

THE CARCINOGENIC POTENTIAL OF CADMIUM ON GINGIVA OF RATS (HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Introduction: Cadmium is a toxic, heavy industrial metal that causes serious environmental health hazards. Cadmium containing compounds have been classified as known human carcinogens and epidemiological data show causal associations to different types of cancers. This study designed to assess the carcinogenic effects of Cadmium on the gingival tissue of rats.

Material and methods: Sixteen male albino Rats (age: 8 to 10 weeks and about 150 to 180 g body weight) from Animal Center of Faculty of Medicine, Mansoura University. The animals were divided into two equal groups, group I (control) and group II (cadmium chloride) rats were given 2 ml dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride orally by gavage needle for 24 days. By the end of experiments, gingival epithelium around the teeth were carefully dissected, processed and stained using Haematoxyline & Eosin and immunohistochemical evaluation by iNOS. Statistical analysis was done to observe the statistical significant differences between the Cd group and control group in relation to dysplastic changes and iNOS immunoreaction.

Results: The gingival tissue of Cd group revealed several dysplastic changes as, drop shape rete pegs, loss of polarity of basal cells, nuclear hyperchromatism, pleomorphism and increased mitotic pattern. Mild dysplastic changes were observed in 4 cases (50%), moderate dysplastic changes in 2 cases (25%), however no dysplastic changes in 2 cases (25%). a statistical significant difference regarding iNOS immunoreaction was found between control group and Cd group.

Conclusion: This study showed that Cadmium chloride at low concentration and longtime of exposure can cause dysplastic changes in gingival tissue of rats.

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INTRODUCTION

Cadmium (Cd) is a heavy metal, it arises from environmental and industrial wastes and carry great risk on animal and human health. It has a cumulative toxic effect due to its long biological half-life^(1,2,3). Cd deposits can exist in air, water and soil and can lead to dangerous effects on human and other organisms^(4,5,6).

Cadmium is used extensively in various industries and produced as a by-product of metals melting. It is also a contaminant in cigarette smoke. Cadmium is classified as a human carcinogen by the Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC). Several reports revealed the association between chronic exposure to Cd and cancer developments especially in lung, liver and kidney⁽⁷⁾. Chronic low-level intake of these metal ions or their excessive levels in the body, entering through inhalation of dust and especially ingestion of bioaccumulated metals in food chain as the main route of exposure, can be of health concern⁽⁸⁾, also changes in oral epithelium and salivary gland tissue due to chronic Cd exposure are also assessed^(9&7).

Different mechanisms are involved in Cd carcinogenesis that include; abnormal gene expression, inhibition of DNA damage repair, induction of oxidative stress, and inhibition of apoptosis. In addition, the ability of Cd to cause aberrant DNA methylation, endocrine disruption and cell proliferation may assume minor importance with respect to its carcinogenic potential^(10,11).

Nitric oxide (NO) is a bioactive free radical with short-life. It acts as a messenger molecule for various physiological and pathological operations including strong oxidative activity that contributes to the killing of microorganisms^(12&13). In contrast, NOS (iNos) is induced by a variety of immunologic stimuli including bacterial lipopolysaccharides (LPS) and proinflammatory cytokines contributing to the killing of microorganisms^(12&14). Activation of iNOS leads to the production of large amounts

of NO from inflammatory cells and also gingival cells for sustained periods of time⁽¹⁵⁾. As iNOS is expressed almost exclusively under inflammatory condition, this has led to the hypothesis that iNOS promotes the inflammatory response⁽¹²⁾.

The aim of this research was to assess the carcinogenic effects of Cd, on the gingival tissue of albino rats, using histological and immunohistochemical examination.

MATERIAL AND METHODS

Study design

The current study was done on sixteen male Rats (age: 8 - 10 weeks and about 150 to 180 g body weight), they were housed in Experimental Research Center (MERC), Mansoura University, Egypt. The animal handling and experimental protocols were approved according to ethical committee for animal care and followed to the roles defined the controlling principle for the animals laboratory procedure in faculty of Dentistry, Mansoura University (the code number A 24120219). They were given the commercial soft diets and water regimens under standard temperature (22–25°C), good ventilation and hygienic conditions. The cadmium chloride was gained from Sigma Chemical Company (St. Louis, MO, USA) with the following characters: hydrate minimum 96% and water content approximately 2.8 mole/mole).

Grouping of animals and treatment

After one week, the Rats were divided randomly into 2 equal groups (n=8). Group I (control negative) and group II cadmium chloride (Cd cl), both groups were given a standard rat chow and water. Rats in group II were given daily 2 ml dose of a solution containing 10 mg/kg body weight of monohydrated cadmium chloride orally by gavage needle for 24 days⁽¹⁶⁾. Similarly, rats in group I (control group) were given daily 2 ml dose of distilled water orally by gavage needle for 24 days.

