



# CHATTAGRAM INTERNATIONAL DENTAL COLLEGE

# Journal

"Flourish Your Stylus"

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Associate Professor & Head  
Department of Orthodontics and Dentofacial Orthopedics  
Chattagram Internal Dental College  
206/1, Haji Chand Meah Road, Shamserpara, Chandgaon  
Chattogram, Bangladesh.  
Cell : 01753 20 03 23, Phone : (031) 2573119-23  
E-mail : shahique\_jpni@yahoo.com, www.cidch.edu.bd
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Cell : 01819 80 30 50



## Chattagram International Dental College

206/1, Haji Chand Meah Road, Shamserpara, Chandgaon, Chattogram, Bangladesh.  
Cell : 01753200323, Phone : (031) 2573119-23, E-mail : info.cidchbd@gmail.com  
Website : www.cidch.edu.bd

### Information to Authors

Chattagram International Dental College (CIDC) started its historical and memorable journey in the 2003 year. CIDC is the only Private Dental College in Chattogram which is smoothly running under the guidance of Chittagong University.

CIDC is approved by the Government of the Peoples Republic of Bangladesh and is recognised by the Bangladesh Medical and Dental Council (BMDC). CIDC is representing pioneer and exemplary academic and clinical oriented research institute of Bangladesh. About 65 Dental students completed their graduation from CIDC per annum.

Chattagram International Dental College commenced to publish a peer reviewed Journal from 1st January 2018. The journal intend to publish article of authors from any part of the globe, but has a special interest in publishing research articles of authors from Bangladesh and of relevance to developing countries. It interested in Editorial, Original (Research) articles, Special articles, Review articles, Short Communications, Case report and letters on new findings of Medical Science.

Chattagram International Dental College Journal is published in english, biannually eg. January and July with prior approval of Editorial board.

Appropriate measures has been taken to make the journal indexed / abstracted in major international indexing systems including the PubMed/MEDLINE, Index Medicus, Google Scholar, DOAJ, Hinari and Scopus etc. The theme of Journal of Chattagram International Dental College is

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A structured abstract should not be of more than 250 words. It should be a factual description of the study performed organized with the heading of Background (Includes aim or objectives) Methods (Includes patient population, procedures and data analysis) Result and Conclusion. The abstract should contain the data to support the key findings or conclusions of the study and this should be self explanatory without references to the text. the first time an abbreviated term is used it should be spelled out in full form and follow with the abbreviation in parentheses for example :- CKD (Chronic Kidney Disease). Please do not cite any references in the abstract.

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#### Types of Manuscripts

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## Department of Orthodontics and Dentofacial Orthopedics

### Chattagram International Dental College

206/1, Haji Chand Meah Road, Shamsarpara,  
Chandgaon, Chattogram, Bangladesh.  
Cell : 01753200323, Phone : (031) 2573119-23  
E-mail : shahique\_jpni@yahoo.com  
Website : [www.cidch.edu.bd](http://www.cidch.edu.bd)

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# Stem Cells: A New Horizon in Regenerative Dentistry

Shahiqul Jabbar<sup>1\*</sup>

## Introduction

The regenerative capabilities of certain lower vertebrates has always been an interest for the researchers. Regeneration is a remarkable physiological process in which remaining tissues organize to reform a missing body part but mammals only have partial regenerative capacity, perhaps for more proficient wound healing ability. Stem cells or progenitor cells are the common denominator for nearly all types of regeneration. Stem cells are defined as “unspecialized human or animal cells that can produce mature specialized body cells and at the same time replicate themselves”<sup>1</sup>. When a stem cell divides, the daughter cells can either enter a path leading to the formation of a differentiated specialized cell or self-renew to remain a stem cell, thereby ensuring that a pool of stem cells is constantly replenished in the adult organ. This mode of cell division, a unique characteristic of stem cells is asymmetric and is a necessary physiological mechanism for the maintenance of the cellular composition of tissues and organs in the body.

The human body is made up of three basic categories of cells: germ cells, somatic cells and stem cells. The materials required for tissue engineering include stem cells, morphogens (or growth factors) and a scaffold to guide cell growth. Scientific study on cell-based therapies has identified tremendous potential for the use of these stem cells to treat a number of diseases and disorders.

It is now accepted that progenitor/stem cells reside within orofacial region. Stem cells residing in the orofacial

region is classified as the Mesenchymal Stem Cells (MSCs) /Adult Stem Cells (ASCs) / Tissue Stem Cells (TSCs)<sup>2</sup>. Studies have identified several niches of multipotent mesenchymal progenitor cells, known as dental pulp stem cells, which have a high proliferative potential for self-renewal. These progenitor stem cells are now recognized as vital for dentine regeneration process following injury. More recently, researchers have discovered that stem cells harvested from deciduous teeth may be a source of tissue regeneration and repair<sup>3</sup>.

## Effectiveness of the Stem Cells

A stem cells Potency its capacity or efficiency specifies to differentiate into different cell types and accordingly the cells can be divided into several categories as follows:

- i) Totipotent stem cells
- ii) Pluripotent stem cells
- iii) Multipotent stem cells
- iv) Oligopotent stem cells
- v) Unipotent cells<sup>4</sup>.

## Sources of the Stem Cells in Orofacial Region

The first type of dental stem cell was isolated from the human pulp tissue and termed as Dental Pulp Stem Cells (DPSCs). Subsequently, four more types of dental-MSC-like populations were identified:

- Stem Cells from Exfoliated Deciduous Teeth (SHED)
- Periodontal Ligament Stem Cells (PDLSCs)
- Stem Cells from Apical Papilla (SCAP)
- Dental tissue from human third molar.

These dental stem cells are derived from the neural crest, and thus have a different origin from bone marrow-derived MSCs, which are derived from mesoderm<sup>5</sup>.

## Dental Applications of Stem Cells

Stem cells from dental sources has found applications in treatment of various diseases and defects which involves craniofacial regeneration,

1. Associate Professor of Orthodontics and Dentofacial Orthopedics  
Chattagram International Dental College  
Chattogram.

\*Correspondence to :

**Dr. Shahiqul Jabbar**

Cell : 01753 20 03 23

Email : shahique\_jpni@yahoo.com

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dentin regeneration, periodontal regeneration, cementum regeneration, pulp regeneration, cleft lip, cleft palate, salivary gland regeneration, TMJ reconstruction, whole tooth regeneration, cancer therapy, cancer models for biology of cancer, oral mucosa models for studying oral biology, cell and organ models for studying molecular physiology behind processes like tooth eruption, forensic dental profiling, correlation and collection of ante-mortem and postmortem data.

#### Challenges of Stem Cell Therapy

A major difficulty with stem cell therapy is to identify the stem cells within a culture of real fabric. The cultures contain many different cells and is a challenge to identify specific cell types. When stem cells are identified and then isolated from tissues, appropriate solutions must be used to trigger these cells into the desired cell types.

