Abnormal Soft Palate, Lingual Mucosa and Intestine of Neonates Maternally Fed on Diet Containing Fried Potatoes Chips or Received Acrylamide-Treatment

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Abstract

Recently, several studies have been documented the presence of acrylamide residues in fried food stuffs such as fried potato chips, snacks etc., due to Maillard reaction for its contents of glucose and asparagine amino acid. The present study was carried out to illustrate the effect of maternal dietary intake of fried potato chips on the development of soft palate, lingual papillae and duodenum of their neonates (1, 7, 13 and 21 days) compared to acrylamide-treatment. Eighty pregnant Wistar albino rats arranged into three groups were used. These are control, fried potato chips (50% standard diet) and acrylamide-treatment (10 mg/kg body weight). Experimentation of acrylamide-treatment or dietary intake of fried potato chips was carried out from 6th day of gestation till 21 days post-partum. Neonates of the mentioned ages were sacrificed and their soft palate, dorsum tongue surface and duodenum were examined histologically, scanning electron microscopically and DNA damage by using comet assay and DNA fragmentation. The present findings revealed several deformations of lingual papillae and taste bud bodies in soft palate. The experimental treated neonates exhibited histological abnormalities such as decreased cornification of the filiform papillae, degeneration of the apical taste buds of fungiform papillae and deformed taste buds in soft palate. The mucosal layer of duodenum possessed a marked damage of epithelial lining cells, reduction of goblet cells and comparative atrophy of the villi. Apparent atrophy of the fungiform papillae and decreased keratinization of the filiform papillae were detected at scanning electron microscopic level. Single and double strand DNA damage was markedly observed in lingual and duodenal cells.

The authors finally concluded that maternally dietary intake of fried potato chips caused marked damage of soft palate, lingual papillae and duodenum of neonates coincides with DNA damage.

Keywords: Acrylamide; Fried potato chips; Neonate; Soft palate; Lingual mucosa; Duodenum; Scanning electron microscopy; Comet assay; DNA fragmentation

Introduction

The findings in April 2002 of elevated levels of acrylamide in a variety of foodstuffs were unexpected and led scientists to do additional research on acrylamide [1]. Recently, high levels of acrylamide (ACR) were unexpectedly detected in widely consumed food items, notably French fries, potato crisps, and bread [2]. French fries and potatoes chips are the common food items of children in fast food restaurants, over the past 30 years. It is now well documented that acrylamide is formed as a result of the reactions of reducing sugars with free amino acids, especially asparagine at high temperatures [3-5].

Different studies have been reported that the fresh food materials contain L-asparagine (L-Asn) and reducing sugars which after overheating in preparing the processed food materials developed varying amounts of acrylamide according to their contents of the mentioned primary nutrients [6-8].

Dietary intake of fried potato chips comparing with acrylamide-treatment have been found to develop neuropathies of developing spinal cord [9], mal-differentiation of both cerebellar neuronal cells during postnatal life [10] and retinal cells [11] and congenital malformation of fetuses with massive histological abnormalities of maternal liver, kidney and femoral bone [12].

The digestive system is the highly affected organ through ingestion and metabolism of these food materials. There is a great concern of acrylamide with decreased BrdU incorporation, expression of cytosolic beta-catenin and cyclin D1; and increased apoptosis in the colon mucosa associated with development of preneoplastic lesions of rats [13]. Two-year acrylamide-treatment was found to develop tumors in Harderian gland and lung in male and female B6C3F1 (1) mice [14]. Mammary gland, thyroid tumors and scrotal mesothelioma of experimental animals and human ovarian and endometrial, renal cell, breast, and oral cavity are highly susceptible to produce cancer in relation with acrylamide exposure [15,16]. Also, acrylamide is closely associated with development of cancer in human especially in oral cavity and pharynx, esophagus, large bowel, colon, rectum, larynx, and prostate [17].

