Arch. Bas. App. Med. 3 (2015) 1 - 8

ARCHIVES OF BASIC
& APPLIED MEDICINE

Research Article

Persistence of Medial Edge Epithelium in the Palate of Neonatal Mice following Brief In-Utero Hyperthermia

Femi-Akinlosotu O. M. and *Shokunbi M. T
Department of Anatomy, College of Medicine, University of Ibadan

Received: 26th June 2014

Abstract

The purpose of this study was to evaluate the process of palatal fusion in neonatal mice exposed *in utero*, during palatal development, to brief hyperthermia. Two groups (experimental and control) of pregnant albino mice studied. In the experimental group in which there were four subsets, the animals were exposed to hyperthermia for either 10 or 15 minutes on each of embryonic days 10 and 11. Morphological indices were measured and were compared between the neonates in the two groups. Tissue sections from the neonatal palate stained with Haematoxylin and Eosin (H&E) were examined for palatal fusion and persistence of the medial edge epithelium. There were no obvious clefts. Hyperthermia-exposed neonates were smaller in birth weight, litter size, head length and palatal width but larger in head width compared to the controls (p value < 0.05). Light microscopy revealed persistence of the medial edge epithelium of the palate in neonates exposed to hyperthermia on day 11 of gestation. Brief in-utero hyperthermia alters the craniofacial dimensions of neonatal mice and delays palatal fusion. The precise mechanisms for these changes are presently unknown.

Keywords: Hyperthermia, teratogen, medial edge epithelium, craniometry

INTRODUCTION

The palate separates the mouth from the nasal cavity and consists of two parts, the anterior bony hard palate and the posterior fleshy soft palate. It functions with the rest of the mouth to hold food, swallow it and also to produce certain sounds and in humans, words. Palate development begins on embryonic day (E) 11.5 in mice (Ferguson, 1998).

It consists of two parts: the primary palate and the secondary palate. The primary palate appears earlier than the secondary palate. The secondary palate completes the palate posterior to the incisive fossa. The infero-medial edges of the maxillary process form the palatal shelves. These palatal shelves are formed from mesenchyme mainly of neutral crest origin as well as mesoderm associated with craniopharyngeal arches (Hillard et al.,2005). Palatogenesis culminates when the shelves make contact, adhere and fuse along the midline to form the definitive palate. The fusion occurs at an epithelial seam that is later replaced by mesenchyme. Humans and rodents share great similarities in palatogenesis. Mouse models are very useful in unveiling the mechanisms for palatogenesis in mammals[2].

Hyperthermia is an elevation of body temperature (Graham et al., 1998) and is thought to be a teratogen in many mammalian species including humans (Sasaki et al., 1995). The causes of hyperthermia include infections, hot/humid environments and heavy exercise (especially in conditions of high heat and humidity). The average body temperature of most mammalian species lies between 37^0C - 40^0C and the usual maximal diurnal variation is approximately 1^0C (Graham et al., 1998).

Cleft palate occurs 1 in 2,500 births (Sasaki et al., 1995). Approximately 70% of the unilateral clefts and 85% of the bilateral clefts of the orofacial region involve the palate (Lettieri, 1993). In Nigeria, orofacial clefts occur with a frequence of 1 in 2,703 live births, and isolated cleft palate at a rate of 1 in 20,000 (Iregbulem, 1982). The possibility of an episode of hyperthermia occurring during pregnancy is not remote (Edwards, 1986). In the Nigeria, maternal fever due to febrile illnesses and infection is rife (Orogade et al., 2008; Enato and Okhamafe, 2006; Dunmade et al., 2007). In a cross-sectional study on antenatal determinants of orofacial clefts in southern Nigeria (Omo-Aghoja et al., 2010), 54.4% of the study subjects had a positive history of maternal illness during pregnancy with 25% being febrile illness/malaria followed by upper respiratory tract infection (19.1%). The study also reported 4.4% and 5.9% incidence of isolated cleft in palates of offsprings of mothers with malaria and upper respiratory tract infections respectively.

Previous studies (Zeiler et al.,1964; Hart et al.,1969) have shown that the relationship between the craniometric parameters of the head, such as the palatal length, palatal width, head length and head height during closure of the secondary palate is complex and that excessive width of the head may be a factor in cleft palate aetiology (Fraser, 1970). The entire spectrum of the consequences of hyperthermia on palatal development is not known.

