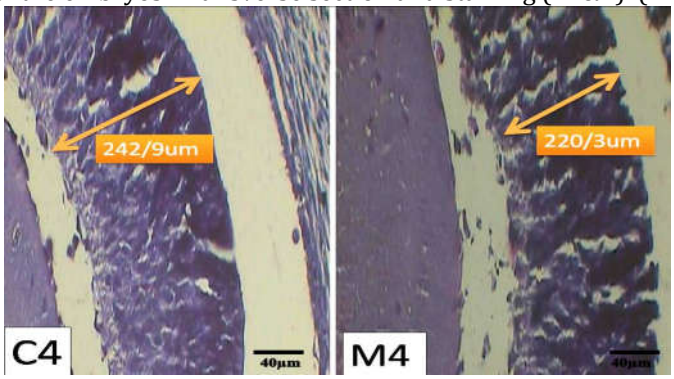

Figure 5: Effect of oral morphine consumption on the development of the thickness of the brain cortex of pregnancy (10-day and retina 17-day pregnant mothers) and a comparison between the experimental (M) groups and the control (C) groups reveals the increase of the brain cortex and reduction of retina in the experimental group, compared to that of the control groups (*) *P* value of <0.05 indicating a significant difference).

DISCUSSION

Development and formation of visual system begins from diencephalon of forebrain as optic vesicles. During early growth of the vertebrate embryo, a longitudinal groove on the neural plate gradually gets deeper and the ridges on either side of the groove (the neural folds) get elevated, and eventually meet, converting the groove into a closed tube, the ectoderm wall which shapes the rudiment of the nervous system. This tube in the beginning differentiates into three vesicles (pockets): the prosencephalon at the front, the mesencephalon, and, between the mesencephalon and the spinal cord, the rhombencephalon. (By six weeks in the human embryo) the prosencephalon then splits moreover into telencephalon and diencephalon; and the rhombencephalon splits into metencephalon and myelencephalon [16, 17]. These vesicles, as vertebrate grows, still differentiate more. The telencephalon differentiates into the striatum, the hippocampus and the neocortex, among other things, and its cavity develops into the first and second ventricles .Diencephalon elaborations contains the subthalamus, hypothalamus, thalamus, and epithalamus, and their cavities shape the third ventricle [18-20]. The tectum, pretectum, cerebral peduncle, and other structures develop out of the mesencephalon, whose cavity grows into the mesencephalic duct (cerebral aqueduct). The metencephalon becomes, among other things, the pons and the cerebellum, the myelencephalon shapes the medulla oblongata, and their cavities change into the fourth ventricle [21, 22].When the visual system develops, especially retinal axons extend from the eye to the optic chiasm at the ventral surface of the diencephalon .Eye is a complicated biological instrument . The functioning of a camera is usually compared with the operation of the eye, this is mostly because both of them focus light from external objects in the field of view onto a light-sensitive medium. With respect to the camera, this medium is film or an electronic sensor; With respect to the eye, it is an array of visual receptors. With this simple geometrical resemblance, according to the laws of optics, the eye functions as a transducer, as does a CCD camera. The light which enters the eyes is refracted as it goes through the cornea. It then goes through the pupil (controlled by the iris) and is further refracted by the lens. The cornea and lens work together as a compound lens to project an inverted image onto the retina [23, 24] .Retina is the neural part of the eye and its proper development has a major role in visual system functioning [25]. And it includes a large number of photoreceptor cells which contain special protein molecules, called opsins . Retinal thickness reduction, not formation of retinal layers, and delay in retinal cells differentiation are the effects of oral morphine consumption by pregnant women on visual system functioning [24, 26].Moreover, the overall size of fovea region and its central thickness that is connected to the optic nerve is reduced [27].In addition, morphine administration during pregnancy impairs fetal normal development of the lens which are made of two cell types, epithelial and fiber cells, and are covered in a collagenous basement membrane (the capsule). The lens capsule shapes the outermost layer of the lens and the lens fibers shape the bulk of the interior of the lens. The cells of the lens epithelium, which are positioned between the lens capsule and the outermost layer of lens fibers, are exclusively found on the anterior side of the lens [14, 24]. The lens themselves lack nerves, blood vessels, or connective tissues. The lens, by changing shape, function to change the focal distance of the eye, therefore they can focus on objects at different distances, and thus they make a sharp real image of the object of interest to be formed on the retina [27, 28] .The is visible as a decrease in the lens length and an increase in the eyelid thickness. Morphine also induced of delay in migration of lens cells from around toward the center. Regarding morphine-induced increasing of plasma corticosterone, the anomalies of visual system development resulted from morphine administration can be attributed to corticosterone intervention .Corticosterone has a very broad impact on development, proliferation, and migration of cells. Corticosterone causes excessive cellular proliferation, impaired development, and delay in cell migration during embryo development [14, 29-31].

CONCLUSION

Malfunctioning in the development of prosencephal bulb will bring about a delay in the natural growth of the important following parts, such as the embryo visual system. Since the visual system receives the external stimulations, it plays an important role in the survival of most of the beings. Visual system is a complicated system with various kinds of tissues and cells which is in connection with the other senses. In addition, delay in the growth of rhombencephalon will create problems in the balance center, including the cerebellum, and also visual disorders and the ability to connect the sense to other senses such as olfactory and the sense of taste.

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