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The Detection of Human Papilloma Virus- 16, in Oral Squamous Cell Carcinoma by in Situ Hybridization

Dr. Maha. M. Al-Sened, B.D.S., H.D.D. Oral surgery,
Dr. Ban A. Abdul. Majeed, MB. ChB. M.Sc, Ph.D (Ph.D. Molecular Pathology)

Abstract

Background: Squamous cell carcinoma of the oral cavity constituted a major health problem and can be a leading cause of death. Several studies link high risk Human papillomavirus -16 to oral squamous cell carcinoma as an important etiological factor. Several molecular markers have also been the subject of an intense research work, trying to clarify their role in oral carcinogenesis. Of these, the cancer suppresser gene P53 has been extensively studied.

Aim of the study: to detect the association of the molecular markers Human papillomavirus -16, in the oral Squamous cell carcinoma using in situ hybridization technique and To correlate Human papillomavirus -16 with clinical data (age, sex, risk factors grade &stage).

Material & Methods: Tumor tissues from 33 patients with newly diagnosed as oral squamous cell carcinoma & who were surgically treated collected, formalin fixed & paraffin embedded. Sections on charged slides were made from each tissue block and submitted to in situ hybridization technique utilizing Human papillomavirus -16, cDNA probe.

Results: Human papillomavirus -16 was localized by in situ hybridization specifically within the nuclei of cancer cell it was detected in (27) (81.8%) of 33 oral squamous cell carcinoma patients. Most of Human papillomavirus -16 positive patients were within the age group (50-59) years. However, there was no significant correlation between Human papillomavirus -16 positive &age. No significant correlation was found to sex &risk factors (smoking &alcohol consumption) either. Most of cases were moderately differentiated squamous cell carcinoma (79%). however no significant correlation was detected regarding histopathological grading or clinical staging with Human papillomavirus -16.

Conclusions: Oral HPV-16 infections are strongly associated with OSCC. Among subject with or without the established risk factor of tobacco& alcohol use

Keywords: Oral squamous cell carcinoma, Human papillomavirus -16, in situ hybridization

Introduction

More than 95% of the carcinomas of the oral cavity are of squamous cell type, in nature. According to World Health Organization, carcinoma of oral cavity in males in developing countries is the sixth commonest cancer [1]. Oral squamous carcinogenesis is a multistep process in which multiple genetic

events occur that alter the normal functions of oncogenes and tumor suppressor genes. [2]. Tobacco smoking and chewing and alcohol consumption are the main risk factors for HNSCC and have been estimated to account for the vast majority of the disease burden worldwide [3].High-risk human papillomavirus (HPV) is known to be tumorigenic in human epithelial tissues. Over 120 types of HPV have been identified, HPV (16, 18) types are often described as high-risk (oncogenic) or low-risk (nononcogenic) [4]. Two viral oncoproteins of high-risk HPVs, E6 and E7, promote tumor progression by inactivating the TP53 and retinoblastoma tumor suppressor gene products, respectively[5]

Materials and Methods

Tumor tissues from 33 patients with newly diagnosed as oral squamous cell carcinoma & who were surgically treated collected from 2006-2008, formalin fixed & paraffin embedded. Sections on charged slides were made from each tissue block and submitted to in situ hybridization technique utilizing Human papillomavirus -16, cDNA probe. OSCC patients and control subjects reported information on lifetime smoking and alcohol consumption.

Results

In this study most of the OSCC cases were moderately differentiated SCC (79%) (grade II) (Figure-1 A) OSCC lesions most clinically presented as an ulcer in (88%) of cases. All tongue lesion (Table-1) which is the most affected site, were presented as an ulcer (55%) of ulcerative lesions (Figure-1 B).

OSCC showed positive ISH signals for HPV-16 in (27) cases (81.8%) (Figure-2 A). Fourteen cases (42.4%) of these involved the tongue (Table -1).

Koilocytosis (Figure -2 B) was seen in 30 (90.9%) cases of OSCC .Twenty-five (75.7%) cases of them were positive for HPV-16 ISH in sections. Of the remaining 6 (18.1) negative for HPV-16 signals 4 (12%) revealed Koilocytosis (Table -2). There were a significant correlation between OSCC &positive HPV-16 ISH signals in sections with koilocytosis ($P<0.001$). %)

Regarding the association between the positive HPV-16 ISH signals& risk factors ,among OSCC cases (15) were smokers&12(80%) of them were with positive HPV-16 ISH signals. Nine of OSCC patients were alcoholic &six of (66.7%) them with positive HPV-16 ISH signals. There was no significant correlation the positive HPV-16 ISH signals& risk factors (smoking &alcohol consumption) (Table-1)

ISH signal intensity of HPV-16 was low in (45.4%), intermediate in (24.2%) and high in (12%) cases (Table -3). Regarding ISH scoring HPV-16 (51.51%) revealed low score, (15%) intermediate score & (15%) high score (Table -4).There was no significant correlation between HPV-16 positive ISH signals but surprisingly there was significant correlation between the score & intensity of HPV-16 ISH positive cases ($P>0.05$).