In this study, we investigated palatal fusion in neonatal mice exposed to brief in-utero hyperthermia during the sensitive stage of palate development, and related our findings to cranial dimensions in neonatal mice.

**Author for correspondence:* +2348022912220
E-mail: temitayoshokunbi@comui.edu.ng

MATERIALS AND METHODS

Experimental Animals: Sixty female and 20 male albino mice, weighing 18-30g were obtained from the animal house of the department of Anatomy, University of Ibadan. They were allowed to acclimatize separately in male and female cages. Water and food pellets were provided *ad libitum*. The females were divided into two groups: experimental and control. The experimental group consisted of 40 females while the control group consisted of 20 females.

Matings: The animals were mated at ratio of approximately one male to three females in the dark for a period of three hours and the females were checked at the end of this period for vaginal plugs. The day on which a plug was seen was taken as day 0 of pregnancy.

Hyperthermia Induction: Hyperthermia was induced in the pregnant dams on embryonic days 10 and 11 (E10 and E11) using a laboratory oven (GRX-9023A Hospibrand, USA) set and maintained at 40˚C (Arora et al., 1979). The animals were kept in the oven for 10 and 15 minutes to raise the core temperature by 2-3^{0}C. A pilot study earlier carried out in our laboratory had shown that a rise in core temperature more than 2-3^{0}C is lethal to the mice. The core temperature of the pregnant dams was measured with a rectal thermometer before and after exposure to hyperthermia. The control group was left at room temperature throughout the period.

Animal sacrifice and dissection: The animals in the experimental group and the control group were allowed to deliver spontaneously. The pups were weighed at birth on a digital laboratory weighing scale (My Weigh i201; Cardinal Scale Manufacturers, Webb City, Missouri, USA)). The pups were euthanized with chloroform in a Coplin jar and decapitated. The heads were examined with a stereomicroscope for gross abnormalities in the palate (non-fusion of the palate). Craniometric assessment was performed by measuring the following parameters of the decapitated head: weight, length of head, width, palatal length and palatal width. The heads were then fixed in10% buffered formalin for 72 hours.

Tissue processing for histological studies: The heads were decalcified in formic acid and then dehydrated in 50%, 70%, 90% and two changes of absolute alcohol for one hour each. They were cleared in two changes of xylene for one hour each and infiltrated in four changes (of 1 hour each) of molten paraffin wax at 60^{0}C. The heads were embedded in paraffin wax. The tissue blocks were mounted, trimmed, and sectioned on a rotary microtome at 7μ thickness. The sections were floated in a water bath at temperature of 30-40^{0}C, mounted on albuminized glass slides and allowed to dry on a hot plate. The sections were deparaffinised and placed in xylene for 20 minutes. They were then passed through two changes each of descending grades (100%, 90% and 70%) of alcohol and water for 5 minutes each. They were thereafter placed in haematoxylin solution for 15 minutes, differentiated in 1% acid alcohol for 4 seconds and washed for 15 minutes in running tap water. They were counterstained in eosin for 1 minute and passed successively for 2 minutes each through increasing grades of alcohol (70%, 90% and 100%), mounted with cover-slip and left to dry overnight.

Photomicrography: The slides were examined with an Olympus microscope (Olympus Manufacturing Systems Inc.4703N Calumet Avenue, Valparaiso, Indiana, USA), and photomicrographs taken with a Kodak digital camera, M340 (Kodak, Seoul, South Korea). The slides were examined for fusion of the palatal shelves and the status of the medial edge epithelium (MEE).

Statistics and data analysis: The cranial dimensions of the neonates of the experimental group were compared with the control groups using means and standard deviations (GraphPad Prism4 software). The level of significance was set at $p < 0.05$.

RESULTS

There was no cleft palate in both experimental and control groups.

Core temperature measurements and temperature profile following exposure to hyperthermia: There was no significant difference between temperature profile of animals exposed for 10 minutes and those for 15 minutes. The initial core temperatures of the mice for the experimental groups were 37.17˚C and 36.4˚C. A 10 and 15 minutes heating periods in 40˚C laboratory oven brought the core temperatures to 39.37˚C and 40.11˚C respectively. These decreased to the initial core temperatures over 18minutes after removal from the oven (Figure 1).