Finally, even though the cells may be identified, isolated and grown, there are supplementary issues like immune response and efficiency. A person's immune system can identify the transplanted cells as foreign bodies and thereby it can generate an immune reaction that results in refusal of the new cells<sup>6</sup>.

#### Conclusion

The prospect of stem cell therapy is to ease the suffering of human beings and to dramatically influence disease has provoked scientists to investigate ways to augment current therapies for stem cell and develop new ones. Dental stem cells can grow not only dental tissues but also other non-dental tissues. They are not only investigated in the

field of Medicine but also found their place in the field of Dentistry as Forensic Odontology as well opening newer insights and avenues for research in this hitherto less ventured arena of Forensic dental investigations using stem cells. In future dental stem cell banking will be an easy way to store one's own stem cells. An extracted tooth is more valuable than a diamond. Nevertheless, challenge for the dental professional in the anticipated era of stem cells and tissue engineering is a growing field of tremendous interest.

#### Disclosure

The author declared no competing interest.

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# Serum Antioxidant Nutrient Status and Dietary Effects Among the Patient with Oral Precancerous Lesion

Monoj Kumar Barua <sup>1\*</sup>

## Abstract

**Background:** To evaluate the level of trace elements in serum and dietary frequency of antioxidant vitamins and mineral containing foods by patients with oral precancerous lesions for early prediction diagnosis & treatment of those patients.

**Materials and methods:** Serum copper & zinc level was estimated by using standard method in thirty nine (39) patients with histologically diagnosed as oral precancerous lesion. A questionnaire was developed to obtain relevant information of the patient. An arbitrary score was designed to evaluate the frequency of some specific food items. **Results:** Data analysis revealed serum zinc level decreased in 86.9% male & in 87.5% Female patients. Serum copper level increase in 30.8% patients & normal in 28.2% pts. Citrus fruits taken daily by 7.7 % patient. 50 % of the patient do not take green leafy vegetables daily. None of the patient takes milk & milk product daily or weekly. Daily intake of Fish, meet and egg by the patient was respectively 51.3 %, 17.9% & 5.1 %. As expected in oral precancerous conditions serum zinc level is reduced & dietary intake of vitamins & minerals also poor. These phenomenons are contributing in the increase of serum copper zinc ratio (Mean 3.62). **Conclusion:** This study shows that vitamins & minerals status may be associated with the pathogenesis of oral precancerous lesions & their progressions. Concerted efforts would therefore help in early detection management & monitoring the efficacy of treatment.

## Key words

Antioxidant; Free radical; Zinc; Copper; Vitamins; Serum; Diet; Oral precancerous lesions.

## Introduction

Various oral mucosal lesions, specially red and white lesions have a potential for malignant change. Pre-malignancy is distinguished from malignancy only by the later invasiveness and metastases<sup>1</sup>. The oral precancerous lesions are leukoplakia, erythroplakia, lichen plunas, sub mucous fibrosis, etc. This oral precancerous lesions caused by some personal habits like cigarette, smoking, tobacco chewing, alcohol, betel nut chewing and dietary intake like vitamin deficiency, nutrient deficiency, taking of hot foods and red chillies contain food. These personal habits irritated the oral mucosa and also induce free radicals in our body<sup>2</sup>. Some prerequisites appear to be necessary before confirming a role of free radicals for molecular injury to specified tissue. These are: 1st there

must be a defined source of free radical species. 2nd the free radicals scavenging systems are either deficient or have not dynamically active<sup>3</sup>. To remove free radicals and maintain tissue integrity there is an essential pathway for the clearances of excess radicals known as Antioxidant system<sup>4</sup>. Some antioxidant such as glutathione and uric acid are produced by normal metabolism. Some are acts by preventing the initiation of peroxidation & this is primarily accomplished by maintaining structure of tissues and cells and architecture within the cell. In this respect zinc, vitamin A, vitamin B complex & vitamin C are considered as antioxidant<sup>5</sup>. Others are the radical quenching antioxidant, which acts through radical propagation & chain elongation. Among them vitamin E, vitamin C and beta carotene are the main nutrients obtain from diet<sup>6</sup>. Enzymes such as catalase, glutathione peroxidase and superoxide dismutase transform the free radicals into less reactive substance. Since selenium, copper, zinc and manganese are the component of these enzymes so they treated as antioxidants nutrients<sup>7</sup>. The superoxide anion an active free radical may efficiently be removed by superoxide dismutase enzyme which is zinc and copper dependent<sup>8</sup>. Zinc influences in all five phases of the immune response include, recognition, activation, proliferation, effector function and memory. Zinc has a polyclonal activity and adjuvant like amplification effects on immune functions<sup>9</sup>. Zinc is needed for many

1. Associate Professor of Oral & Maxillofacial Surgery  
Rangamati Medical College, Rangamati.

\*Correspondence to :

Dr. Monoj Kumar Barua

Cell: 01817 702711

Email : drmonojbarua16@gmail.com

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biological functions including synthesis of DNA and RNA, protein synthesis, cell division and gene expression. It protect against DNA damage and cell fragmentation caused by various toxins and by tumor necrosis factors<sup>10</sup>. Timothy (1997) shows that zinc status is a better indicator of tumor burden and stage of the disease in head and neck cancer patients than the patients overall nutritional status<sup>11</sup>. Prasad et al. (1997) found that zinc deficiency and zinc dependent immunological dysfunction are present in more than half of the patient with head neck cancer<sup>12</sup>. Krishnaswamy et al. (1995) found that clinical complete remission of oral precancerous lesion was seen in 57% of subjects on supplements by zinc<sup>13</sup>. Gupta et al (1999) shows that among patients with oral precancerous lesion 20% reduction in risk occur by taking zinc therapy per day<sup>14</sup>. Varghese et al. (1987) found significant reduction of serum zinc level among the patients with oral precancerous lesion<sup>15</sup>. Though copper act as an antioxidant by some enzyme but excess copper can induce oxidative stress that causes cell and organ damage<sup>16</sup>. As a free ion, copper ion can induce formation of reactive oxygen species that can damage biomolecules, including unsaturated lipids and DNA, probably via Fenton like chemistry<sup>17</sup>. As a transition metal copper can generate the reactive oxygen species including hydroxyl radicals<sup>18</sup>. As copper and zinc share similar chemical properties and compete for similar binding sites in cells serum copper concentration are increased in zinc deficient state<sup>19</sup>. Animal food source is the very good source of zinc, vitamin A and vitamin E . This includes meat, fish, milk, egg, cheese, yogurt etc<sup>20</sup>. Green & colored vegetable & fruits like tomato, pumpkin, carrot, beet, sweet potato, mango, papaya, banana etc. are the very good source of carotenoid and vitamin E<sup>21</sup>. Citreous fruits & green vegetables are rich source of vitamin C<sup>22</sup>.

### Materials and methods

This study was conducted in out-patient Department of Oral and Maxillofacial Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka, Bangladesh. The duration of study was from January 2006 to June 2007 consenting newly diagnosed patients with histologically proven oral precancerous conditions were included in this study. Patients who did not consent and those on vitamin or mineral supplement at the time or within last six months of enrollment were excluded.