There is no available work on acrylamide on soft palate, lingual papillae and duodenum of neonates as well as little of information’s are reported on the digestive tract of adult human. Hence, the present study focused on illustrating the suspected alterations of the mentioned organs of rat neonates maternally fed on fried potato chip comparing with acrylamide-treatment.

Materials and Methods

Eighty virgin female and fertile male albino rats 160 g-180 g b.wt (1 male/3 female) obtained from Hellman Breading Farm, Ministry of......
health, Egypt were used during experimentation. They were maintained under good ventilation and 12 h light and dark cycles with free access of food and water ad libitum.

**Acrylamide treatment**

Acrylamide obtained from Sigma-Aldrich chemical company with highest purity of 99.9% was used. Each pregnant rat dosed orally 10 mg/kg body weight from 6th day of gestation till the end of the 3rd week after parturition. The applied dose was chosen according to Olstorn, et al. [18] and Bowyer, et al. [19].

**Fried potatoes chips supplementation with food**

Fried potatoes chips were brought from the market and mixed with standard diet at a concentration of 50% and used for feeding of pregnant rats throughout the experimental period as previously mentioned.

**Experimental Work**

This work was carried out under the supervision of Ethical Committee of Faculty of Science, Mansoura University. Sixty fertile virgin females and fertile males of Wistar albino rats (Rattus norvegicus) were obtained from Hellman Animal Breeding farm, Ministry of health, Cairo, EGYPT and used for experimentation. Their body weight was nearly 150 g-160 g. Virgin females were mated with male at ratio of 1 male/3 female for overnight and the next morning examined for vaginal plug and sperm to precise the onset of gestation. The housing lab for rats is good aerated with 12 h light-dark cycle and approximately 23°C. Free access of standard diet and water were supplied ad libitum.

The pregnant were divided into three groups (n=16) such as control fed on standard diet, acrylamide-treatment (Each received oral doses of 10 mg/kg body weight from 6th day of gestation till 1, 2 and 3 week post-partum) and fried potato chips-supplemented group (Each pregnant fed on standard diet containing 50% fried potatoes chips during the previously mentioned experimental period). Neonates at 1, 7, 14 and 21 day-old of both control and experimental groups were weighed and scarified and their soft palate, tongue, and duodenum were dissected and processed.

**Histological studies**

Soft palate, tongue and duodenum were separated and fixed in phosphate buffered 10% formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten paraplast 58°C-62°C. Histological sections five µm were carried out, stained with haematoxylin and eosin and examined under bright field light microscope.

**Scanning electron microscope**

Specimens of soft palate and tongue specimens of the studied groups were fixed in 2.5% phosphate buffered glutaraldehyde, dehydrated in ascending grades of ethyl alcohol, critically drying in carbon dioxide apparatus, coating with gold and examined under a Joel 5300JSM (Musashino3-chome Akishima Tokyo 196-8558, Japan).

**The single cell gel electrophoresis (Comet assay)**

Tongue and duodenal specimens of 1, 7, 14 and 21 days-old neonates were separated and immediately stored at-20°C. The gel layer was carried out by adding 100 µL of normal melting point agarose (0.7%) onto a charged slides and cover slipped until the agarose solidified at 4°C, then removed. The samples were conjugated with the low melting-point agarose and 100 µL of the mixture was run to the gel layer. The process of slide covering was repeated and the cover slide was removed after solidification. Lysis of cells was carried out by 100 mL of fresh lysis buffer (2.5 mol/L NaCl, 100 mmol/L EDTA, 1% sodium hydroxide, 10 mmol/L Tris, 1% Triton X-100, 10% DMSO (pH 10) at 4°C for 1 h. After draining, slides were treated with DNA unwinding solution (300 mmol/L NaOH, 1 mmol/L EDTA, pH 13) for 30 min at 4°C, and placed directly into a horizontal gel electrophoresis chamber filled with DNA-unwinding solution. Gels were run with constant current (300 mA at 4°C) for 30 min. After electro phoresis, the microgels were neutralized and stained with 20 µL ethidium bromide (10 µg/mL) and visualized at 400 × magnification using an inverted fluorescence microscope (Olympus, Tokyo, Japan) attached to a video camera (Olympus). Damaged cells characterized a brightly fluorescent head and a tail to one side formed was photographed and counting as 100 cells per slide to calculate the percent of damage. The tail moment is assayed from the equation of “Tail moment=Tail length × % of DNA in the tail”. Both tail length and DNA intensity are measured automatically by image analysis software [20].

