Immunohistochemical study of PDGF, IGF of radiated tooth rat embryo

Rasim Mahdi Salih, B.D.S, D.O.D. [(1)]
Athraa Y Al-Hijazi, B.D.S., M.Sc., Ph.D. [(2)]

ABSTRACT

Background: Exposure to microwaves radiation from microwave oven may be harmful for users especially for the one who have highest contact with microwave oven. Because the body is electrochemical in nature, any force that disrupts or changes human electrochemical events will affect the physiology of the body by destabilization and interruption of many chemical body substance including growth factors.The insulin-like growth factors (IGFs) are a family of mitogenic proteins that control growth, differentiation, and the maintenance of differentiated function in numerous tissues. It fulfils an important role in growth and development of teeth, mandible, maxillae, and tongue. Platelet derived growth factors (PDGF) are proteins that regulate cell growth and division. In particular, it plays a significant role in blood vessel formation (angiogenesis). It seems that IGF and PDGF share in much tissue developmental process. Therefore they included in the present study in correlation to tooth growth & development. This study Illustrates the expression of insulin like growth factor and platelet derived growth factor by dental cells of rat embryos at periods of gestation 16th,18thday intrauterine life(I.U.L) and one day neonatal life.

Materials and Methods: Animal model: Thirty-six female rats were used in this study .Starting from zero days (time of gestation that recorded) the pregnant rats were divided into three groups. Group A serve as a control, groups B exposed to microwave oven radiation For (15 /minutes; 5/min /hour for 3 hours continuously) daily and C exposed to EMF radiation for (45 minutes ;15 min /hour for3hours continuously)daily starting from zero day of gestation till the last day. The embryo of rats at 16thday and 18th day of intrauterine life and one day old rat (new born rat) were studied immunohistochemically for localized of platelet derived growth factor (PDGF) and insulin growth factor (IGF) markers.

Results:The results showed that experimental group (B) exposed to short duration of radiation (5/ min.) stimulates the development of tooth germ and faster tooth growing in comparison to control with immunohistochemical results show strong to moderate intense stain for positive expression of growth factors(PDGF,IGF) by dental tissue.For long exposure period of radiation(group C) , it showed retardation in the tooth growth withimmunohistochemical findings record weak to negative intense stain for the expression of growth factors(PDGF,IGF) by dental tissue.

Conclusion: Exposure to microwave (oven) radiation during pregnancy may play a role in the expression of IGF and PDGF by cells of tooth germ thatinfluence on cell differentiation and physiological activity of specialized dental cells, depending on exposure time.

Key words: PDGF, IGF, Immunohistochemical study. (J Bagh Coll Dentistry 2013; 25(1):110-115).

INTRODUCTION

Radiofrequency (RF) electromagnetic waves may interact with biological tissue through a number of mechanisms [(1)].

Radiofrequency interaction can take place through thermal or non -thermal mechanisms. Thermal mechanisms are those resulting from the temperature change of the tissue caused by the RF fields. All interactions between RF fields and biological tissue are likely to result in energy transfer to the tissue and this will ultimately lead to an increase in its temperature. But non-thermal mechanisms are those that are not directly associated with this temperature change but rather to some other change produced in the tissue by the electric or magnetic field [(2)].

Sensitivity to electromagnetic fields varies between people, due to known and unknown reasons, including past exposures; cumulative exposure; duration and intensity of exposures; presence of heavy metals, chronic infections, dental amalgams and other stressors; differences in detoxification capacities; etc.

(1) Master Student, Ministry of Health.
(2) Professor, Department of Oral Histology and Biology, College of Dentistry, University of Baghdad.

Overtime, and with increasing exposure, more people feel the effects of electromagnetic fields [(3)]. Oral tissues are important part of the body that absorbs radiation. Many studies illustrate the effect of RF on the role of growth factors that concerned with oral tissue such as the role of IGF system and PDGF in growth regulation of salivary gland cell, periodontium and in tooth growth and development [(4)].