In relation to other clinicopathological features statistical analysis revealed no significant correlation between HPV-16 positive ISH signals in tissue & site ($P =0.43$) (Table -1) age ($P=0.09$) sex ($P=1.0$) (Table -1) & the positivity of HPV-16 in tissue was independent of cancer histological differentiation ($P=0.37$), or clinical staging ($P=0.081$), (Table -1).

Discussion

Establishing the link between HPV and a subset of OSCC has been difficult because of the heterogeneity of OSCC, findings by Syrjänen *et al*. 1983[6] was the first hint that HPV may be involved in the pathogenesis of a subset of HNSCC. HPV sequences have been repeatedly detected in a variable proportion of HNSCC, from as few as 10% to 100%. [7] In the present study HPV-16 DNA was detected in (81.8%) which is similar to the study of Patiman, 2001[8] who found it in (81.6%) using PCR &to that of WHO 2005 (86.7%) from different geographic regions using (PCR) [9], percentages were variable, (74%) by Zhang 2004 [10], and it was (68%) HPV-16 out of HPV-positive group by Koh 1998 [11], and (38.3%) in a study by Gillison 2008 on 240 patients of HNSCC [12], by (34.1%) in Hungary, [13] and (33.6%) in India [14] . This difference might explain the regional differences in the distribution of risk factors other than HPV infection, and difference in the sensitivity of methods of detection [9]. Although oral—genital contact may be responsible for HPV transmission, transmission through direct mouth-to-mouth contact or other means could not be excluded. [15] An increased risk of HPV-associated OSCC in individuals with a history of HPV-associated anogenital cancers and in husbands of women with *in situ* carcinoma and invasive cervical cancer also suggests that sexual transmission of HPV infection to the oral cavity can occur [16,17]

Correlation between HPV-16 and clincopathological parameter

HPV-16 and site of OSCC lesions

Most of HPV-16 positive signals detected in tongue (42.4%) Close to findings by Ruzica 2004 (64%) [18]. However, in India detected it in higher percentage (99%). [19] Increasing of percentage of HPV infection in tongue suggested of role of viral carcinogenesis. [20] There was no correlation between HPV-16 ISH signals and the site in agreement with Ruzica 2004 [18]. While other studies found correlation with oropharyngeal and tonsilar SCC [5,21]. That these regions of the mouth are covered by a thinner, nonkeratinized mucosa, which provides less protection against carcinogens. [22].

HPV-16 positive signals in males under 60 years in age [10,23]. Which in many studies indicating relation to sexual behavior which confirmed HPV infection as risk factors [24]. Koilocytosis is regarding a definite finding in HPV-16 infection of squamous epithelium whether benign or malignant [25]. This study confirmed the relation of HPV-16 to koilocytosis since it was present in most of the cases. This is in turn confirm the etiology of OSCC being mediated via a pathway associated with HPV-16 infection [12]

Correlation between HPV-16 and risk factors

While tobacco &alcohol are primary risk factors for HNSCC development, In this study the non-smokers were more likely to be in the HPV-16 positive group than smokers 15(83%) the same thing was true for the alcoholic patients 21(82.5%). However there was no significant association between the HPV-16 & risk factors including tobacco and alcohol use. This in agreement with several studies worldwide [5, 10, 13, 15, 26] suggesting asynergistiy with tobacco and alcohol exposure [5, 24].

a study by Applebaum 2007 found that HNSCC risk associated with smoking, alcohol, and HPV16 differs by tumor site and The strongest risk factors by tumor site were smoking for

laryngeal cancer, alcohol for cancer of the oral cavity while. Alcohol or tobacco use does not further increase risk of HPV16-associated cancer. [27]

D'Souza *et al*. [28] showed that OSCC was significantly associated with oral HPV infection and with HPV-16 L1 seropositivity among patients both with and without a history of heavy tobacco and alcohol use. In a Swedish study, oral high-risk HPV infection was associated with dramatically increased risk of OSCC development, after adjustment for alcohol and tobacco use.[29] In a separate, nested case-control study, HPV-16-seropositivity conferred a greater than 14-fold increased risk of subsequent development of OSCC.[30]

Conclusion

Oral HPV-16 infections are strongly associated with OSCC. Among subject with or without the established risk factor of tobacco& alcohol use& Lack of significant association between the HPV-16 & P53 detection confirm the presence of wild-type P53 with HPV-16 infection.