**DNA fragmentation assay**

Freshly isolated specimens were suspended in 100 ml of lyses buffer (10 mM Tris HCl/10 mM EDTA/0.5% Triton X-100, pH 8.0), vortex-mixed, sonicated, and incubated on ice for 20 min. After centrifugation for 20 min at 4°C 14, 000 3 g), the supernatant containing fragmented (soluble) DNA were treated with RNase A (0.5 mg/ml) for 1 hr at 37°C and then with proteinase K (Sigma, 0.4 mg/ml) for 1 hr at 37°C to remove RNA. After adding 20 mL of 5 M NaCl and 120 ml of isopropanol, the samples were incubated overnight at 20°C, and the fragmented DNA was investigated with a ladder-like appearance [21].

**Biostatistics**

Statistical analysis was carried out between both the control and experimental groups. Means and standard deviations were calculated from ten replicates per each case and student T test was determined and the differences were considered significance from the control when P<0.05.

**Body weight gain**

Figure 1 illustrates that the neonates at 1, 7, 14, and 21 days-old maternally-treated with either acrylamide (10 mg/kg b.wt) or supplemented food containing 50% fried potatoes chips possessed significant decrease of the mean body weights compared to the control. Neonates of acrylamide-treated mothers were highly affected.

![Figure 1: Mean body weight (g) of neonates maternally fed on fried potato chips or received acrylamide-treatment.](Image 327x94 to 553x229)
Scanning and Light Microscopic Observations

Soft palate

At scanning electron microscopic level, the control neonates of 1 and 7 day older possessed regularly arranged dome-shaped depression of taste buds surrounded by thin filaments structure semi-like filiform papillae. Its outermost zone is wrinkled and the intermediate one become smooth (Figures 2A and A1). Neonates maternally treated with acrylamide or fed on fried potato chips exhibited retarding differentiation of their structural organization of taste buds (Figures 2B and B1). Light microscopic observation of 1 day-old neonate revealed that the control neonate one-day old exhibited thin mucosal layer of soft palate with regularly oriented fungiform papillae with its characteristic taste buds. There is a large amount of elastic tissue present in the lamina propria, arranged in the form of interlocking bundles Blood vessels are also distributed (Figure 2A2).

On the other hand, the soft palate of neonates maternally treated with acrylamide or fed on fried potatoes chips showed abnormal developing structures with malformed taste buds and abnormal distribution of blood vessel (Figures 2B2 and C2).

Tongue

At scanning electron microscopic level, the control neonate 1 day-old possesses numerous fungiform papillae at the anterior and middle dorsum tongue surface. Keratinized filiform papillae of either rounded or conical breaded free ends are densely distributed around the gustatory papillae compared to the control. (Figure 3A). On the other hand, there is a considerable missing of ordinary normal structures and deformation of both fungiform and filiform papillae in neonate maternally treated with acrylamide or fed on fried potatoes chips (Figures 3B and C). In control 14 and 21-day old neonate, the filiform and fungiform papillae are well differentiated. Taste bud pores are clearly observed in the apical surface of the fungiform papillae (Figures 3A1 and A2). However, in those neonates maternally treated with acrylamide or fed on fried potato chips, there was a marked increase in deformations of both the fungiform and filiform papillae (Figures 3B1, 3B2, 3C1 and 3C2).