MATERIALS AND METHODS

Thirty six pregnant albino female rat (2-3 months of age, 900 -1000 gram of weight) were used in the present experiment, divided into three groups:

1-control group A: consist of 12 pregnant rats, not exposed to microwave oven radiation.

2-experimental group B: consist of 12 pregnant rats exposed to microwave oven radiation for (15 minutes; 5 min /hour for 3 hours continuously) daily at specific time during (lightperiod) starting from zero gestation till the day of scarifying.

3-experimental group C: consist of 12 pregnant rats exposed to micro wave oven radiation for (45 minutes; 15 min /hour for 3 hours continuously) daily on specific time during (light period)

starting from zero tome of gestation till the day of scarifying.

Premaxilla (contain incisor teeth) of rat embryos at 16th day, 18th day IUL and one day neonatal rat were fixed in 10%buffered formalin and studied for immunohistochemical localization of **Platelet derived growth factor-A and Insulin-like growth factor I**(E-10)SANTA CRUZ BIOTECHNOLOGY,INC. With

Detection KitSanta Cruz Biotechnology, using of primary antibodies, then Staining Systems include :Normal blocking serum 1.0 ml.,Biotinylated secondary antibody 250 mg. ,Avidin and Biotinylated horseradish peroxidase(AB reagents) 0.5 ml each .,Peroxidase substrate 1.0 ml 50x., DAB chromogen 1.0 ml 50x .,Substrate buffer 3.0 ml 10x.

RESULTS

Immunohistochemical results Insulin growth factor (IGF).

At 16th day IUL Positive expression of IGF was detected in the dental lamina and oral epithelia in control (group A) and group C While group B shows positive expression in dental sac too**,** figures (1,2,3).

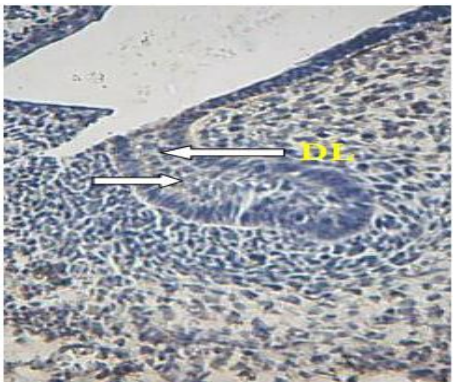

Figure1: Immunohistochemical view for tooth germ of rat (16thday I.U.L) control.Shows positive expression for IGF in dental lamina (DL) and mitotic cell(arrow).DAB with counter stain hematoxylin×100

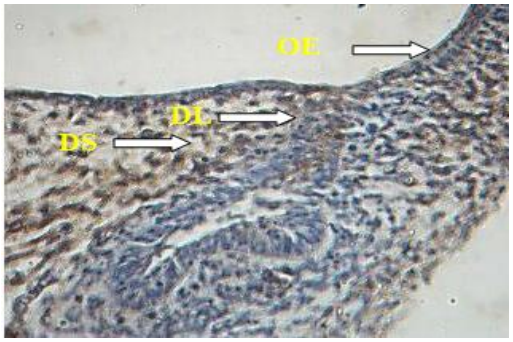

Figure 2: View for positive expression of IGF in tooth germ of rat (16th day I.U.L) treated (group B) shows positive brown color in dental lamina ,oral ectoderm cell(OE)and dental sac (DS). DAB with counter stain hematoxylin×100

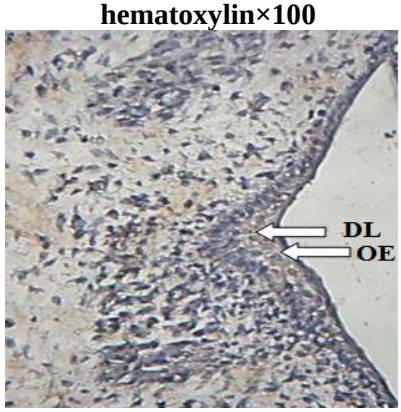

Figure 3: Immunohistochemical view for express IGF in oral ectoderm and dental lamina of tooth germ in bud stage of rat(16th day I.U.L) treated (group C).DAB with counter hematoxylin×100

At 18 day IUL: Positive expression of IGF was illustrated by inner enamel epithelium, outer enamel epithelium ,dental lamina and dental sac **in groups A and B**. **While group C** shows weak expression of IGF in dental lamina and negative expression in dental papilla .Figures (4,5,6).