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Table 1: The association of selected independent variables with positivity rate of paraffin section HPV-16 among cases (OSCC).

	Total	**Positive HPV-16 Paraffin section**		**P-value**
	N	**N**	**%**	
Age group (years)				**0.71*[NS]***
30-39	3	3	100	
40-49	8	6	75	
50-59	12	9	75	
60	10	9	90	
Total	33	27	81.8	
Gender				**1*[NS]***
Female	8	7	87.5	
Male	25	20	80	
Alcohol consumption				**0.31*[NS]***
Negative	24	21	87.5	
Positive	9	6	66.7	
Smoking habit				**1*[NS]***
Non smoker	18	15	83.3	
Smoker	15	12	80	
Tumour grading				**0.37*[NS]***
Grade-I	4	4	100	
Grade-II	26	20	76.9	
Grade-III	3	3	100	
Site				**0.17*[NS]***
Tongue	16	14	87.5	
Buccal mucosa	4	4	100	
Floor of mouth	4	4	100	
Palate	4	2	50	
Alveolar mucosa	5	3	60	
Tumour staging				**0.56*[NS]***
Stage (I)	1	1	100	
Stage (II)	4	4	100	
Stage (IV)	28	22	78.5	
Total	33	27		

Table (2): Correlation between HPV-16 positivity & Koilocytic changes observed in OSCC cases

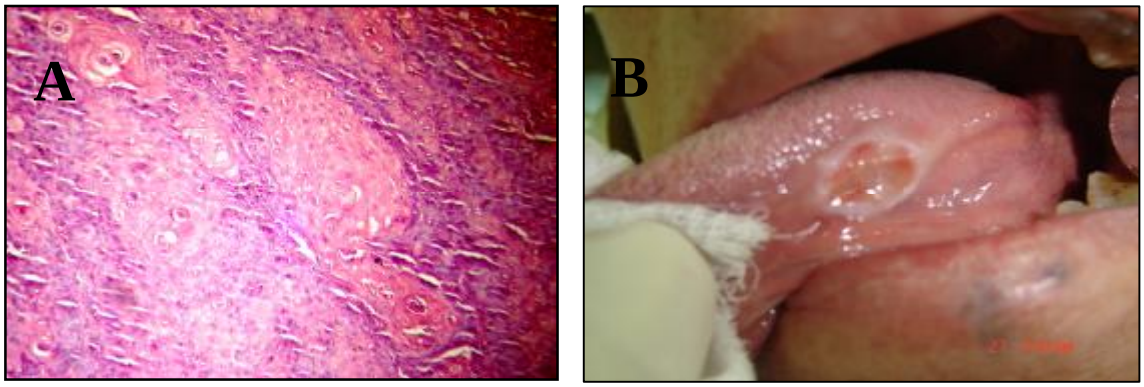
Variable	Koilocytosis				Total	P-value
	Positive		**Negative**			
	No	**%**	**No**	**%**		
OSCC	**30**	**90.9**	3	9	33	P<0.001
Positive HPV-16 in paraffin section	**25**	**75.7**	2	6	27	P<0.001
Negative HPV-16 in paraffin section	4	12	2	6	6	

Table 3: The association between alcohol consumption and smoking habit with HPV-16 positive ISH signals intensity.