Ethical permission was obtained from the ethical committee.

A questionnaire was developed to obtain relevant information from the subjects. It aimed to collect data on socio-economic status, oral & general health, clinical and histopathological findings of oral precancerous lesions and a dietary habit.

With all aseptic precaution five ml of venous blood was drawn from the subjects arm, collected in a metal free tube and allowed to stand for two hours at room temperature. sample was centrifuged at 1,000 revolution per minute for 10 minutes. After separation, serum was collected in a di-ionized vial then frozen and stored at - 30<sup>o</sup>c.

The serum zinc and copper levels were determined using an atomic Absorption Spectrophotometer (AAS), by Graphite Furnace (Pyrolytic coated). Stock zinc & copper standard solution was WAKO chemical company, Japan. Reference value for serum zinc was 700 to 1400 ppb for male and 800 to 1550 ppb for female. Reference serum copper level was 700 to 1200 ppb. The normality of the distributions of the data was assessed using the K-S goodness of fit test. Means and 95% confidence intervals were calculated. Correlation analysis was performed to assess the relationship between various antioxidant nutrient intake and status. The statistical package SPSS 11.5 was used for analysis.

### Results

This cross sectional descriptive study reveals that 28 (71.8%) patients with oral precancerous condition was suffering from lichen planus. Next frequently occurring lesion was leukoplakia (17.9%) followed by oral submucous fibrosis (10.3%).

In oral cavity buccal mucosa was the commonest site of the lesion having frequency 34 (87.2%). The next common site was the tongue 12 (30.8%). Five cases had lesion on the vestibule, 4 (10.3%) on inner lip and 3 (7.7%) on the palate. A good number (46.2%) of the patients had lesion on multiple sites.

**Table I :** Distribution of the patients by habit influencing formation of precancerous conditions.

Habit	Present no. (%)	Absent no. (%)	Total no. (%)
Red chili contain food taking	29 (74.4)	10 (25.6)	39 (100.0)
Betel nut chewing	24 (61.5)	15 (38.5)	39 (100.0)
Smoking	18 (46.2)	21 (53.8)	39 (100.0)
Hot food taking	12 (30.8)	27 (69.2)	39 (100.0)
Tobacco chewing	10 (25.6)	29 (74.4)	39 (100.0)

The patients were asked about the habits influencing in formation of precancerous conditions. Red chili contain food taking topped having frequency 29 (74.4%). Betel nut chewing habit 24 (61.5%); followed by smoking 18 (46.2%), hot food taking habit 12 (30.8%) and tobacco chewing habit 10 (25.6%) (Table I).

**Table II :** Distribution of the patients by serum zinc level.

Serum Zinc level (ppb)	no.	%	Statistics (group)	Statistics (Combined)
<b>Male</b>				
90 - 399	7	30.4	Mean 501.70	Mean 474.33
400 - 699	13	56.5	Median 500.00	Median 450.00
*700 - 1400	2	8.7	Mode 500	Mode 500
1401 - 1500	1	4.3	Std. Deviation	Std. Deviation
Total	23	100.0	306.091	295.875 Minimum 90 Maximum 1500
<b>Female</b>				
90 - 399	9	56.3	Mean 435.00 Median 300.00	
400 - 699	2	12.5	Mode 110(a) Std. Deviation	
700 - 799	3	18.8	285.587 Minimum 110 Maximum 1000	
**800 - 1550	2	12.5	(a) Multiple modes exist. The smallest	
Total	16	100.0	value is shown	

\* Normal serum zinc level for male

\*\* Normal serum zinc level for female

Serum zinc levels of the patients are shown in table II. Among the male patients (23) only 2 (8.7%) had normal serum zinc level. 1 (1.43) of the patients had serum zinc level above normal range (1500 ppb). The rest 20 (86.9%) had reduced serum zinc level. On the other hand among 16 female patients only 2 (12.5%) patients had normal serum zinc level. The rest 14 (87.5%) had reduced serum zinc level (Table II).

**Table III :** Distribution of the patients by serum Copper level.

Copper level (ppb)	Frequency	Percent (%)	Statistics
200 - 499	8	20.5	Mean 1073.67
500 - 699	8	20.5	Median 800.00
*700 - 1200	11	28.2	Mode 650
1201 - 1499	4	10.3	Std. Deviation 752.922
1500 - 1999	2	5.1	Minimum 200
2000 - 2499	3	7.7	Maximum 3600
2500 - 2999	2	5.1	
3000 - 3600	1	2.6	
Total	39	100.0	

Serum copper level shown in table III. Normal serum copper level is 700 – 1200 ppb. It was normal in 11 (28.2%) patients, below the normal level among 16 (41.0%) of patients and increased in 12 (30.8%) of patients. Among the patients 5 (12.8%) of patients had serum copper level 2000 – 3000 ppb. One had 3600 ppb.

**Table IV :** Distribution of the patients by copper/zinc ratio group.

Copper-zinc ratio	Frequency	Percent	Statistics
0.80 – 1.00	4	10.3	Mean 3.6272
1.01 – 2.00	21	53.8	Median 1.6667
2.01 – 3.00	5	12.8	Mode 1.30(a)
3.01 – 6.00	3	7.7	Std. Deviation
6.01 – 15.00	5	12.8	4.67626 Minimum 0.80
15.01 – 21.05	1	2.6	Maximum 21.05
Total	39	100.0	(a) Multiple modes exist. The smallest value is shown

Table IV shows the copper-zinc ratio of the patients. As expected in oral pre-cancerous conditions serum zinc level is reduced. The phenomena are contributing in the increase of serum copper-zinc ratio of the patients. In this study the mean ratio was found 3.6272 with standard deviation  $\pm 4.67626$ . Three patients had Cu-Zn ratio 3.01–6.00 and 5 (12.8%) had 6.01– 5.00. One patient was found to have serum copper-zinc ratio 21.05.

**Table V :** Distribution of the patients by Dietary frequency of some selected food items n=39.

Selected food items	Daily	Weekly	Fortnightly	Monthly	Never
	no (%)	no (%)	no (%)	no (%)	no (%)
Green leafy vegetable	20 (51.3)	19 (48.7)	-	-	-
Colored vegetable	17 (43.6)	17 (43.6)	1 (2.6)	-	4 (10.3)
Colored fruit	7 (17.9)	26 (66.7)	6 (15.4)	-	-
Citrus fruit	3 (7.7)	27 (69.2)	9 (23.1)	-	-
Fish	20 (51.3)	19 (48.7)	-	-	-
Meat	7 (17.9)	-	25 (64.1)	7 (17.9)	-
Egg	2 (5.1)	12 (30.8)	18 (46.2)	7 (17.9)	-
Milk & milk product	-	-	8 (20.5)	31 (79.5)	-

Dietary frequencies among the patients were enquired for some selected food items. Nearly 50% of the patients do not take green leafy vegetables daily. 9 (23.1%) took citrus fruit twice a month and only 3 (7.7%) took it daily. Consumption of animal sources of zinc were found to be very poor among the patients. Daily consumption of meat was present only in 7 (17.9%), Fish was consumed daily by 20(51.9%) of the patient while that of egg was taken only by 2 (5.1%) of the patients. None of them was found to be take milk and milk product daily or weekly (Table V).