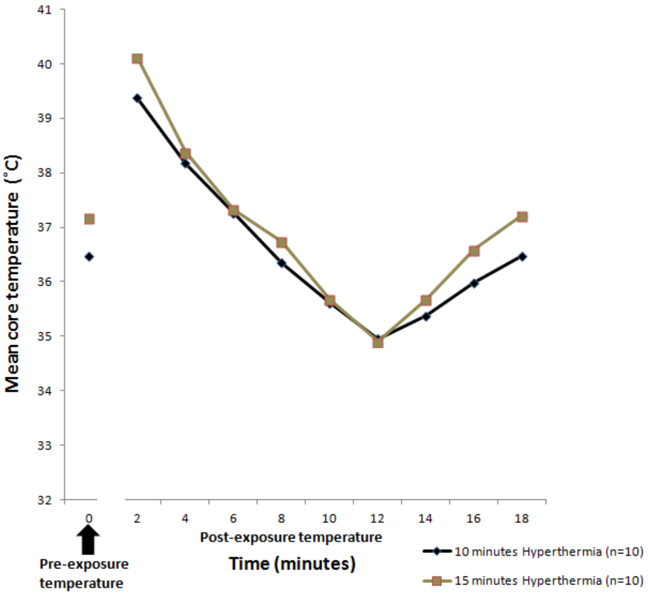

Figure 1
Temperature profile of pregnant mice exposed to hyperthermia

General observations: The mean litter size was reduced in all the experimental groups compared to the control group. The mean litter size from pregnant dams exposed to 10 minutes hyperthermia on E10 was 8.20±0.50 while that for E10 dams exposed to 15 minutes of hyperthermia was 5.60±1.44, (control 10.80±0.58, p value < 0.05 and $p< 0.05$ respectively; Table 1) The mean litter size from E11 dams exposed to 10 and 15 minutes hyperthermia were 7.00±0.32 and 4.40±1.81

respectively (p value <0.01 and p value <0.03 respectively; Table 1). The mean birth weights of neonatal mice from E10 dams exposed to 10 and 15 minutes hyperthermia were 1.11 ± 0.03g and 1.07 ± 0.04g respectively and were lower than the those of controls (1.26 ± 0.01g, p<0.01; table 1). Similarly, the mean birth weights of neonatal mice from E11 dams exposed to 10 and 15 minutes of hyperthermia were also lower than those of the controls (1.16 ± 0.01g and 0.99 ± 0.02g respectively).

Table 1
Litter size and birth weight in mice following in utero hyperthermia

	Hyperthermia – 10 minutes	**Hyperthermia – 15 minutes**	**Control**
Litter size			
E 10	8.20±0.50 (n=10;p<0.05)	5.60±1.44 (n=10;p<0.02)	10.80±0.58 (n=10)
E 11	7.00±0.32 (n=10;p<0.01)	4.40±1.18 (n=10;p<0.03)	10.80±0.58 (n=10)
Birth weight (g)			
E 10	1.11±0.03 (n=79;p<0.01)	1.07±0.04 (n=54;p<0.01)	1.26±0.01 (n=105)
E 11	1.16±0.01 (n=70;p<0.01)	0.99±0.02 (n=55;p<0.01)	1.26±0.01 (n=105)

(E – Embryonic day; n – Sample size)

Craniofacial measurements: The mean head length of neonatal mice from both E10 and E11 dams exposed to 10 and 15 minutes hyperthermia were all significantly smaller (p<0.01) than those of the control group (Table 2) while the mean head widths of neonatal mice from E10 dams exposed to 10 and 15 minutes hyperthermia were 6.48±0.07mm and 7.27±0.12mm respectively. The neonates from E11 dams exposed to 10 and 15 minutes hyperthermia were 6.88±0.10mm and 6.95±0.08mm. The values were all significantly higher (p<0.01) than those in the control group (5.92±0.07mm; Table 2).