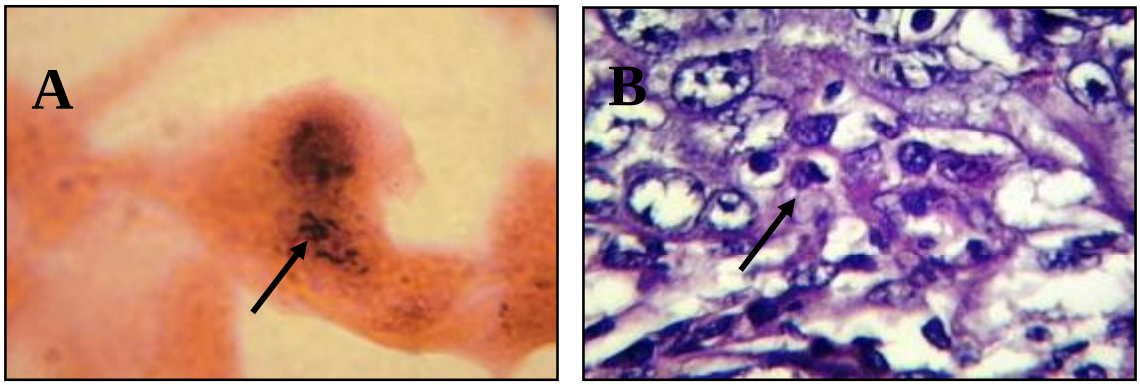
HPV-16 intensity											
Variable	**1**		**2**		**3**		**Total**		**Median**	**Mean rank**	**P**
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**			
Alcohol consumption											
Negative	11	45.8	5	20.8	4	16.7	24	100	1	18.21	**0.21**[NS]
Positive	4	44.4	2	22.2	0	0	9	100	1	13.78	
Smoking habit											
Non smoker	7	38.9	4	22.2	4	22.2	18	100	1	19.28	**0.12**[NS]
Smoker	8	53.3	3	20	0	0	15	100	1	14.27	

Table 4: The association between alcohol consumption and smoking habit with HPV-16 positive ISH signals scores.

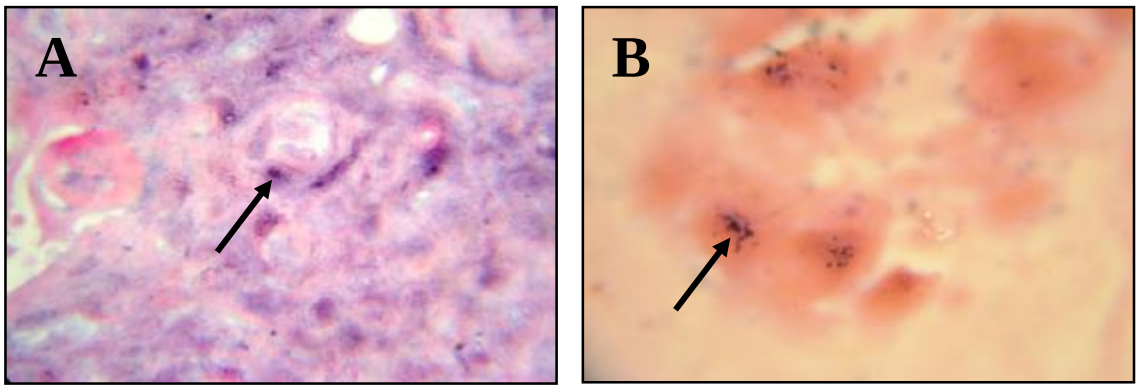
HPV-Score											
Variable	**1**		**2**		**3**		**Total**		**Median**	**Mean rank**	**P**
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**			
Alcohol consumption											
Negative	13	54.2	3	12.5	5	20.8	24	100	1	18.27	**0.18**[NS]
Positive	4	44.4	2	22.2	0	0	9	100	1	13.61	
Smoking habit											
Non smoker	9	50	2	11.1	4	22.2	18	100	1	17.86	**0.54**[NS]
Smoker	8	53.3	3	20	1	6.7	15	100	1	15.97	



(Figure-1) (A) Representative histopathological features of moderately differentiated OSCC X1000. (B)Clinical presentation of OSCC of lateral border of tongue presented as an ulcer.



(Figure-2(A) HPV-16 positive ISH signals in the nucleus moderately differentiated SCC X1000. (B) Koilocytosis in moderately differentiated SCC X1000



(Figure-3) HPV-16 positive ISH signals (B) Intermediat intensity in Moderatly differnetiated OSCC X400 (arrow) (C) Low intensity, intermediat scoring of moderatly differnetiated OSCC X 1000(arrow)

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Hyalinization of dental pulp tissue due to occlusal trauma (Experimental study)

Dr. Athraa Y. Al-Hijazi M.Sc., ph.D. in oral histology and biology
Dr. Inas Fadhil B.D.S.,M.Sc. in oral histology and biology
Dr. Nada M.Hasan AL-Ghaban MSc.,ph.D. in oral histology and biology

Abstract

Dental pulp tissue is a highly specialized conneclive tissue contains a formative and protective cells that can be affected easily by stimuli such as occlusal trauma subjected by occlusal overhang filling.