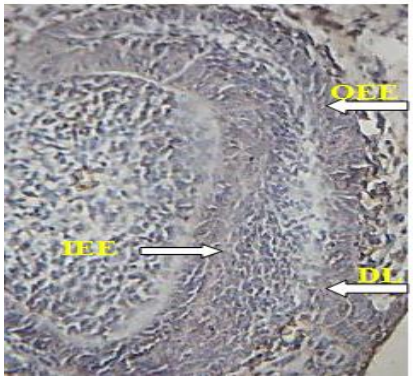

Figure 4: Immunohistochemical view for tooth germ of rat (18th day IUL) control shows positive expression of IGF in inner enamel epithelium(IEE), outer enamel epithelium (OEE), dental lamina(DL).DAB with counter stain hematoxylin×200.

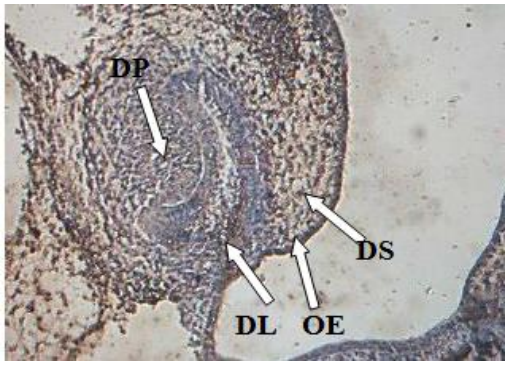

Figure 5: Immunohistochemical view for tooth germ of rat (18th day IUL)(group B) shows moderate positive expression of IGF by oral ectoderm(OE),dental sac (DS) and dental papilla(DP). DAB with counter stain hematoxylin×100.

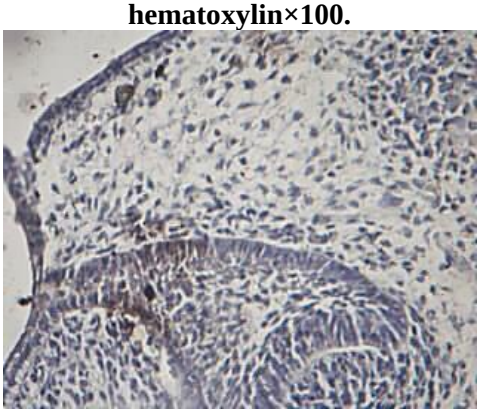

Figure 6:Immunohistochemical view of tooth germ of rat (18th day IUL)(group C) shows weak expression of IGF in dental lamina. DAB with counter stain hematoxylin×100

At One day neonatal life Positive expression of IGF was illustrated in stratum intermedium ,odontoblast ,ameloblast and dental pulp**in groups A and B** . While **group C** shows faint stain for IGF that is hardly expressed by odontoblast and in bone overlying tooth germ, figures(7, 8, 9).

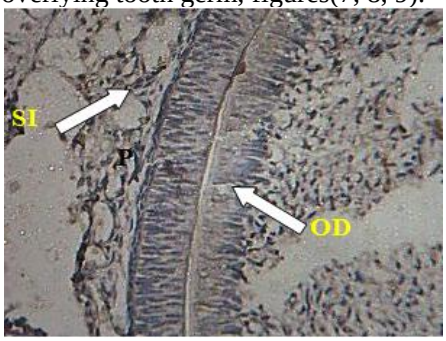

Figure 7: Immunohistochemical view for tooth germ of rat (1 day old) control. Shows positive expression of IGF by stratum intermedium (SI),odontoblast (OD),ameloblast (AB) and dental pulp(P).DAB with counter stain hematoxylin×200