At light microscopic level, the lingual mucosa of control neonate 1 day-old possessed few numbers of the fungiform papillae all over the dorsal lingual surface. The filiform papillae take the form of finger-like projections with rounded or blunted tips. The entire core of the papillae was formed of a fine collagen network and fibroblasts (Figure 4A). However, in neonates maternally treated with acrylamide or fed on fried potatoes chips, there was detected comparative reduction of lingual keratinization. Atrophied of fungiform papillae with characteristic degeneration of their taste buds were observed. Leukocytic infiltrations were abundant in the collagenous network of lamina propria (Figures 4B and C).

In control 14, 21 day-old neonates, there are abundant distribution of fungiform papillae with marked differentiation of taste buds. Keratinization of the filiform papillae were markedly increased (Figures 4A1, A2). However in those neonates maternally treated with acrylamide or fed on diet contained fried potatoes chips; there was a marked decrease of keratinization and degeneration of apical taste buds of the gustatory fungiform papillae. Their connective tissue core possessed leukocytic infiltrations (Figures 4B2, 4C2).

Intestine

The more sensitive region of the duodenum is its mucosal layer. It is appeared in the form of villi with short crypts (glands), lined by a simple columnar epithelium and goblet cells are regularly interval in between the epithelial cells. In control newly born, the duodenum possesses...
tortuous arrangement of villi having numerous supranuclear vacuoles in between the epithelial lining cells and fairly less numerous of goblet cells. Alcian blue staining possesses few numbers of goblet cells (Figures 5A, A1). In neonates maternally fed on diet containing 50% fried potatoes chips, or received acrylamide-treatment, there was a marked loss of many of the supranuclear vacuoles as well as degeneration of lining epithelial cells. The size and depth of the villi were comparatively decreased. Few numbers of goblet cells were detected. The intensity of damage was detected post-acrylamide-treatment (Figures 5B, B1, C, C1). In control 7d-old neonate, the villi are more elongated associated with widening of their terminal free borders. Crypts of Lieberkuhn are more developed. Abundant goblet cells are detected in between the lining epithelial cells after alcian blue staining (Figures 5A2, A3). On the other hand, there was a detected massive damage of epithelium and goblet cells lining the villi. Leukocytic infiltrations were remarkably increased in crypts. Highest atrophy of villi and degenerative lesions were observed in neonates maternally fed on fried potatoes chips or received acrylamide-treatment, (Figures 5B2, B3, C2, C3).

Genomic DNA Damage

DNA fragmentation

Lingual mucosa and intestine of neonates at 1, 7, 14 and 21 days old maternally fed on fried potato chips or received acrylamide-treatment possessed genomic DNA fragmentation. Higher incidence of genomic DNA fragmentation was markedly increased in duodenal tissues of those maternally treated with acrylamide (Figures 6A and B).

Comet assay

There was a detected increase of tail length and DNA concentration of lingual mucosa and duodenal cells of neonates maternally fed on diet containing fried potato chips or received acrylamide-treatment (Figures 7, 8A and B).

Discussion

Body weight

Breast feeding neonate represents the main period of active growth and differentiation of the body organs. The present findings revealed that the developing neonates maternally treated with acrylamide or fed on fried potatoes chips showed a decrease of their mean body weights and are highly affected with increasing age. The present findings confirmed the work of Friedman, et al. [22] and Garey, et al. [23] in rat neonate maternally treated with acrylamide throughout lactation. Wise, et al. [23] Friedman, et al. [22] attributed the depletion of body weight to the reduction of feeding habit explained by decreased or missing milk.