The mean lengths of the palate for neonates exposed on E10 were 5.74±0.11mm and 5.38±0.16mm for 10 and 15 minutes exposure respectively. The corresponding values for neonates exposed on E11 were 4.46±0.05mm and 4.51±0.10mm. With the exception of neonates from 10 minutes exposure on E10, the values were reduced compared to controls (5.73±0.07mm, p<0.03, p<0.01; table 2) The palatal length for neonatal mice exposed on E10 for 10 minutes was not significantly different from the controls. The mean widths of the palate for neonates exposed on E10 were 3.77±0.09mm and 2.79±0.06mm for 10 and 15 minutes of exposure respectively. The corresponding values for neonates exposed on E11 were 3.04±0.07mm and 2.69±0.06mm. The width of the palate was significantly reduced in exposed animals compared to controls (3.49±0.0mm, p<0.01; table 2), with the exception of neonates exposed for 10 minutes on E10, in which the width was significantly higher (p<0.01, table 2).

Table 2
Head and palate dimensions in neonatal mice following *in utero* hyperthermia

	Hyperthermia – 10 minutes	**Hyperthermia – 15 minutes**	**Control**
Head width (mm)			
E 10	6.48±0.07 (n=79;p<0.01)	7.27±0.12 (n=54;p<0.01)	5.92±0.07 (n=105)
E 11	6.88±0.10 (n=70;p<0.01)	6.95±0.08 (n=55;p<0.01)	5.92±0.07 (n=105)
Head length (mm)			
E 10	10.99±0.16 (n=79;p<0.01)	11.63±0.07 (n=54;p<0.01)	12.35±0.05 (n=105)
E 11	10.45±0.08 (n=70;p<0.01)	11.67±0.04 (n=55;p<0.01)	12.35±0.05 (n=105)
Palate length (mm)			
E 10	5.74±0.11 (n=79;p=0.96)	5.38±0.16 (n=54;p=0.03)	5.73±0.07 (n=105)
E 11	4.46±0.05 (n=70;p<0.01)	4.51±0.10 (n=55;p<0.01)	5.73±0.07 (n=105)
Palate width (mm)			
E 10	3.77±0.09 (n=79;p<0.01)	2.79±0.06 (n=54;p<0.01)	3.49±0.04 (n=105)
E 11	3.04±0.07 (n=70;p<0.01)	2.69±0.06 (n=55;p<0.01)	3.49±0.04 (n=105)

(E – Embryonic day; n – Sample size)

Microscopic Observations: Microscopic examination of the palatal epithelial lining of neonatal mice in the control group revealed a cartilaginous septum between the palatal epithelium and the nasal epithelium (Plate 1).

Plate 1
Photomicrograph of palatal section of control mice showing the cartilaginous septum (*) between the palatal epithelium (c) and the nasal epithelium (d) with cavitations (arrow heads); a, nasal cavity; b, oral cavity.

Plate 2
Photomicrograph of palatal section of mice exposed to hyperthermia for 10 minutes on day 10 of gestation;. The palatine epithelium is relatively uniform in height (*). The cartilagenous septum is well defined and sits atop the palate (star).

The palate was completely fused with no trace of the medial edge epithelium, MEE. The stratified squamous epithelium of the palate was relatively uniform in thickness. The cartilaginous septum in-between the palatal epithelium and the nasal epithelium showed evidence of early ossification i.e. cavitations (Plate 1).

In the neonates from dams exposed at E10 to 15 minutes of hyperthermia the palatal epithelium was stratified squamous but not uniform in thickness. The cartilage appeared condensed in the mid-line while the epithelium overlying it seems relatively thinner. The palatal section of neonatal mice exposed to hyperthermia for 10 minutes at E11 showed persistence of MEE (Plate 2). The stratified squamous epithelium was also not uniform in thickness, being thicker on either side of the medial edge epithelium. However, the palatal cartilaginous septum was uniform in density with no evidence of ossification. In mice exposed to hyperthermia for 15 minutes at E11, there was bilateral invaginations of the palate together with epithelial proliferation into the cartilage septum and persistence of the MEE (Plate 3). These form pits in the oral cavity with the epithelial proliferation extending into the nasal cavity. The invaginations were equally observed to be covered by grossly uniform palatal epithelium. In some of the sections however, there are fairly dense cartilage with no sign of ossification (Plate 4). The persistent MEE appeared to be thrown into irregular folds of non-uniform thickness. In the palate of neonatal mice from E11 dams exposed to hyperthermia for 15 minutes, there was epithelial proliferation into the cartilage septum merging with the nasal epithelium and making it appear to have undergone metaplastic change, with similar morphology to the oral epithelium. The most superficial portion of the oral epithelium in this section appeared very rough and irregular, especially at the lateral portions (Plate 5).