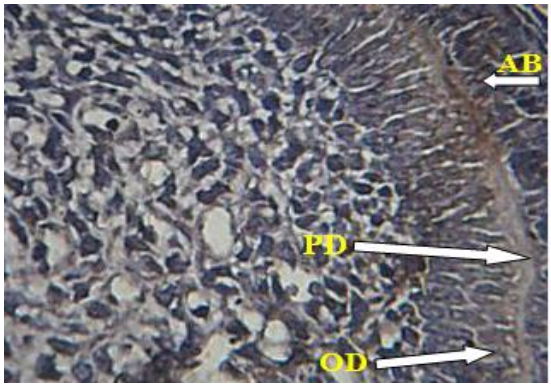

Figure 8: Immunohistochemical view for tooth germ of one day old rat (group B) shows positive expression of IGF by odontoblast (OD),ameloblast(AB) and predentin(PD).DAB with counter stain hematoxylin×400

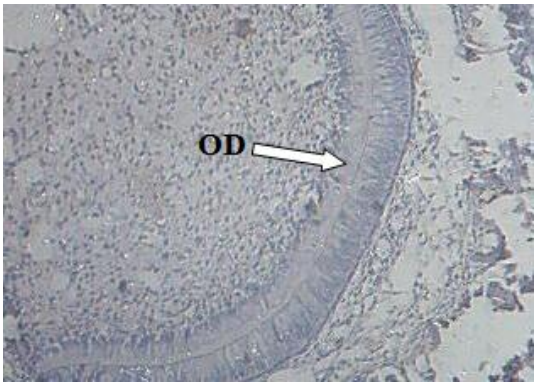

Figure 9: Immunohistochemical view of tooth germ of one day old rat (group C) shows faint DAB stain for localization of IGF,it hardly expressed by odontoblast(OD) .DAB with counter stain hematoxylin×100.

Expression of Platelet derived growth factor:

At 16 day IUL.control**groupA**shows positive expression of PDGF by oral ectoderm and proliferating cells ectomesenchymal cells of tooth germ figure (10).

Group B shows positive expression of PDGF by dental lamina, dental papilla and dental sac,figure (11).While **group C**illustrates faint positive stain in tooth germ figure (12).

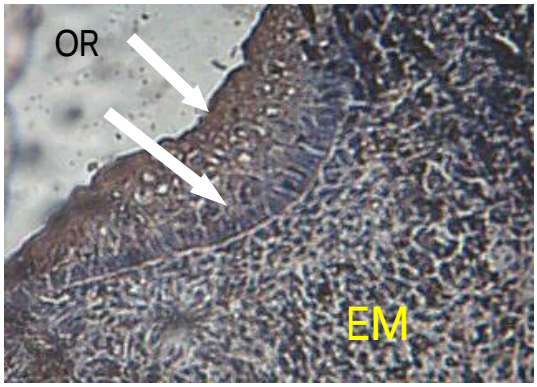

Figure 10:Immunohistochemical view of tooth germ of rat (16th day I.U.L) control. Shows positive PDGF expression by oral ectoderm (OR), proliferation central cell (arrow) and ectomesenchymal cell (EM).DAB with counter stain hemotoxylin× 200.

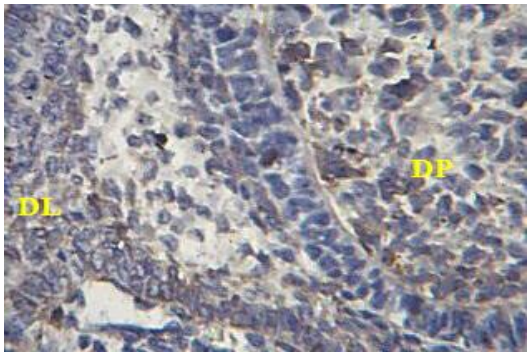

Figure 11: Immunohistochemical view of tooth germ of rat (16th day I.U.L) (group B).shows positive PDGF expression by dental lamina (DL) and dental papilla (DP).DAB with counter stain hematoxylin×200