**Table VI :** Distribution of the patients by scores of taking antioxidants.

Source score	Frequency	Percent	Source statistics
<b>Vit. A source</b>			
6 – 8	5	12.8	Mean 9.64, Median 10.00 Mode 10 Minimum 6 Maximum 11
9 – 11	34	87.2	
<b>Citrus fruit source</b>			
2	9	23.1	Mean 2.85, Median 3.00 Mode 3 Minimum 2, Maximum 4
3	27	69.2	
4	3	7.7	
<b>Animal source</b>			
6 – 8	16	41.0	Mean 8.95, Median 9.00 Mode 9 Minimum 6, Maximum 12
9 – 11	19	48.7	
12	4	10.3	

An arbitrary score was designed to evaluate the frequency of food item for some specific items. The scores are derived from the diet information of individuals.

Never taking = 0, Monthly = 1, Fortnightly = 2, Weekly = 3 and Daily = 4.

Green leafy vegetables, colored vegetables and colored fruits are the sources of carotenoid. And for each source highest score is 4. So for vitamin A expected higher score is 12 (Green leafy vegetable 4, colored veg 4 & colored fruits 4). Again citrus fruits are the source of vitamin C. So expected score of vit C is 4. For zinc fish, meat, egg, milk and milk products are the chief animal sources. So expected higher score of zinc is 16 (4\*4). In table VI shows expected source score of vit A was 12. It was 9 – 11 with 34 (87.2%) of the patients and the score 6 – 8 was among 5 (12.8%) of patients.

Expected source score vit. C was 4. It was 3 with 27 (69.2%) of patients. It was 2 with 9 (23.1%) patients. Full score 4 was among 3 (7.7%) of patients.

Expected score of zinc was 16. The score range from 9-11 among 19 (48.7%) of patients and score 6-8 was found in 16 (41.0%) of patients. None of the patients was found to achieve the full-expected score (16).

### Discussion

This study shows most frequently occurring site of oral precancerous lesion was buccal mucosa 87.2% followed by tongue 30.8% vestibule 12.8% and inner lip 10.3%. Involvement of multiple sites was 46.2%.

Trivedy et al (2002) also found that buccal mucosa and tongue were the most frequently affected sites. He showed it was areca induced<sup>23</sup>.

Vergheze et al (1987) analyzed serum level of copper and zinc in 100 patients with oral precancerous lesion and 50 patients with oral cancer. He found reduction of serum copper and zinc level in both oral precancerous and cancer condition. The copper /zinc ratio was found to be elevated in the patients of the oral precancerous lesion<sup>24</sup>.

Abdulla et al (1979) observed significantly decreased plasma zinc level ( $p < 0.001$ ) and an copper/zinc ratio was high ( $p < 0.001$ ) in the patient with head and neck cancer<sup>25</sup>.

Study also shows consumption of animal sources of zinc was found to be very poor among the patients.

Zinc availability is considerably greater from animal protein in comparison to cereal protein<sup>26</sup>.

Prashed et al found that zinc intake from animal protein was significantly less among the zinc deficient oral cancer patient<sup>27</sup>.

Data also shows that there is a high intake of spicy food, it was 74.4%. It also supported by Yu-Heng Chen<sup>28</sup>.

Chiba 2001 found that tobacco chewing and smoking habits have an effect on oral cancer<sup>29</sup>. My study also shows that 46.2% of the patient have tobacco smoking habit and 25.6% have tobacco chewing habit.

Gupta et al showed that tobacco smoking and tobacco chewing is the primary etiologic factor for oral precancerous lesion<sup>14</sup>.

Gupta et al after working with 226 patients with oral precancerous lesion found that after controlling of tobacco use and increased intake of fruits and vegetables evinced inverse trends in risk, with an average reduction of over 10% part quartile of exposure. He summarized that food and vegetable intake that's mean consumption of vitamin A & vitamin C and several dietary micronutrients have a protective effect against oral precancerous lesion<sup>14</sup>.

### Conclusion

The present study shows patients with oral precancerous lesions having a low serum zinc level and a higher serum copper/zinc ratio. Majority of the patients had a habit of betel nut chewing, tobacco smoking and taking of red chili food. This habit also placed an extra demand on the need of antioxidant nutrient by body.

Increased free radical load from injurious habits combined with lower status of serum antioxidant micronutrient may result in a free radical - antioxidant imbalance, which when crosses a critical limit may initiate a depreciation at site of local irritations of injurious habits & develop pre-cancerous lesions.

#### Disclosure

The author declared no competing interest.

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# Endogenous Pain Modulation of Irreversible Pulpitis and Persistent Endodontic Pain

Md. Abu Saeed Ibn Harun<sup>1\*</sup> Muslim Uddin<sup>2</sup> Shahiql Jabbar<sup>3</sup> Md. Ali Hossain<sup>4</sup> Zenia Chowdhery<sup>5</sup>

## Abstract

**Background:** The most common location of orofacial pain in intra oral which encompassed Endodontic Pain (EP) and remain persistent even after successful endodontic treatment named Post Endodontic persistent Pain (PEPP). The aim of this study was to compare the modulation and dimension of primary Endodontic Pain and Post Endodontics Persistent pain. **Materials and methods:** 32 patients with pain were included in this study. Among those 16 patients were diagnosed as Irreversible pulpitis and 16 with PEPP. If any possible endodontic involvement of other teeth in same quadrant were excluded from this study. All Patients were evaluated with preformed questionnaire and examined with dynamic and static sensory test. **Results:** The result showed that Facial Pain Scale rating of endodontic pain were significantly more than post endodontic persistent pain. Conditioning pain modulation of PEPP was less efficient in affected site, contralateral site and forearm. The response to 26 grams monofilament in forearm of EP was significantly more than PEPP. **Conclusion:** PEPP is the more inhibitory arm rather than facilitatory arm of pain modulation but EP is more facilitatory. The dimension of both type of pain were Sensory-discriminative and Affective-evaluative. But EP is more Sensory-discriminative rather than EP.

## Key words

Endodontic; Persistent Pain; Sensory test; Dimension of Pain.