Sixteen albino rats aged range (6-8 months) weight range (0.5-0.75 kg) were subjected the occlusal overhang filling made for upper first molar for 2 periods, and according the periods, rats divided in the 2 groups. Groups I (8rats) subjected for occlusal trauma for 2 weeks duration group II (8rats) subjected for occlusal trauma for 8 weeks duration. Histological evaluation have been done for 2.8 weeks using hematoxyline and eosin stain.

Occlusal trauma for 2 weeks in group I showed wide irregular predentin formation will displacement in odontoblast cell.

While in group II for 8 weeks duration, histological feature for pulp tissue showed hyalinization (50-95 μm) in length.

Occlusal trauma for long period cause hyalinization of pulp tissue that negatively influence on endodontic treatment in future.

Key words: dental pulp, hyalinization occlusal trauma.

Introduction

Tooth and its surrounding structures are continually challenged by microbial flora and restorative dentistry[1]. Glickmen in 1996 [2] reported that a month with a healthy status may be affected by restorations of poor quality, and a restoration of highest quality may be fail in a mouth with dentogingival disease. The presence of caries, broken, missing over restorations and open or light contacts may lead the altered chewing pattern due the food impaction[3], or an unstable occlusal relationship. Occlusal trauma represented by over hang dental restorations are a major dental health problem and may promote dental tissue change[4].

A review of articles summarized on over hang fillings concern with over extended margins. Affecting periodontium system included bone loss, pocket formation, attachment loss and inflammation. They showed significantly greater severity of disease associated with overhangs, compared to homologous teeth without overhanging dental restoration [5, 6].

Larg et al (1983)[7] reported in a study of dental students where

restoration temporarily placed for a limited period with overhangs. A change in oral flora with presences of specific periodontal pathogens may damage the embrasure by impinging on interproximal space and the biologic width. Excessive occlusal forces showed to enhance the rate of tissue destruction in periodontal disease. No report was found up to our knowledge studied changes in pulp tissue due to overhang restoration therefore the present study was designed.

Histological feature of dental pulp:

The dental pulp is a loose connective tissue and made up of a combination of cells embedded in an extracellular matrix of fibers in a semi fluid get[8]. The extracellular matrix made up of a versatile group of polysaccharides and protein secreted by the cells of the tissue and assembled into a complex frame work closely associated with pulp cells (odontoblast, fibroblast, undifferentiated mesenchymal cell) collagen type I is the predominant extracellular matrix of pulp it is present as a fibrils thiny scattered through pulp. There are also a large amount of type III have collagen similar pattern to type I collagen .Small amount of type V and type VI collage are also present [9].

Hyalinization

A degenerative process resulting from long-continued occlusal trauma in which fibers become hyalinized in to homogeneous mass. It appears as an acellular avascular glassy area illustrated in periodontal ligament results in orthodontic forces in which ligament subjected to compression [10].

Aim of the study

To study the effects of Occlusal overhang filling on dental pulp tissue (histologically)

Materials and Methods

Sixteen albine rats, aged range (6-8) mouths, weight range (0.5-0.75 kg) were included in the present study. Amalgam occlusal CI fillings, for upper first molar teeth with, overhang, up to anatomic contours of the tooth being restored.

Histological preparation

Two groups of rats subjected to overhang filling for 2 periods group I (8rats) for 2 weeks. Group II (8rats) for 8 weeks. Histological study have been done for 2 groups under light microscope using hematoxyline and cosine stain [11].

Results

Histological feature of dentin- pulp tissue response to occlusal overhang filling for a period of 2 weeks showed wide irregular pre dentin. Odontoblast cells showed displacement and congested blood vessels illustrated in the pulp figures (1, 2).

For a period of 8 weeks duration dentin pulp tissue complex responded occlusal overhang filling and illustrated resorption in dentin, showing odontoctast cell as a mullincleated giant cell Hyalinization of pulp appears as a cellular glassy mass scattered in pulp tissue figures (3,4,5).

Table (1) shows width of predentin (25.4-308 μm) in 2 weeks duration wider in comparison to 8 weeks duration (6.7-10.3 μm) hyaline length also estimated in pulp tissue of group II (50-75μm) in length.

Discussion

Dental restorations (fillings, inlays, crowns, etc) should be made to blend smoothly with the contours of tooth being built with restoration, other wise it may cause a damage to dental tissue if the restoration being beyond the border of the cavity [(1)].

Excessive occlusal forces have been implicated in the development of dental problem, and enhance the rate of tissue destruction [(2)].

All studies indicated the necessity of were organized successful restoration treatment and the one that could not adapt would lead a destruction of dental tissue and bone loss [(12)].