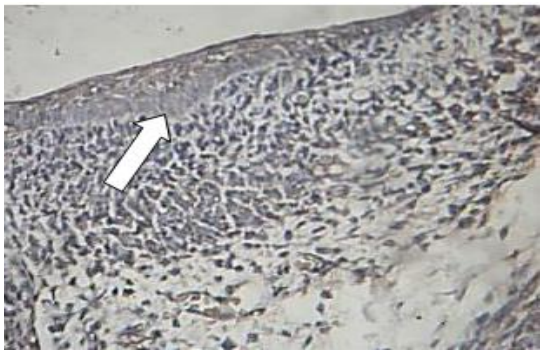

Figure 12: Immunohistochemical view of tooth germ of rat (16th day I.U.L) (group C).Shows faint (weak) positive reaction of tooth germ in bud stage(arrow) for PDGF.DAB with counter stain hematoxylin×100.

At 18thday I.U.L.control **group A** illustrates positive reaction in dental lamina ,apical loop and strong positive in bone formation area of dental sac and outer enamel epithelium,figure (13).**Group B** shows positivity in oral ectoderm,outer enamel epithelium, inner enamel epithelium, dental lamina, dental sac area and newly bone ,figure(14).While **group C** shows positive stain at cusp region ,figure (15).

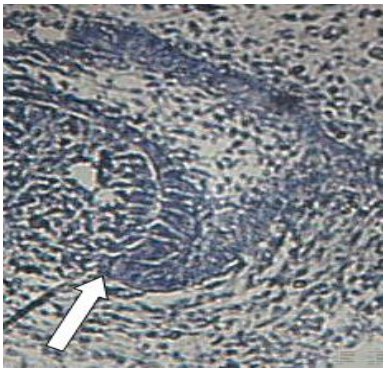

Figure 13: Immunohistochemical view for detection of PDGF expression in tooth germ (bell stage) of rat (18thday I.U.L) control .Show positive of DAB stain in dental lamina and apical loop (arrow).DAB with counter stain hematoxylin×200.

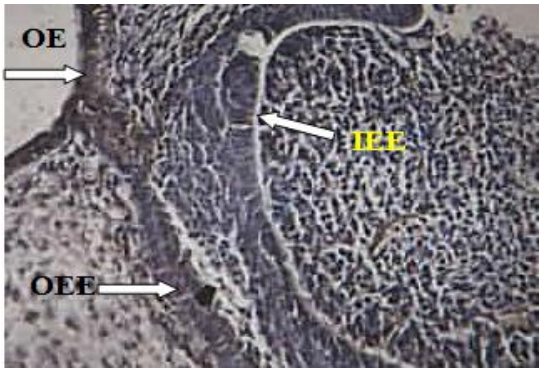

Figure 14: Immunhistochemical view for detection of PDGF expression in tooth germ (at bell stage) of rat 18thday I.U.L (group B).Shows positivity for oral ectoderm (OR), outer enamel epithelium (OEE) and inner enamel epithelium (IEE).DAB with counter stain hematoxylin×200.

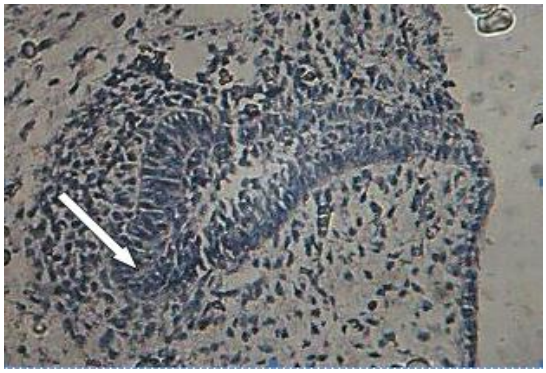

Figure 15: Immunohistochemical view for detection of PDGF expression in tooth germ of rat 18th day IUL (group C).Shows positivity of cusp region (arrow).DAB with counter hematoxylin×200.