## Introduction

Endodontic pain causes suffering and reduced daily activities and is a major component of oral health and quality of life<sup>1</sup>. The survival of teeth could be related to the absence of pain symptoms. But pain of endodontic origin is widely feared by the patients<sup>2</sup>. As a result the number of people with untreated oral conditions reached 3.5 billion in 2015<sup>3</sup>. A major biological consequence of untreated oral condition is endodontic pain. Current models of endodontic pain view it as complex event. By its very nature, pain is

no longer considered a single entity. Instead it involves many overlapping components<sup>4</sup>. Because of modulation and crossover in the central neural pathways, its may be difficult for object when describing their pain. Modulation can intensity or suppress pain, giving it a multidimensional character. The pain process begins in the periphery, where specialized nerve fibers receive a painful stimulus. This nerve fibers transmit this information to the spinal cord and ultimately to the brain where the information is interpreted and recognized as pain<sup>4,5</sup>.

The are major psychological dimension of pain : Sensory-discriminative, motivational-affective and cognitive- evaluative<sup>5</sup>. Short form of McGill Pain Questionnaire (SF-MPQ) and Visual Analogue Scale (VAS) and other form of pain assessing tool named Pain Assessment in Advanced Dementia (PAINAD) Scale used in patient who are cognitively impaired with advanced dementia, who as a result of their condition can experience more pain or prolonged pain due to its under treatment. Facial Pain Scale- R (FPS-R) can also be used for assessment of pain severity<sup>5</sup>.

Most common source of orofacial pain are odontogenic especially from pulp. Root Canal Treatment (RCT) are the procedure to remove the cause of pain with restore the diseased tooth<sup>6</sup>. RCT a surgery that involves the distal aspect of the second and third branches of the trigeminal nerve innervating teeth<sup>7</sup>. About 3-7% of patients remain with chronic pain after surgery<sup>8</sup>. When affecting in dentate region,

1. Associate Professor of Conservative Dentistry and Dental Radiology Chattagram International Dental College, Chattogram.
2. Professor & Principal Chattagram International Dental College, Chattogram.
3. Associate Professor of Orthodontics and Dentofacial Orthopedics Chattagram International Dental College, Chattogram.
4. Associate Professor of Oral and Maxillo facial Surgery Chattagram International Dental College, Chattogram.
5. International Credit to Oral and Maxillo facial Surgery, USA.

\*Correspondence to :

**Dr. Md. Abu Saeed Ibn Harun**

Cell: 01711 15 75 86

Email : drharunpg7@icloud.com

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the pain may mimic a toothache and may lead to irreversible and unnecessary dental procedures, with no resolution of the pain<sup>9</sup>. Some evidence suggested that persistent pain 6 months after RCT is fairly common and it's about 10% which is higher than previously reported<sup>10</sup>. Analgesics or narcotic do not relieve the symptoms<sup>9,10</sup>. Pain modulation is altered in patients with various chronic pain conditions and it has been suggested that the pain modulatory system can define susceptibility to develop chronic pain disorders<sup>11</sup>.

Although psychophysical studies in patient with Post Traumatic Trigeminal Neuralgia (PTTN) have reported, their modulatory pain system has not been examined<sup>11-13</sup>. Dynamic psychophysical testing can assess pain facilitation or inhibition and provide information on the status of the modulation system. The two available tests in the laboratory are Temporal Summation (TS) and Conditioned Pain Modulation (CPM). TS testes the facilitatory modulation process, usually performed by measurement of the change in pain perception as a result of series of repeated, constant noxious stimuli<sup>14</sup>. It is believed to be the psychophysical correlate of windup of second-/third-order neurons in the spinal cord and higher up, which may contribute to central sensitization<sup>15</sup>.

Conditioned Pain Modulation (CPM) represents the inhibitory modulation process, it reflects the efficiency of endogenous analgesia exerted through the descending pain-modulatory system<sup>16</sup>. It can be studied in the laboratory using 2 remote noxious stimuli, the "conditioning" stimulus that typically inhibits the "test" stimulus<sup>16</sup>.

An improved understanding of the underlying mechanisms leading to persistent pain after RCT could assist in developing interventions to prevent or reduce these adverse outcomes, while at the same time improving our understanding about modulation of endodontic pain. The aim of the study was to compare the pain modulation between irreversible pulpitis and Post Endodontic Persistent Pain (PEPP).

### Materials and methods

All patients with acute irreversible pulpitis including in this study due to irreversible damage of pulp and post endodontic persistent pain after endodontic treatment without odontogenic cause and were not under pharmacological treatment. If any possible endodontic involvement of other teeth in same quadrant were excluded from this study. Patient's severity of Pain were measure by Visual Analogue scale, FPS-R and PAINAD. Pain description and Pattern were evaluated by short-form McGill pain questionnaire.

### Sensory Testing

Total 32 subject included in this study for sensory testing. Among those 16 patients with irreversible pulpitis pain, 16 patients had been suffering pain more than 6 months even after successful endodontic treatment. All test were performed in random order on the volar part of dominant forearm, nonkeratinized gingival apical to the affected tooth and contralateral tooth. To clarify randomization, all the sensory test were performed first in randomized order, and subsequently, all tests involving immersion into hot water were performed in randomized order with 10-minute interval between test.

### Dynamic Sensory Testing

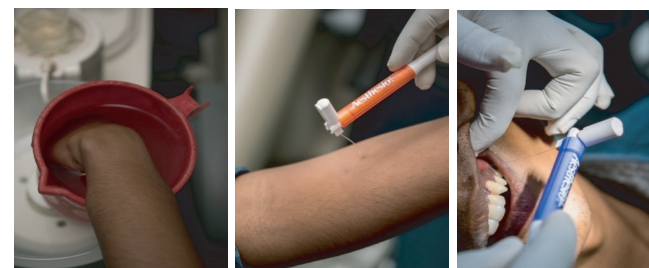
#### Temporal Summation

Mechanical Temporal Summation (TS) was assessed with a 5.46 von Frey filament inducing 26 gram of force with a single stimulus than a train of 30 successive stimuli in 1Hz frequency. Patients were asked to rate the resulting sensation on a Numerical Pain Scale (NPS) of 0 to 10. The difference between the scores after the 10th and 30th stimuli and the scores after a single stimuli were calculated and represent TS (Last minus first score).

### Conditioning Pain Modulation



**Figure 1 :** Application of von frey. **Figure 2 :** Affected side-5.46 (26gm).  
(Filament on patient)



**Figure 3 :** Immersion of hand in hot water. **Figure 4 :** Volar Part of dominant forearm. **Figure 5 :** Non affected side 4.31 (2gm).  
(Hand to the wrist level in hot water)

Immersion of non dominant hand to the wrist level in hot water ( 46.5 degree for 60 seconds). The hot water was circulated during the test to maintain homogeneous water temperature. Thirty one seconds after the hand immersed in the water, TS stimuli were applied using 5.46 von Frey filaments as a single stimulus than train of 30 stimuli. The subject were asked to report a number reflecting the level of stimulus intensity on VAS as above. Data were collected after a single stimulus and then at the end of every 10<sup>th</sup> stimulus. The difference between the 30<sup>th</sup>TS before immersion of the hand into water bath and 30<sup>th</sup>TS during hand immersion was calculated and represent CPM. Negative values indicate more efficient pain reduction.