![Figure 4](image_url)

**Figure 4:** (A-C3) Photomicrographs of lingual mucosa of 1 14 and 21day-old neonate maternally fed on fried potato chips (C-C2) or received acrylamide-treatment (B-B2) compared to the control (A-A2). Note decreased cornification and damaged lingual mucosa of filiform papillae of neonates maternally fed on fried potato chips (C and C1) or treated with acrylamide (B and B1). Also the fungiform papilla of the control possesses apical differentiation of taste bud of fungiform papillae and atrophied filiform papillae surrounding the gustatory papillae compared to the control. Also, the control possess fungiform papilla with well differentiated apical taste bud (A2) compared to deformed ones in those of mother fed on fried potato chips (C2) or treated with acrylamide (B2).

![Figure 5](image_url)

**Figure 5:** (A-C3). Photomicrographs of transverse histological section of duodenum 1 and 7 day-old neonate maternally fed on fried potato chips (C-C3) or received acrylamide-treatment (B-B3) compared to the control (A-A3). Note atrophy of villi and degeneration of both epithelial lining cells and disorganized crypts of Lieberkühn in those of mothers treated with acrylamide (B-B2) or fed on fried potato chips (C-C2). A-A2, B-B2 and C-C2 HX-E. Alcian blue staining histological sections (A3-C3) exhibited regular orientation pattern of alcian positive staining goblet cells compared to marked loss of goblet cells in those of mother fed on fried potato chips (C3) or treated with acrylamide (B3).
in their stomach as a result of losing of their appetite. Also, hypoxia developed from formation of acrylamide DNA adduct [24] may disrupt the body metabolism as well as inhibition of protein synthesis.

**Soft palate**

Normally, the oral cavity is the proximal part of digestive tract important for swallowing and mechanical transmission of the food items to the esophagus. Scanning electron microscopy and histological examination revealed that that soft palate of 1 and 7-day old neonates possessed normally oriented large spherical taste buds scattered in the soft palate basal lamina. The gustatory function of soft palate taste buds is important for consuming soft nutrient materials in suckling neonate. Similar findings were recorded by Hall and Bryan [25], El-Sharaby, et al. [26] and Rashwan et al. [27]. Also, neonates maternally fed on fried potatoes chips or intoxicated with acrylamide exhibited altering of the palate surface with marked retardation of taste buds at SEM level and massive damage of taste buds at the light microscopic level. The damage of neonatal taste buds may be attributed to the increased acrylamide level in blood and secretion in milk, the nutritive source of neonates. It is known that acrylamide produced neurotoxicity by forming irreversible Michael-type adducts with nucleophilic sulfhydryl thiolate groups on cysteine residues of nerve cell proteins [28] and interfering with different pattern of neurotoxicity [9,29]. The observed deformation of palatal taste bud may be associated to direct cytotoxicity of acrylamide or its metabolite on cell differentiation leading to deformation of taste bud.

**Tongue**

Also, the lingual mucosa carries different forms of the lingual papillae. Filiform papillae perform mechanical function facilitating habit. The control neonates possess regular distribution of keratinized filiform papillae. The present findings agree with Dhouailly, et al. [30], Iwasaki, et al. [31] and Wannaprasert et al. [32]. Nagatoet, al. [33] mentioned that the filiform papillae facilitated uptake of food items and thermoregulation of body temperature. On the other hand, the control fungiform papillae are detected throughout the dorsum tongue surface. Taste buds and taste pores are clearly detected at scanning electron microscopic level and apically distributed in histological investigation of the studied control neonates. Similar findings were reported by Silva, et al. [34] after studying the surface structures of the lingual papillae in the rabbit.

Neonates of mother fed on diet containing fried potato chips or received acrylamide treatment exhibited atrophied gustatory fungiform papillae with almost missing of their apical taste buds. There is a considerable decrease of keratinization in filiform...

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**Figure 6:** Genomic DNA fragmentation of lingual mucosa (A) and duodenum (B) of neonate 1, 7, 14 and 21 days old maternally fed on fried potatoes chips or received acrylamide-treatment showing increased DNA fragmentation.