At One day neonatal life control **groupA**shows positive reaction in odontoblast and bone ,figure (16). **Group B** shows positive reaction in odontoblastpredentine, ameloblast, stratum intermedium , stellate reticulum ,Tom's process and pulp cell expressed positive PDGF by fibroblast and mesenchymal cell figure (17). **Group C** illustrates weak reaction in odontoblast and ameloblast cell layer and negative expression in dental pulp, figure (18).

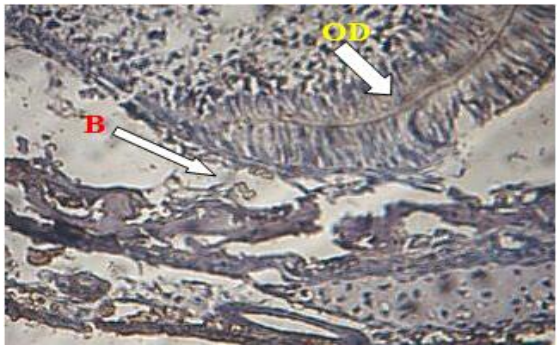

Figure 16: Immunohistochemical view for PDGF expression in tooth germ of one day old rat (control group).Show positive reaction inodontoblast(OD) ,bone (B).DABwith counter stain hematoxylin×200.

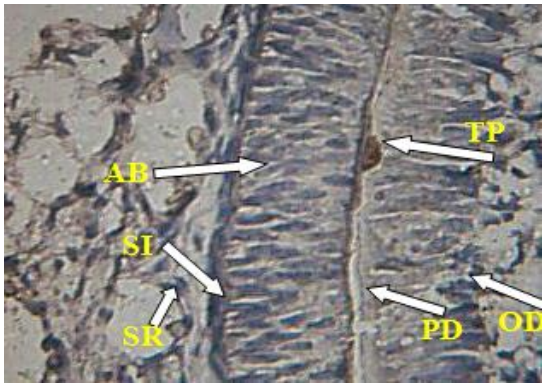

Figure 17: Immunohistochemical view for PDGF expression in tooth germ of one day old rat (group B).Shows positivity in odontoblast (OD), predentine(PD),ameloblast(AB),stratum intermedium (SI), toms process(TP) and stellate reticulum(SR).DAB with counter stain hemayoxyline×400.

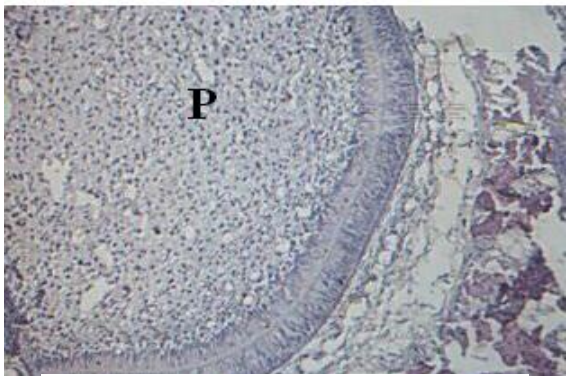

Figure18Immunohistochemical view of PDGF expression in tooth germ of one old rat (group C).Shows weak reaction in odontoblast and ameloblast cell layer with negative expression in dental pulp (P), except bone (B) shows positive reaction.DAB with counter stain hematoxylin×100.

DISCUSSION

Expression of IGF in developing dental tissue of control and experimental groups.

The IGF system plays a role in the formation of the mandible and teeth[5] and participates in the regulation of bone metabolism. It has been proposed that IGF-I functions as an autocrine/paracrine regulator of tooth development and influences cell differentiation and the physiological activity of specialized dental cells[6].

In the present study expression of IGF appeared as strong and weak intensity in different study groups and in different periods that matched the level of the expression of IGF marker.