Histological preparation

Animals of each group were sacrificed by exposure to ether anesthesia. Gingival tissues around the teeth were carefully dissected and fixed in 10% neutral buffered formalin. Gingival tissues were mounted in paraffin wax to prepare paraffin blocks and 4 μ m sections were cut and stained using

1. Haematoxyline and Eosin (H&E) stain for histological examination.
2. iNOS stain for immunohistochemical examination.

Immunohistochemical technique: 4 μ m thick sections were cut from each block; slides were immersed in 0.5% methanolic hydrogen peroxide for 15 min to block endogenous peroxidase. Citra (Biogenex) was used for antigen retrieval. The sections then were incubated with the primary antibody (anti-iNOS, C-terminal, rabbit polyclonal antibody), sc-651, Santa Cruz Biotechnology, Inc.), diluted 1:100 in PBS overnight in a specific chamber at humidity 4°C. Antibody binding sites were visualized using a streptavidin-peroxidase detection kit (Kit Multilink, Biogenex). Slides with Hematoxylin counterstain were mounted. As negative control for antibody specificity tissue samples were included in which the primary antibody was neglected. The presence of brown staining in the cytoplasm was considered positive provided it could be clearly distinguished from the negative reaction of the nucleus in preparations without counterstaining. Immunohistochemical evaluations: reaction was considered negative if the reaction less than 5% positive cells mildly reaction 5-10%; moderate 10-25% and high reaction <25%.⁽¹⁷⁾.

Computer Assisted digital image analysis (Digital morphometric study)

Quantification of Immunohistochemical staining, slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 400 x objective. The re-

sult images were analyzed on Intel® Core I3® based computer using VideoTest® Morphology® software (Russia) with a specific built-in routine for area measurement and stain quantification.

Two slides from each rat were prepared and five random fields from each slide were analyzed.

Statistical analysis

Fischer exact test was done to observe the statistical significant differences between the Cd group and control group in relation to dysplastic changes and iNOS immunoreaction p value was significant at 0.05> .

RESULTS

Clinical observations: Gingival changes in Cd group in the form of leukoplakic (white patches) in the gingiva of 2 rats (25%) and erythroleukoplakic (red and white patches) in 4 rats (50%), however 2 (25%) rats have no changes.

H&E results

Group 1 (control group): the gingival tissue appeared normal. It is formed of surface epithelium and underlying connective tissue lamina propria. The surface epithelium is keratinized stratified squamous epithelium with different 4 types of cells. Basal cell layer appeared columnar in shape with oval nuclei, spinous cell layer appeared polyhedral in shape with rounded nuclei and superficial granular cell layer with flat nuclei covered with keratin layer. The underlying lamina propria is formed of papillary and reticular layers. The papillary layer revealed tall, numerous and slender papillae (rete pegs). It contains fine collagen fibers arranged in a loose network. The reticular layer contains coarse closely packed collagen fibers (Fig 1, A).

Group 2 (cadmium group): the gingival tissue revealed basal and parabasal hydropic degeneration and some dysplastic changes as, drop shape rete pegs, loss of polarity of basal cells, nuclear hyperchromatism, pleomorphism and increased mi-

totic pattern. Mild dysplastic changes (dysplastic changes in basal third of tissue) were observed in 4 cases (50%), moderate dysplastic changes in 2 cases (25%) (Dysplasia till the middle third of the epithelium), however no dysplastic changes in 2 cases (25%) (Fig 1, B).

A statistical significant difference was present between group 1 and group 2 regarding to epithelial dysplastic changes (Table 1).

TABLE (1) Dysplastic changes in Cd group versus control group.

| | Cd group | Control group | Test of significance |
|-----------------------|----------|---------------|----------------------|
| | N=8 | N=8 | |
| Dysplastic changes | 6 (75%) | - | FET |
| NO dysplastic changes | 2 (25%) | 8 (100%) | P=0.04* |

*F.E.T: Fischer exact test p: probability * statistically significant at (p>0.05).*

Immunohistochemical results

All cases of group 1 revealed negative reaction for iNOS (Fig 2, A), while in group 2, 6 cases(75%) revealed positive reaction, it range from mild in 2 cases and moderate reaction in 4 cases while 2 cases had negative reaction (Fig 2, B-D). There was a statistical significant differences regarding iNos immunoreaction was found between control and Cd group, (Table 2).