**Figure 7:** Comet assay of (A) lingual mucosa and (B) duodenum of neonate 1 and 7 days old maternally fed on fried potatoes chips (C-C3) or received acrylamide-treatment (B-B3) showing detached cells with increased tail length manifesting DNA damage compared to intact nuclei of control (A-A3).

**Figure 8:** (A and B) Chart illustrating tail length (µm) of lingual (A) and duodenal (B) cells of neonates maternally fed on fried potato chips or received acrylamide-treatment. Note increased tail length in those of treated mother rats.
papillae. These may be attributed to the increased level of acrylamide in blood of treated mothers (median 21 pmol/g globin, range 18-104 pmol/g globin) as well as in the umbilical cord blood of neonates (median 10 pmol/g globin, range 6-43 pmol/g globin) as a result of its higher affinity of forming N-terminal hemoglobin adducts. These may alter the oxygen transport leading to hypoxia, vascular disturbances and release toxic metabolites such as nitric oxide interfering with mitochondrial energy metabolism and liberation of free radicals [24,35] as well as forming adducts with glutathione, proteins, and DNA directly or after metabolized to its epoxide, glycidamide (2, 3-epoxy-1-propanamide), the more toxic metabolite [36]. The concentration of acrylamide adducts in the blood of neonates reached almost to 50% of the adduct level found in the blood of the mother. Although acrylamide had a very shorter life span in neonatal erythrocytes, the incidence of acrylamide adducts in neonates was equal to that of the mother. The trans-placental exposure of neonates to acrylamide may increase fetal defects [37].

Duodenum

The observed findings revealed the neonates maternally treated with acrylamide or fed on fried potatoes chips showed marked degeneration of both epithelium and goblet cells lining the villi which become atrophied. Disorganization of villar epithelium led to mal-absorption of food metabolites and consequently affected the neonate. Similar intestinal damage was reported in small intestine of male mice of the BALB/c [38] and small and large intestine of rat [39] subjected to acrylamide-treatment at different dose levels. The present findings supported the work of Tomaszewska, et al. [40] in jejunum of guinea pig maternally dosed daily orally doses of 3 mg/kg body weight of acrylamide in drinking water for 35 days during intrauterine life. The authors reported decreased thickness of both crypt Liberkuhn and villi epithelium coincides with apparent reduction of goblet cells in the duodenum. Also, there is a marked increase of DNA fragmentation of both lingual and intestinal cells of neonates maternally treated with acrylamide or received diet containing fried potato chips. Higher intensity was detected in intestinal cells. Similar DNA damage was reported in sperm of mice [41,42], testis [43,44], liver of rat received 20 mg/kg-40 mg/kg body weight [45,46], as well as in keratinocytes exposed to 2.00 mmol/L. Acrylamide was found to metabolized into glycidamide, the active binding DNA molecule forming DNA adduct [47-49] leading to chromosomal breaking [50].

Epidemiological studies revealed that although the level of acrylamide in the fried food stuffs, it is sufficient evidence for its carcinogenicity. Data from Italian and Swiss hospital clarify that dietary acrylamide intake and cancers of the oral cavity and pharynx (749 cases, 1, 772 controls), esophagus (395 cases, 1, 066 controls), large bowel (1, 394 cases of colon, 886 cases of rectal cancer, 4, 765 controls), larynx (527 cases, 1, 297 controls), breast (2, 900 cases, 3, 122 controls), ovary (1, 031 cases, 2, 411 controls) and prostate (1, 294 cases, 1, 451 controls).

Conclusion

The authors finally concluded that pregnant must be aware of feeding on fried potato chips due to its content of acrylamide residues. Acrylamide and its toxic metabolites were reaching to fetus via transplacental passage as well as secreted in milk and affected neonates. Hazard damage was reported in soft palate, tongue and intestine of neonate. It is recommended to overheating of slices of potato chips to a steam of water vapor before processing to reduce acrylamide formation.

References

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