### Static Sensory Test

“Mechanical Stimuli” were applied with 2 calibrated von Frey filaments 5.46 and 4.31 inducing 26g and 2g force respectively. Patients were asked to rate the resulting sensation on a Numerical Pain Scale (NPS) of 0 to 10. The response was calculated as the average of 3 stimuli applied by each filaments to slightly different sites.

“Cold Stimuli” was applied to the alveolar mucosa of testes area for three seconds with cotton swab(5mm diameter). The subjects were asked to report the type of sensation felt, whether either cold or hot that Paradoxical Hot Sensation (PHS).

### Data Analysis

Mechanical TS for each tested sites was calculated as the score in response to the 10<sup>th</sup> stimulus minus the score after the first stimulus (TS= Response to the 10<sup>th</sup> stimulus-Response to the First stimulus) and the score in response to the 30<sup>th</sup>stimulus minus the score after the first stimulus ( TS= Response to the 30<sup>th</sup> stimulus - Response to first stimulus). Conditioned Pain modulation was calculated as the 30<sup>th</sup> stimuli mechanical TS during the hand immersion in hot water minus the 30 stimuli mechanical TS performed without hand immersion ( CPM= TS with conditioning – TS without conditioning). All data were posted into data sheet and send for data analysis. Graph-Pad (2018) software were used for data analysis.

### Results

A study of 32 patients were included in this study, 16 in the Post Endodontic Persistent Pain (PEPP) Group and 16 in the Endodontic Pain (EP) Group. The mean age of the patients in the PEPP Group was 41.87 ± 13.70 and 40.87 ± 6.74 years in the EP Group. PAINAD Pain Score, Visual Pain Score and Total Pain

Rating Index was 1.12 ± 1.80, 2.12 ± 1.45; 5.75 ± 2.25, 7.37 ± 2.26; 12.25 ± 7.62, 10.12 ± 3.77 in PEPP and EP in respectively. The dimension of the Pain was sensory 5.87 ± 5.75, 7.25 ± 3.69; Affective dimension 4.12 ± 3.64, 4.37 ± 3.69 in PEPP Group and EP Group respectively. All was statistically non significant ( p value > 0.05). Facial Pain Scale Rating was 1.5 ± 1.41 in PEPP Group, 3.5 ± 1.41 in EP Group. Those was statistically significant.

*Temporal summation* : Temporal summation was present in both groups in all sites( p value > 0.05). However no significant difference were found between the TS scores after 10 and 30 stimuli of the PEPP and EP Groups in the affected area ( Table II: TS10: p=0.598, TS30: p=0.427), contralateral side (Table II: TS10: p=0.263, TS30: p=0.184), or dominated forearm (Table II: TS10: p=0.080, TS30: p=0.259).

*Conditioned Pain Modulation*: Patient with PEPP demonstrated less efficient CPM compared with EP Group but not statistically significant in affected area (Table II: CPM- p=0.09), in contralateral side ( Table II: CPM- p=0.081) and in dominated forearm ( Table II: CPM- p=0.889).

*Response to 26 grams*: The PEPP and EP Groups response to 26g intraoral stimuli were not significantly different in the affected side ( Table II: 26g: p=0.328) and in the contralateral side ( Table II: 26g: p=0.207). The EP Group's response to 26g stimuli was significantly elevated compared with the PEPP group ( Table II: 26g: p=0.031).

*Response to 2g*: The response to 2g stimuli between PEPP and EP Groups was non significantly different in affected ( Table II: p=1.00), contralateral side (Table II: p=0.276) and dominated forearm (p=0.555).

*Paradoxical Heat Sensation (PHS)*: 4 patients with PEPP were felt hot ever after apply the cold in affected side.

**Table I:** Comparison of Patients and pain characteristic between PEPP and EP.

	Persistent Endodontic Pain	Endodontic pain	p value (0.05)
Age	41.87 ± 13.70	40.87 ± 6.74	0.855
PAINAD pain score	1.12 ± 1.80	2.12 ± 1.45	0.241
FPS- R	1.5 ± 1.41	3.5 ± 1.41	0.013 S
Visual Pain Scale score	5.75 ± 2.25	7.37 ± 2.26	0.172
Sensory dimension	5.87 ± 5.74	7.25 ± 3.69	0.576
Afferent Dimension	4.12 ± 3.64	4.37 ± 3.96	0.897
PRI-T( Pain Rating Index- Total)	12.25 ± 7.62	10.12 ± 3.77	0.324

**Table II:** Comparison of pain modulation of Persistent Endodontic Pain and Endodontic Pain.

Variables	Persistent Endodontic Pain	Endodontic Pain	p= Value (0.05)
Intra Oral Affected side			
TS 30th	1.43 + 1.6	2.5 + 3.34	0.427
TS 10th	1.25 + 0.8	1.8 + 2.77	0.598
CPM	-0.37 + 2.0	1.06 + 1.05	0.095
26grm	0.56 + 0.5	1.16 + 1.60	0.328
2grm	0.12 + 0.35	0.12 + 0.35	1.000
PHS	4	No	
Intra Oral Contralateral Side			
TS 30th	1.18 + 0.92	2.68 + 2.9	0.184
TS 10th	0.81 + 0.65	1.68 + 2.01	0.263
CPM	-0.5 + 1.6	1.00 + 1.6	0.081
26grm	0.22 + 0.26	1.12 + 1.91	0.207
2grm	0	0.08 + 0.2	0.276
PHS	No	No	
Dominated Forearm			
TS 30th	1 + 1.1	2.06 + 2.3	0.259
TS 10th	0.62 + .74	1.56 + 1.2	0.080
CPM	-0.375 + 2.01	-0.25 + 1.5	0.889
26grm	0.18 + 0.34	1.53 + 1.56	0.031. S
2grm	0.19 + 0.35	0.5 + 1.41	0.555
PHS	No	No	0

## Discussion

Pain is viewed from a clinical perspective, it is not the inevitable consequence of the activation of a unique pain system but is, instead an expression of dysfunction in the somatosensory system<sup>17</sup>. Somatosensory changes are important clinical features of neuropathic pain. Pain modulation is altered in patients with various chronic pain modulatory system can define susceptibility to develop chronic pain disorders<sup>18</sup>.

Temporal summation is an experimental protocol demonstrating pain facilities through obtaining pain reports along a series of identical stimuli. The common response along the series representing the physiological phenomenon of wind up<sup>19</sup>. The TS10 and TS30 in affected area, contralateral side and forearm of PEPP less than EP. It is probably due to activation of silent nociceptors, leading to a state of peripheral hypersensitivity and hyperalgesia. Because in acute pain activation of silent C fibers with reduction of pain threshold and increased excitability<sup>20</sup>. In our pilot study VAS score was indicated that EP was severe rather than PEPP was moderate.