Clinical consideration for restorative dental treatment specially for proximal caries must be compatible with periodontal heath, so therefore many researches studied the inter-relation ship between restoralive dentistry and periodontics. They found that poorly contoured dental restorations may progress gingivitis that affected gingival attachment fibers to bone destruction [(4)].

Other study showed that colonization of prophyromonas gingivitis bacteria, is one of a major causative agent of adult periodontitis due to accumulation of subgingival dental plaque as a result of proximal overhang filling [(3)].

Von et al in (2004)[(6)] evaluated the histological changes in the periodontal structures of beagle dogs after using high and low force during tooth movement small patch of hyalinization were found at the pressure side while other area showed long areas of necrotic tissue.

Von et al in 2009[(16)] aralyzed literature was concerning hyalinization in relation to experimental tooth movement in animals and humans. They found that all studies considered hyalinization as undesirable side effect of orthodontic tooth movement and it needs for well-designed to elucidate the role of hyalinization.

Miyoshi et al (2001)[(13)] studied response of periodontal tissue to orthodontic force, histologically. Using two groups one subjected to light period force second group subjected to long period.

The light group showed less extensive hyalinization of periodontal ligament than the long period group.

Some authors studied dental pulp morphology and distribution of acid and neutral mucopolysaccharides in teeth subjected to orthodontic therapy.

They found that number of collagen fibers increased, densely packed and a certain degree of hyalinization was observed and they suggested that these changes in dental pulp are irreversible and correspond to those of aging [(14)].

Vier et al 2007 [(15)] evaluate the effects of radiotherapy on the dental pulp and they illustrated nuclear alteration with hyalinization status in dental pulp tissue of rats. The present results showed a changes in dentin pulp tissue included odontoblast displacement which may be reversible to hyalinization of pulp which is a irreversible response depending on the period of subjection to occlusal trauma illustrated by overhang filling. The present study suggested of negatively influence of long period occlusal trauma to endodontic treatment in future and to a fact that a restoration may preclude a permanent dental damage if it not done carefully and correctly.

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Table (1): Morphometric parameter of dentin-pulp tissue in study groups.

Groups	**Parameter**
Rang-Dentin width μm	
Group I	136-146
Group II	130-133
Range per dentin width μm	
Group I	25.4-30.8
Group II	6.7-10.3
Range hyalinized one length (μm)	
Group I	Negative
Group II	(50-95)

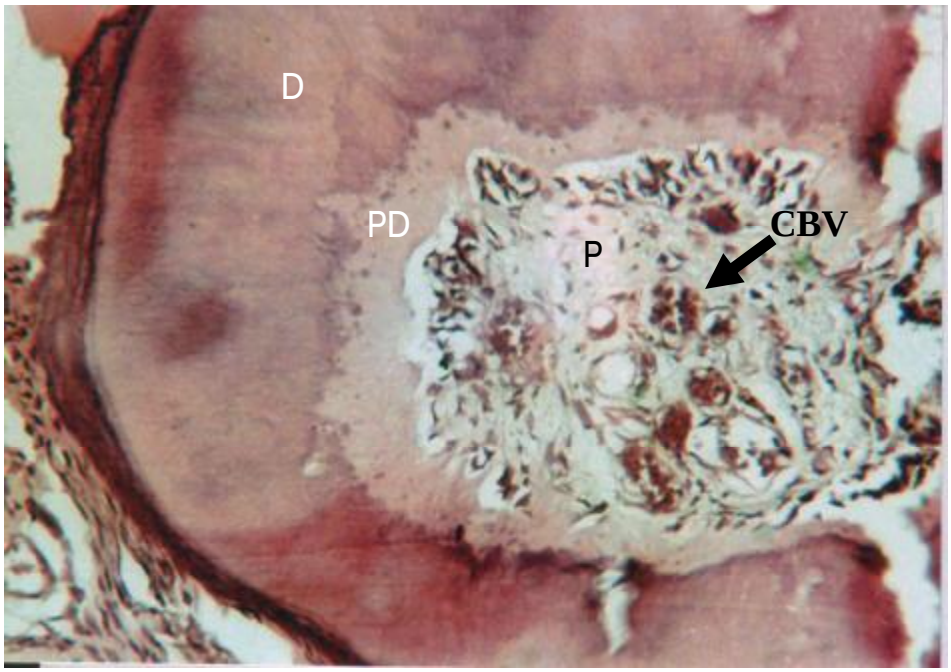

Fig: 1: Microphotograph view for rat's dentin- pulp tissue subjected occlusal amalgum hang filling for period of 2 weeks the view shows wide irregular predentin [PD], dentin [D], pulp [p], congested blood vessels [CBV] H&E x100.