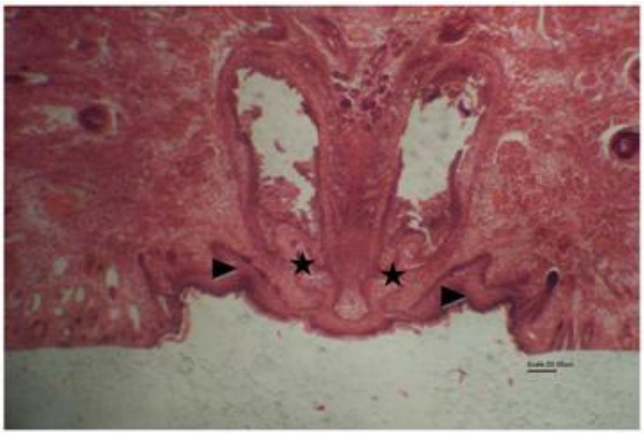

Plate 3
Photomicrograph of palatal section of mice exposed to hyperthermia for 10 minutes on day 10 of gestation;. The palatine epithelium is relatively uniform in height (*). The cartilagenous septum is well defined and sits atop the palate (star).

Plate 4
Photomicrograph of palatal section of mice exposed to hyperthermia for 15 minutes on day 10 of gestation. The epithelium of the palate is stratified squamous and is of variable thickness. It shows areas of hyperplasia, extending upwards to either side of the cartilage of the nasal septum (stars) with a thin superficial layer of keratin (arrowheads). There is also epithelial proliferation on the floor of the nasal cavity (β)

Plate 5
Photomicrograph of a section of the palate from mice exposed to hyperthermia for 15 minutes at day 11 of gestation showing epithelial proliferation into the cartilage septum which merged with the nasal epithelium (*). δ, medial edge epithelium; a, nasal cavity; b, oral cavity; c, palatal epithelium.

Plate 6
Photomicrograph of a section from the palate of mice exposed to hyperthermia for 15 minutes at day 11 of gestation showing epithelial proliferation into the cartilage septum which has merged with the nasal epithelium (*) (δ, medial epithelial edge; a, nasal cavity; b, oral cavity; c, palatal epithelium)

Table 3

Summary of changes in head and palate dimensions in mice following *in utero* hyperthermia

Embryonic Stage (Days)	Exposure (Minutes)	Head Width	Head Length	Palate Length	Palate Width
E 10	10	↑	↓	↓	↑
	15	↑	↓	↓	↓
E 11	10	↑	↓	↓	↓
	15	↑	↓	↓	↓

E – Embryonic day

Increase - ↑ Decrease - ↓

DISCUSSION

The mammalian palate is formed by the union of three elements: the primary palate from the frontonasal process and the two lateral maxillary palatal shelves from the first branchial arch that will form the secondary palate (Mossey et.al., 2009). Palatal fusion involves three events: laterally, the palatal shelves of the maxillae (the future secondary palate) fuse across the midline to form the roof of the oropharynx (Brown and Sandy, 2002). Superiorly, the palatal shelves in the midline fuse with the inferior border of the nasal septum. In the anterior region, the anteromedial borders of the palatal shelves fuse with the primary palate (Cui et al.,2005). Palatal fusion is completed within a very short period of time and it is achieved by outgrowth of bilateral palatal shelves from the medial part of the maxillary processes at embryonic day 12 in mice. By embryonic day 13, these palatal shelves have grown vertically down the sides of the tongue. At embryonic day 14, the shelves elevate to a horizontal position above the dorsum of the tongue, approximate to each other, and come together in the midline at embryonic day 14.5. These sequence of events give rise to a multilayer epithelial seam, formed by the fusion of the opposing epithelia covering the tips of the palatal shelves: medial edge epithelium (Martinez-Alvarez et al.,2000).

The disappearance of the medial edge epithelium is a key event during palatal development and the mechanisms underlying this phenomenon are still controversial (Sani et al.,2005). Cell death is a prominent feature in many organs that are damaged by heat (Miller et al.,2002).