Thesedata suggest that the IGF system likely participates in more than one process during tooth development [7]. This result could be explained:
1-During tooth development each mitotic cells related to enamel organ ,dental papilla or dental sac were under go repeated changes in the morphology and function that obviously detected in life cycle of specialized cells include ameloblast and odontoblast ,these modulation process that occur normally may implicated by IGF system .
2-Short exposure of microwave radiation in 5/min.group B suggest to enhance IGF system which considered a pleiotropic acting as both mitogen and differentiation factors promoted the acceleration in the differentiation of development of ameloblast, endothelial cells.

In group C ,dental cells appear weak (faint) to negative reaction due to retardation and decrease the mitogen role of IGF with poor differentiation and specialization of these cells, or may be retarded by growth hormone insensitivity or lack of growth hormone receptor response.

Long exposure to radiation may increase the stress status and hormonal changes like oestrogen status, so, effected the secretion and amount of IGF and response to reaction withantibody, therefore, it appears faint or negative.

Expression of PDGF in developing dental tissue of control and experimental groups

Platelet derived growth factor (subunits -A and -B) are important factors regulating cell proliferation, cellular differentiation and cellgrowth, and it plays a significant role in blood vessel formation (angiogenesis)[8].
Positive reaction for PDGF was detected in proliferating central cells, ectomesenchymalcell, oral ectoderm, dental lamina and dental sac in control and group B at 16^{th}day ,18^{th} day I.U.L, these findings illustrated that PDGFs are mitogenic during early developmental stages, drivingthe proliferation of undifferentiated mesenchyme and some progenitor populations. And during later maturation stages, PDGF signaling has been implicated in tissue remodeling and cellular differentiation, and in inductive events involved in patterning and morphogenesis [9].

Strong expression of PDGF in the cusp region during morphogenesis play important role in growth, differentiation and morphogenesis, but, it showed to be negative after complete tooth differentiation and morphogenesis [10].

Furthermore, PDGF positive expression during control and short duration of EMF radiation as these radiation may increase the role of PDGF in angiogenesis and enhance the growth and development .The faint expression of PDGF in group C at development stage of rat tooth germ may due to long duration of radiation that retard the growth by effecting on PDGF receptor response.

REFERENCES

1. Challis LJ. Mechanisms for interaction between RF fields and biological tissue. Bio electromagnetics. 2005; Suppl 7: S98-S106.
2. Foster KR. Thermal and non-thermal mechanisms of interaction of radio-frequency energy with biological systems. IEEE Trans Plasma Science 2000; 28:15–23.
3. Rubin J, Munshi J, Simon W. Electromagnetic hypersensitivity: a systematic review of provocation studies ".Psychosomatic Medicine.2005; 67 (2): 224–32.
4. Sood S, Gupta S, Mahendra A. Gene therapy with growth factors for periodontal tissues.Med Oral Pathol. Oral Cir Bucal 2012; 17(2): e 301-10.
5. Werner H, Katz J .The emerging role of the insulin-like growthfactors in oral biology. J Dent Res 2004. 83: 832-6.
6. Joseph BK, Savage NW, Daley TJ, Young WG. In situ hybridization evidence for a paracrine/autocrine role for insulin-like growth factor-I in tooth development. Growth Factors1996, 13:11–17.
7. Yamamoto T, Oida S, Inage T. Gene expression and localization of insulin-like growth factors and their receptors throughout amelogenesis in rat incisors. J Histochemistry & Cytochemistry 2006; 54(2): 243-52.
8. Joukov V, Pajusola K, Kaipainen A, Saksela O, Alitalo K, Olofsson B, von Euler G, Orpana A, Pettersson RF, Eriksson U. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. Proc Natl Acad Sci U.S.A.1996; 93(6): 2567-81.
9. Hoch RV, Soriano P. Roles of PDGF in animal development. Development 2003; 130(20): 4769-84.
10. Betsholtz C, Raines EW. Platelet-derived growth factor: a key regulator of connective tissue cells in embryogenesis and pathogenesis. Kidney Int 1997; 51: 1361-9.