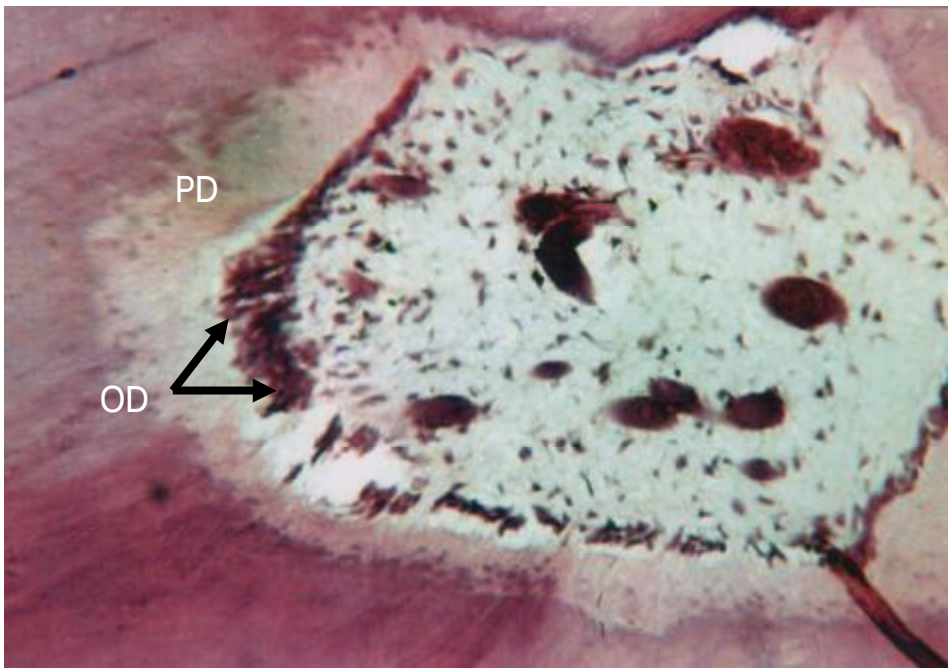

Fig: 2 High magnification view for rats dentin pulp tissue subjected to occlusal amalgam hang filling for 2 weeks duration, showing displacement of odontoblast cells [OD], wide irregular predentin [PD]. H&E x 200.

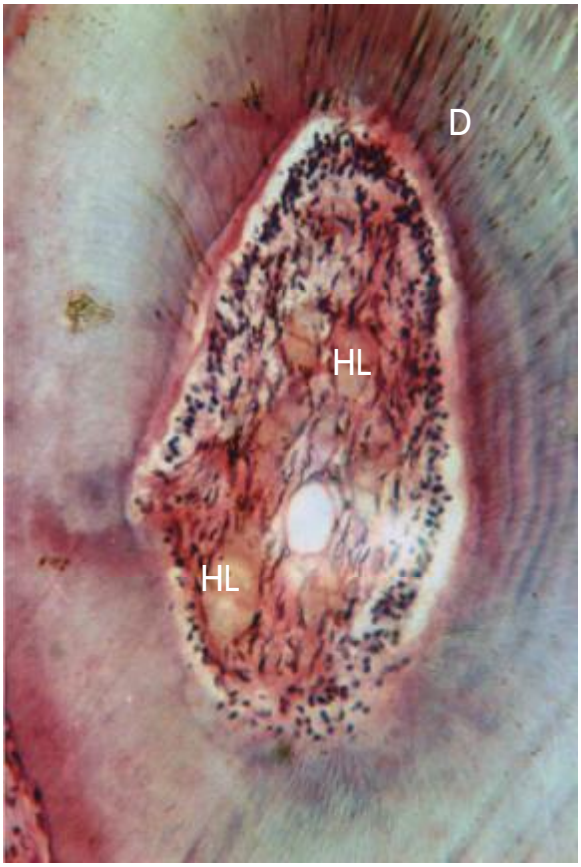

Fig: 3 Microphotograph view for rats dentin pulp tissue subjected to occlusal amalgam hang filling for 8 weeks, shows hyalinization of pulp [HL] H&E x200.