In this species of mice, exposure of pregnant dams to hyperthermia in the laboratory oven raised the core temperature of the pregnant dams by about 3^{o}C above normal. Following removal from the oven, the core temperature responded in a biphasic manner first becoming subnormal, before returning to normal. It is unclear whether the swings in body temperature constitute additional teratogenic stress to the developing conceptus, beyond the mere increase in core temperature. There does not appear to be any difference in the pattern of recovery of body temperature between animals exposed for 10 and those for 15 minutes.

In this study, hyperthermia decreased litter size and birth weight in exposed dams, with the reduction greater in animals exposed for a longer period. These results suggest that hyperthermia is detrimental to intra-uterine growth and development in this species of rodents. The level and duration of hyperthermic insult in this study did not produce clefting of the palate in neonatal mice. This could be due either to the strain of the mice as suggested by Webster and Edwards (1984); Finnell et al. (1996), or the genotype of the embryo (Finnell et al., 1996). The interval between the heating period and the return of the core temperature to the initial level was different from that of the study carried out by Webster et al (1985), where rats were exposed to hot water bath and the animals' core temperatures returned to the initial temperature after about 2 hours. This suggests that the recovery from hyperthermic exposure in wet heat differs from that in dry heat and may have implications for the severity of the insult to the conceptus.

The reduction in the litter size and birth weight in the experimental groups may be due to foetal resorption arising from vascular disruptions of the placenta (Nilsen, 1984) and placenta necrosis (Edwards,1986). In an experimental study carried out by Arora et al. (1979), the effect of high temperature on the placentas of hyperthermia-induced rats resulted in thicker, heavier placentas and degeneration of the decidua basalis of the placentas.

Craniofacial morphology has been postulated as being a possible predisposing factor for orofacial clefts. During embryogenesis, a little deviation in the extent or direction of growth in a critical timeframe can disrupt the fusion of facial prominences. Craniofacial morphological studies (Zeiler et al.,1964; Hart et al.,1969) have shown that there is a complex interrelationship between the various growth parameters of the head, such as the palatal length, palatal width, head length and head height during closure of the secondary palate. Palatal shelf elevation is a rapid movement triggered by both intrinsic forces within the palatal shelves proper and by influences from other craniofacial and oral structures, including the movement of the tongue, and growth of the cranium and mandible (Yu et al.,2009). At the time of palatal closure, the head grows rapidly with different components of the face exhibiting different rates and directions of growth. As head becomes upright and positioned above the heart, the jaw is opened and the tongue is also withdrawn from between palatal shelves, which were originally growing vertically on each side of the tongue. Excessive width of the head has been proposed as a factor in cleft palate aetiology (Fraser,1970).

Studies carried out on the size of the craniofacial complex and palatal development in different strains of mice (Diewert,1982; Gong and Eulenberg, 2001) revealed wider craniofacial parameters. Our results demonstrate the opposite:

narrower craniofacial parameters except for the head width (Table 3). This may be attributed to the strain of the locally bred mice.

This study revealed that hyperthermia on embryonic day 11 has more has a more adverse effect on the palatal development in mice than on embryonic day 10 especially with 15 minutes duration by delaying the elevation of palatal shelves. In mice, the palatal shelves arise from the outgrowing maxillary prominences at embryonic day 11 and by embryonic day 14, there is rapid elevation to a horizontal position and fusion (Ferguson, 1998; Ferglei et al., 2008). For a complete palatal fusion, there must be epithelial-mesenchymal transformation (EMT) of the midline epithelial seam (MES) cells into mesenchymal cells (Fitchett and Hay 1989; Shuler et al.,1992). Alternatively, Medial Epithelial Seam (MES) cells have been thought to disappear by migrating along the midline towards either the nasal or oral epithelia (Carette and Ferguson 1992). All these mechanisms might have been altered by the hyperthermic insults giving rise to the persistence of the MEE. The formation of the mammalian secondary palate which is a highly regulated and complex process can be disrupted at any of the multiple morphogenetic steps (Ferguson, 1998; Hillard et al., 2005; Cohen 2002). The effects of high temperature have been suggested to lead to oxygen deficiency in the cells causing glycolysis followed by acidification of intercapillary spaces and subsequent necrosis resulting from intra and extracellular leakage of lysosomal enzymes (Muhammad Abu-Hussein 2012) could affect the integrity of the MEE.