TABLE (2) Immunoexpression of iNOS in both groups

| | Cd group | Control group | Test of significance |
|---------------|----------|---------------|----------------------|
| | N=8 | N=8 | |
| INOs positive | 6 (75%) | - | FET |
| INOs negative | 2 (25%) | 8 (100%) | P=0.04* |

*F.E.T: Fischer exact test * statistically significant at (p>0.05)*

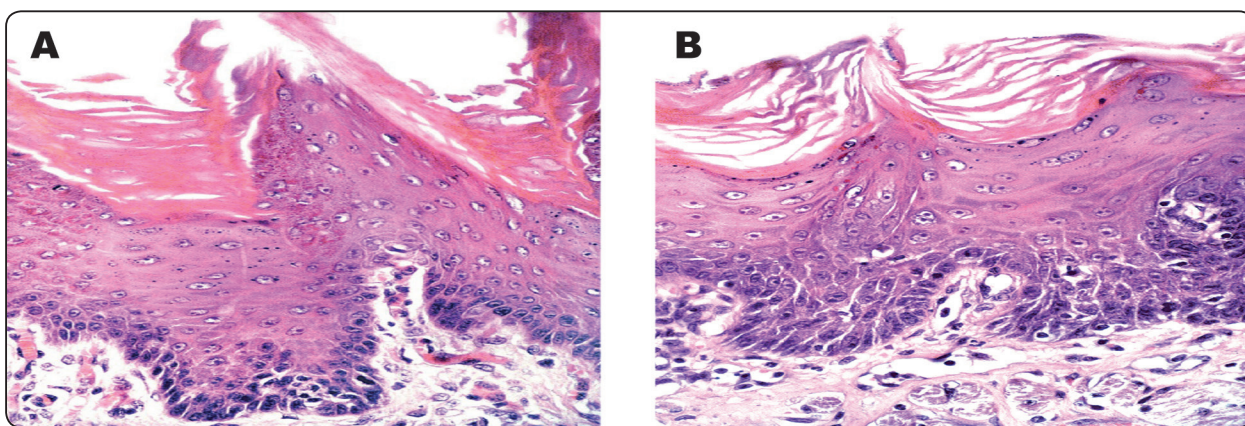


Fig. (1) (A) Group 1 showed normal gingival tissues consisted of keratinized stratified squamous epithelium and underlying connective tissue lamina propria. (B) Goup 2 CD showed mild dysplastic changes in epithelial tissue as nuclear hyperchromatism and increased mitotic figures in the epithelium, large number of dilated blood vessels in the lamina propria (H & E stain, X400)