The static psychophysical profile largely confirmed previous finding that show a mix of sensory gain and loss in patients with Post traumatic trigeminal neuralgia (PTTN) which is typically seen in neuropathic pain<sup>7</sup>. The dynamic sensory testing demonstrated uncton of endogenous modulatory system. Less efficient CPM was found with post endodontic persistent pain but non significant to EP. Possible causes that reduced the endogenous pain modulatory system mainly in patients with PEPP suffering from the condition for more than a year<sup>21</sup>. Because dental injury and inflammation induced microglial activation in the trigeminal subnucleus caudalis (Vc) and activation of microglia may be implicated in the central mechanisms of pain<sup>7,21</sup>. Both peripheral and central mechanisms are probably involved in the pathophysiology of PEPP. Less efficient CPM was found in patients with PEPP at both injury site, contralateral site and forearm demonstrating more widespread extra segmental inhibitory pronociceptive changes in somatosensory processing.

CPM is seems to compared the interaction between physiological pathway on the one hand and psychological-cognitive one on the other. The psychological dimension of PEPP and EP was neither purely sensory nor affective. The response to 26grms monofilament in a EP typically painful and unpleasant than PEPP. When considering signs as allodynia and hyperalgesia, it is important to appreciate that evidence from imaging experiments in human spinal nerve neuropathies suggested a complex CNS involvement<sup>21,22</sup>. Hyperalgesia is associated with neuronal activity that spreads beyond the pain neuromatrix involving cognitive and emotional response and shows significant difference in the pattern of neuronal activity vis-à-vis dynamic mechanical allodynia<sup>22</sup>. In our study results showed that EP was not purely sensory but affective dimension also. Two patients with PEPP in affected site was response to cold as paradoxical heat sensation. The presence of paradoxical heat sensation upon cold stimulation is usually considered indicative of a disturbance in Ad-cold fiber function or of a central sensitization<sup>22</sup>. However, a dysfunctional modulatory system and prolonged painful sensation after intraoral cold stimulus as shown here suggest a significant role for CNS component. Sample size and pain suffering time is the another important factor for reflection of the central nervous system transition from more acute to more chronic phase of the disease. So further research have to conduct in bigger sample size.

**Conclusion**

These findings suggest that PEPP is associated more with the inhibitory rather than the facilitatory arm of pain modulation and that the central nervous system has a role in PEPP pathophysiology. In cases of endodontic pain is associated more with facilitatory arm of pain modulation.

**Disclosure**

All the authors declared no competing interest.

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# Prevalence of Malocclusion Among the Referred Patients of Dhaka Dental College and Hospital

Mohammad Abu Taher<sup>1</sup> Md. Zakir Hossain<sup>2</sup> Layla Rahman Leena<sup>3</sup>  
 Mohammad Hossain Bhuiyan<sup>4</sup> Shahiqul Jabbar<sup>5</sup>

## Abstract

**Background:** Malocclusion plays an important role in the overall oral health of an individual because it is associated with periodontal disease, temporomandibular disorders and may be complicated by an individual's disparity. Careful attention to malocclusion in patients with special needs leads to a considerable improvement in the quality-of-life. The aims of this study was to determine frequencies of different types of malocclusion in different ages of the patients with concerned about their problems. **Materials and methods:** A total of 300 referred patients with permanent dentition stage were evaluated in this study. Angle's classification is used in this study because it is a universally acceptable system. **Results:** In this study the frequencies of class I malocclusion was 52.3%, class II div I was 31.0%, class II div 2 was 4%. Class III malocclusion was observed in 12.7% of cases. Among total population female subjects were slightly more than male. Class III group came earlier for their treatment because they were more aware about their problems. **Conclusion:** Class I were more frequent malocclusion but patient were concerned about Class III malocclusion.

## Key words

Malocclusion; Malocclusion in Bangladesh; Referred patients.

## Introduction

Malocclusion is a manifestation of normal biological variability. This is a continuum ranging from an ideal occlusion to considerable deviation from normal<sup>1</sup>. Malocclusion is one of the commonest esthetic and functional problems in our country. It is not a disease, rather, it is a disability with a potential influence on physical and mental health and appropriate treatment can be important for the patients' well being<sup>2</sup>.

For many years, studies have been conducted to determine the prevalence of malocclusion in different population. The result of these studies, even those studies conducted in a population of the same origin,

may show great variability. Instead of differentiating normal and abnormal occlusion in a population, determining frequencies of different types of malocclusion in a referred population may also give valuable information<sup>3</sup>.

Many classification of malocclusion have been proposed and used according to different criteria. But in our study we used Angle's classification because it is a universally acceptable system that reduce subjectivity and provide an easy way of classifying malocclusion<sup>1-3</sup>.

About 10% of the orthodontically treated patients are motivated by periodontal problems. Therefore, patient with periodontal involvement and dental crowding should receive orthodontic alignment, considering also the high prevalence of periodontal disease in adult population<sup>4</sup>.

In every country, there is need to identify different malocclusions, there incidence and the need for treatment so that appropriate manpower arrangement can be made. The results of present study are useful for public health planning and for the generation of hypothesis for future studies. As the incidence of malocclusion may change or fluctuate in population with time, follow-up studies are required. The aims of this study was to determine frequencies of different types of malocclusions in different ages of the patients and concerned about their problems.

## Materials and methods

A total of 300 patients referred to the Department of Orthodontics Dentofacial Orthopedics, Dhaka Dental

1. Junior Consultant of Orthodontics, Dental Unit Chittagong Medical College Hospital, Chattogram.
2. Professor of Orthodontics (Retired) Dhaka Dental College and Hospital, Dhaka.
3. Junior Consultant of Orthodontics Dhaka Dental College and Hospital, Dhaka.
4. Junior Consultant of Orthodontics, Dental Unit Chittagong Medical College Hospital, Chattogram.
5. Associate Professor of Orthodontics Chattogram International Dental College, Chattogram.

\*Correspondence to :

**Dr. Mohammad Abu Taher**

Cell: 01712 165305

Email : abutaherpavel@gmail.com

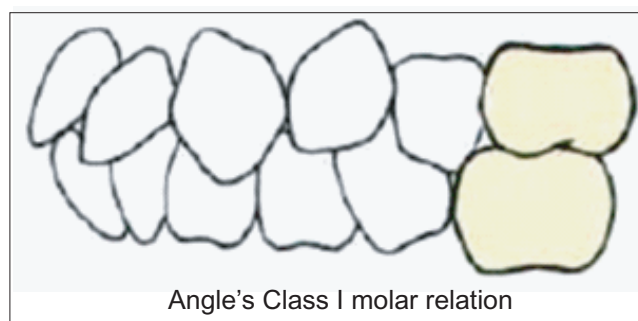
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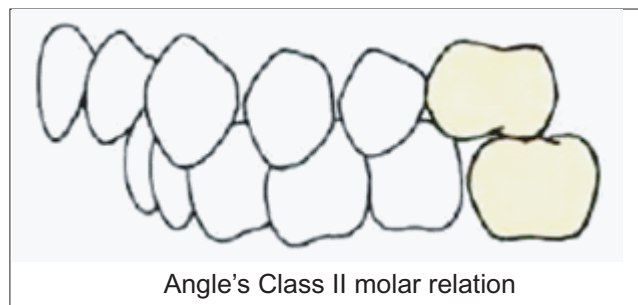
College and Hospital, Dhaka Bangladesh, were evaluated in this study. The patients were in permanent dentition stage. These patients were selected by convenient sampling and none of the subjects had undergone previous orthodontic treatment. Orthodontic examinations of the patients' model were carried out by the investigator. Patients with systemic diseases were excluded from the study.