A common criticism of hyperthermia as a teratogen has been that mammals including humans are homeothermic and that the temperature elevations required to induce defects exceed those which are possible under all but extremes of environmental or febrile conditions. There is little doubt that under less than extreme temperature elevations, embryonic death with resorption or abortion is common. Moreover, an elevation of only 1.5-2.5^{o}C is teratogenic at the right stage in a number of species, including rats, rabbits, guinea pigs, and sheep. Such an elevation is not an unusual event and its occurrence owing to noninfectious agents causes little if any maternal distress or ill health. There is no reason to believe that the human embryo has an abnormally high threshold to heat damage; human malignant and nonmalignant cells in culture appear to be as susceptible to heat as cells from other species (Nilsen 1984). It is important to recognize that cell injury leading to thermal teratogenesis appears to occur at much lower temperature elevations than does the killing of cancerous somatic cells - *in vitro* and *in vivo* (Miller et al., 2002).

CONCLUSION

In-utero exposure to hyperthermia in this outbred mouse strain model interfered with palatal fusion without producing obvious palatal cleft. This supports the results of previous studies on the teratogenic effect of hyperthermia on palatal development and suggests that hyperthermia exerts a spectrum of effects on the developing palate when applied during the sensitive stage of palatal development (Warkany, 1986; Germain et. al., 1985; Edwards, 1986; Bennett, 2010) Although obvious clefts were not demonstrated, the exposure to hyperthermia altered craniofacial dimensions along with the delay of the palatal fusion. Additional studies are needed to explore these findings further

REFERENCES

Arora K.L., Cohen B.J., Beaudoin A.R. (1979). Fetal and placental responses to artificially induced hyperthermia in the rats. *Teratology* ;19:251-60.

Bennett Gregory D. (2010). Hyperthermia: Malformations to Chaperones. *Birth Defects Research* (Part B) 89:279–288.

Brown N.L. and Sandy J.R. (2002). Basic sciences in normal and abnormal palate development. *Braz J Oral Sci.* July/September;(1)2

Carette M.J., Ferguson M.W. (1992). The fate of medial edge epithelial cells during palatal fusion in vitro: an analysis by Dil labelling and confocal microscopy. *Development* ;114:379-88

Cohen M.M. Jr. (2002). Malformations of the craniofacial region: evolutionary, embryonic, genetic and clinical perspectives. *Am J Med Genet* ;115:245-68

Cui, X.M., Shiomi, N., Chen. J. et al., (2005). Overexpression of smad-2 in Tgf-beta-3 null mutant mice rescues cleft palate. *Dev Biol* ;278: 193-202.

Diewert V.M. (1982). A comparative study of craniofacial growth during secondary palate development in four strains of mice. *J Craniofac Genet Dev Biol* ;2:247-63.

Dunmade A.D., Segun-Busari S., Olajide T.G., Ologe F.E. (2007). Profound bilateral sensorineural hearing loss in Nigerian children: Any shift in etiology? *J Deaf Stud Deaf Educ*;12:112-18.

Edwards M.J. (1986). Hyperthermia as a teratogen: A review of experimental studies and their clinical significance. Teratogen Carcinog Mutagen 6:563-582.

Enato E.F.O., Okhamafe A.O. (2006). A survey of antimlarial activity during pregnancy and children’s malaria care-seeking behaviour behaviour in two Nigerian rural communities. *Scand J Infect Dis*;38:474-78

Fenglei H., Xiong W., Yu X., Espinoza-Lewis R., Liu C., Gu S., Nishita M., Susuki K., Yemada G., Minami Y., Chen Y. (2008). Wnt5a regulates directional cell migration and cell proliferation via Rof 2-mediated noncanonical pathway in mammalian palate development. *Development*;135:3871-79.

Ferguson M.W. Palate development. (1998). *Dev Suppl*;103:41-60.

Finnell R.H., Moon S.P., Abbott L.C., Golden J.A., Chernoff G.F. (1996). Strain differences in heat-induced neural tube defects in mice. *Teratology*;33:247-52.

Fitchett, J.E., Hay E.D., 1989. Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. *Dev Biol* ;131:455-74.