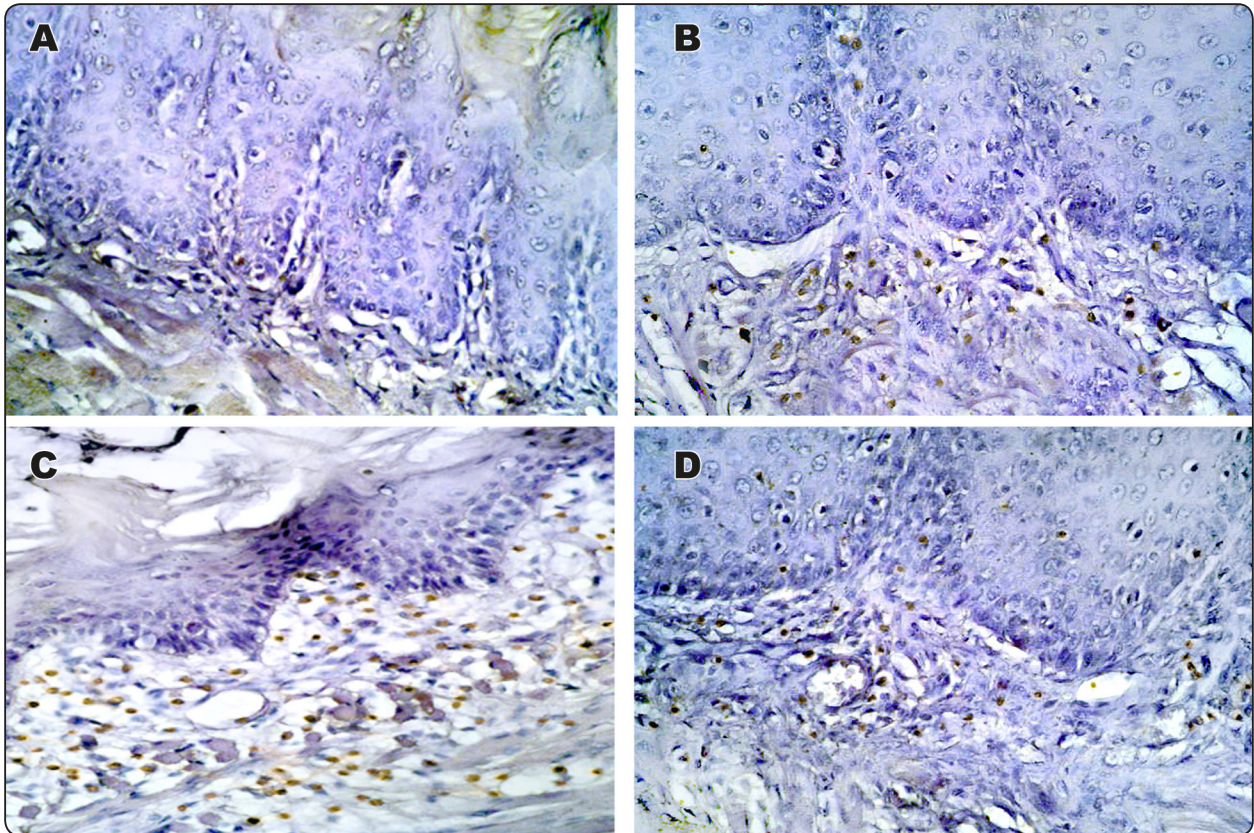


Fig (2) Group 1 (A) revealed negative iNOS immunoreaction in the epithelial cells and mild immunoreaction was existed in the connective tissue. Group 2 (B-D) revealed mild to moderate immunoreaction of iNOS both in epithelium and connective tissue (IHC stain, $\times 100,400$).

DISCUSSION

Cd was considered as a heavy metal, it was existed in the contaminated environment and food, and it causes a serious risk to the human health. Cd affects cell proliferation, differentiation, and apoptotic pathway. A growing attention towards the severely damaging effects of Cd and its relation to different cancers⁽¹⁸⁾.

Cd has been designated a human carcinogen, and is clearly a potent multi-tissue animal carcinogen. In this study, sixteen albino rats were divided into control group and Cd group they were given orally for 24 days to assess its role in carcinogenesis⁽¹⁹⁾.

Several researches were explained the carcinogenic effect of Cd, Martelli BKL et al., 2014 in their study in which Cd was given in the diet to

rats, they stated that Cd might lead to tumors of the prostate, testis and haematopoietic system⁽²⁰⁾. This was interpreted by Kevin Range, 2012 that Cd impairs p53 activity by inhibiting its DNA binding capacity leading to faulty DNA replication causing Cd-induced carcinogenesis. This happens when cells are exposed to concentration of Cd, lower than that can inhibit cell viability⁽²¹⁾.

Regarding to histological changes in gingival tissue in Cd group, we observed dysplastic changes as mild dysplasia was existed in 4 cases (50%), moderate dysplasia in 2 cases (25%). A statistical significant difference was exist between Cd and control group. This can be explained that Cd-induced ROS has been implicated in carcinogenesis⁽²²⁾, resulting in increased lipid peroxidation and DNA

damage⁽²³⁾. Depletion of cellular antioxidants has been suggested as the mechanism by which Cd facilitates exacerbation of ROS related cellular and DNA damage, thus further promoting carcinogenesis⁽²⁴⁾. Cd-exposure, can cause genetic and epigenetic alterations, uncontrolled cell proliferation, and alteration of cellular signaling, all of which are primary mechanisms involved in carcinogenesis^(25,26).

In the current study iNOS was used in assessment of the dysplastic changes in epithelial tissue, a statistical significant difference was found between control and Cd groups, these was in acceptance with Ying and Hofseth, 2007, they noticed that Over-expression of NOS2 has been found in all dysplastic specimens and has also been involved in early cellular changes⁽²⁷⁾. Also Brennan et al., 2000 observed in their study that, the expression of iNOS is assessed by immunohistochemistry in all cases of oral epithelial, the degree of expression of the marker was correlated with the severity of epithelial dysplasia⁽²⁸⁾. All cases of control group in this study revealed negative reaction this might support the role of iNos in assessment of tumor genesis. Morelato et al., 2014 noticed that, in samples of normal buccal mucosa no iNOS protein or mRNA was existed, or in negative controls⁽¹⁸⁾. In the current study iNOS was expressed in normal stromal cells adjacent to dysplastic epithelium, including endothelial and inflammatory cells and this might be explained that the connective tissue cells might be affected by dysplastic changes of epithelial tissue, also further researches are needed for explanations⁽²⁹⁾.

CONCLUSION

Cadmium chloride at low concentration and longtime of exposure can cause dysplastic changes in gingival tissue of rats as tissue abnormalities were found in most of investigated animals.

Histopathological and immunohistochemical examination with INOs could be used as a fast way and effective index to monitor toxicological changes

induced by polluted environment during exposure to pollutants in various tissues and organs.

More researches are required, including molecular pathways to understand the carcinogenic effect of cadmium on oral mucosa and chemo preventive medications that inhibit or block the genesis of iNos is recommended to decrease the tumor genesis.

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