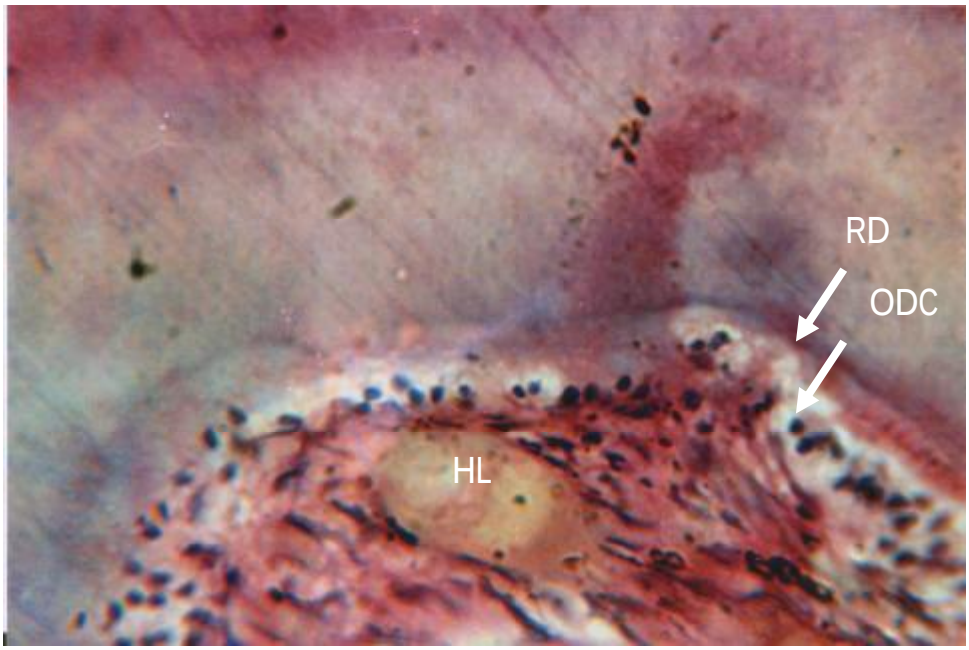

Fig: 4 View for odontoclast [ODC]cell, occupied resorbed dentin [RD] and hyaline mass [HL] can be detected in pulp rat subjected to occlusal amalgam hang filling for 8 weeks duration H&E x200.

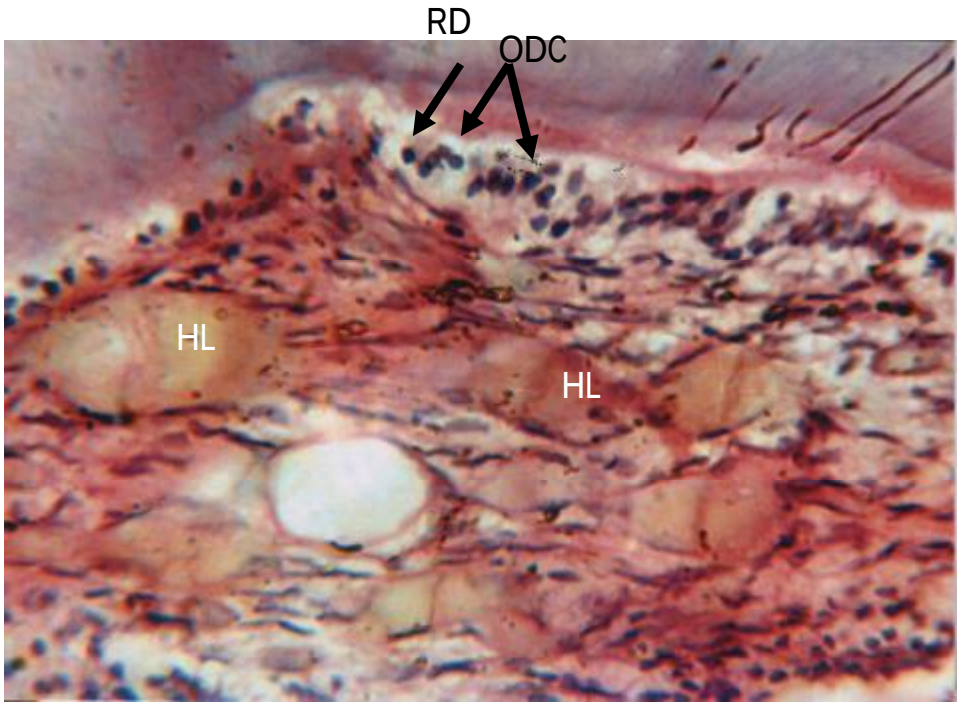

Fig: 5 High magnification view of figure [4] shows odontoclast cell [ODC] in howshipus lacunae, resorbed dentin [RD H&E x400.