Fraser F.C. (1970). The genetics of cleft lip and cleft palate. *Am J Hum Genet*;22:336-52

Germain M.A., Webster W.S., Edwards M.J.(1985). Hyperthermia as a teratogen: parameters determining hyperthermia-induced head defects in the rat. Teratology 31:265-272.

Gong S., Eulenberg B.S. (2001). Palatal development in Twirler mice. *Cleft Palate Craniofac J* ;38:622-28

Graham J.M. Jr, Edwards M.J., Edwards M.J. (1998). Teratogen update: Gestational effects of maternal hyperthermia due to febrile illnesses and resultant patterns of defects in humans. *Teratology* ;58:209-21.

Hart J.C., Smiley G.R., Dixon A.D. (1969). Sagittal growth of the craniofacial complex in normal embryonic mice. *Arch Oral Biol* ;14:995-97.

Hillard S.A., Ling Y., Shuping G., Zunyi Z., Yi R.C. (2005). Regional regulation of palatal growth and patterning along the anterior-posterior axis in mice. *J Anat*;207:655-67.

Iregbulem L.M. (1982). The incidence of cleft lip and palate in Nigeria. *Cleft Palate J*;19:201-205

Lettieri J. (1993). Lip and oral cavity. In: Stevenson RE, Hall JG, & Goodman RM. (Eds.) Human malformations and related anomalies. New York: Oxford University Press:367–74.

Martinez-Alvarez T.C., Perez-Miguelsanz J., O'Kane S., Puerta J., Ferguson M.W.J. (2000). Medial edge epithelial cell fate during palatal fusion. *Dev Biol* ;220:343-57.

Miller M.W., Nyborg W.L., Dewey W.C., Edwards M.J., Abramowicz J.S., Brayman A.A. (2002). Hyperthermic teratogenicity, thermal dose and diagnostic ultrasound during pregnancy: implications of new standards on tissue heating. *Int. J. Hyperthermia*; vol.18:5:361-384

Mossey Peter A., Little Julian, Munger Ron G., Dixon Mike J., Shaw William C. (2009). Cleft lip and palate. *Lancet ;* 374: 1773–85

Muhamad Abu-Hussein. (2012). Cleft lip and palate – etiological factors. *Dent Med Probl* ;49:2:149-156

Nilsen N.O. (1984). Vascular abnormalities due to hyperthermia in chick embryos. *Teratology* ;30:237-51.

Omo-Aghoja V.M., Omo-Aghoja L.O., Ugboko V.I., Obuekwe O.N., Saheeb B.D.O., Feyi-Waboso P., Onowhakpor A. (2010). Antenatal determinants of oro-facial clefts in Southern Nigeria. *African Health Sciences*;10:31-39

Orogade A.A., Falade C.O., Okafor H.U. et al., (2008). Clinical and laboratory features of congenital malaria in Nigeria. *Journal of Pediatric Infectious Diseases*;3:181-87

Sani F.V., Hallberg K., Harfe B.D., McMahon A.P., Linde A., Gritli-Linde A. (2005). Fate-Mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. *Dev Biol* ;285:490-95.

Sasaki J., Yamaguchi A., Nabeshima Y., Shigemitsu S., Mesaki N., Kubo T. (1995). Exercise at high temperature causes maternal hyperthermia and fetal anomalies in rats. *Teratology* ;51:233-36.

Shuler C.F., Halpern D.E., Guo Y., Sank A.C. (1992). Medial Edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation in-vivo. *Dev Biol* ;154:318-30.

Warkany Josef (1986). Teratogen Update: Hyperthermia. *Teratology* 33:365-371

Webster W.S., Edwards M.J. (1984). Hyperthermia and the induction of neural tube defects in mice. *Teratology* ;29:417-25.

Webster W.S., Germain M., Edwards M.J. (1985). The induction of microphthalmia, encephalocele and other head defeats following hyperthermia during the gastrulation process in the rat. Teratology ;31:73-82.

Yu W., Serrano M., Miguel S.S., Ruest L.B., Svoboda K.K. (2009). Cleft lip and palate genetics and application in early embryological development. *Indian J Plast Surg* ;42:35-50

Zeiler K.B., Weinstein S., Gibson R.D. (1964). A study of the morphology and the time of closure of the palate in the albino rat. *Arch Oral Biol* ;9:545-549