Four malocclusion groups were formed according to the following criteria:

**Class I group:** Angle's class I molar relationship in centric occlusion.

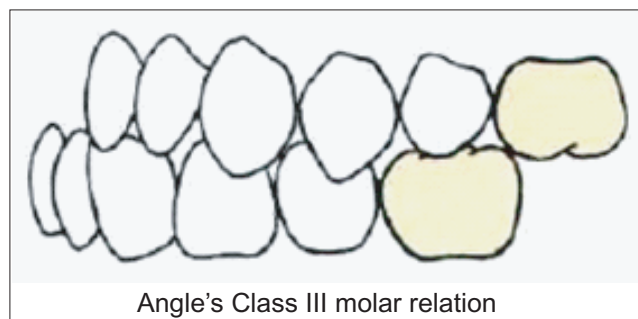


**Class II div-1 group:** Angle's class II molar relationship in centric occlusion.



**Class II div-2 group:** Retroclination of two or more maxillary incisors, Angle's class II molar relationship in centric occlusion.

**Class III group:** Angle's class III molar relationship in centric occlusion



**Results**

Class I malocclusion was found in 157 patients which represented 49.4% of the sample. The frequency of class II division 1 and class II division 2 malocclusion were 31.4% and 4.1%, respectively. Class III malocclusion was present in 15.1%

**Table I :** Distribution of sex by type of malocclusion.

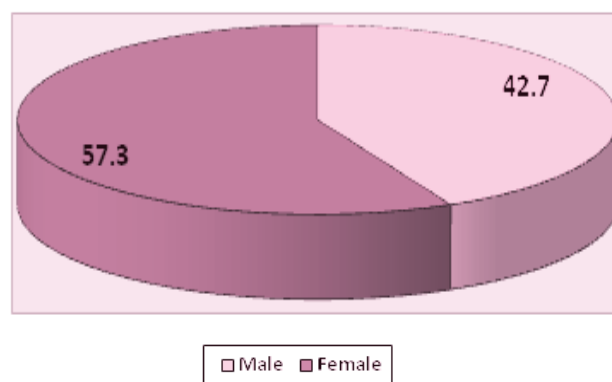
Angle's classification	Total n=300	%	Male n=128	%	Female n=172	%	p-value
Class-I	157	52.3	72	56.3	85	49.4	0.2234
Class-II div-1	93	31.0	39	30.5	54	31.4	0.8676
Class-II Div-2	12	4.0	5	3.9	7	4.1	0.9304
Class-III	38	12.7	12	9.4	26	15.1	0.1421

Table I Shows that Class I malocclusion was found in 157 subject, which represented 52.3% of the sample. The frequency of Class II division 1 and Class II division 2 malocclusions were 31% and 4%, respectively. Class III malocclusion was present in 12.7% of the patients.

**Table II :** Distribution of age in years by sex.

Age in years	Male %(n)	Female % (n)	Total % (n)	p-value
13-17	39.5(34)	60.5(52)	28.7(86)	0.0003
18-22	43.2(63)	56.8(83)	48.7(146)	0.0198
23+	45.6(31)	54.4(37)	22.7(68)	0.1316

Table II Shows that significant difference was found between male and female in (13-17) years and (18-22) years age groups but 23+ years age group was not significant ( $p < 0.05$ ).



**Figure 1 :** Distribution of study population by sex.

Figure 2 : Shows that female subject were slightly more than male which was 57.3% was female and 42.7% in male was male.

**Table III :** Statistical comparisons of mean ages related to malocclusion groups.

Class-I n=157		Class-II div-1 n=93		Class-II div-2 n=12		Class-III n=38		p-value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
21.01	3.51	18.38	5.47	20.17	5.51	18.10	4.18	0.0001

Table III Shows that comparison of the mean ages of the malocclusion groups indicated a statistically significant difference between Class I and Class II division 1 groups ( $p < .05$ ).

### Discussion

Now a days, in modern countries sophisticated attention is given to the development of dentofacial disorders and to the treatment of malocclusions<sup>5</sup>. Appearance of the teeth tops the rank order of the reasons for seeking orthodontic treatment. Parents also reported malalignment of their children as a significant cause of being teased. Even mild form of facial deviation, can predispose toward psychological distress and anxiety<sup>6</sup>.

The demand for orthodontic treatment is increasing in most countries. Therefore, rational planning of orthodontic measures on a population basis is essential in assessing the resources required for such a service. This stresses the importance of epidemiological studies in order to obtain knowledge about the prevalence of different types of malocclusions and the need for orthodontic treatment<sup>7</sup>.

A total of 300 patient's model (Male 42.7 % Female 57.33 %) referred to the department of orthodontics and dentofacial orthopedics, Dhaka Dental College and Hospital were evaluated in this study. The subjects of the study were selected on the basis of inclusion and exclusion criteria. The reported prevalence of malocclusion varies from 39% to 93%<sup>7</sup>. The prevalence of different types of malocclusion may show great variability, even in a population of the same region. Clearly, the evaluation of referred patients and distribution of malocclusion types may give valuable information for planning an orthodontic service<sup>3</sup>.

According to our result the prevalence of class I malocclusion was 52.3% (Male 56.3%, Female 49.4%). Males are higher than females, however, the difference was not statistically significant. Similar trends were found in Danish children with adolescent dentition<sup>8</sup>. Contrary to the present result at West midlands children in Britain and modern Alaskan Eskimo showed female predominance in class I malocclusions<sup>9-13</sup>. This maybe due regional and ethnic variations.

In our study the frequencies of class II div I was 31.0% (Male 30.5 %, Female 31.4%), class II div 2 was 4% (Male 3.9 %, female 4.1 %). Class III malocclusion was observed in 12.7% (Male 9.4 %, Female 15.1%).

The patients treated in the department of orthodontics, Selcuk University Turkey. They reported that 61.7% of the patients had class I, 25.1% had class II division 1, 3.0% had class II division 2 and 10.2% had class III malocclusion. Although their reported frequency of class I malocclusion was higher and class II division I was lower than our study. The differences of between the frequencies of malocclusions can be related to the material differences<sup>3,12</sup>.

Malocclusion and facial types in 132 Saudi Arabian patients referred for orthodontic treatment and reported that 53.8% had class I, 28.8% had class II div 1, 4.5% had class II division 2, and 12.9% had class III malocclusion<sup>13</sup>. The result of this study is almost similar to us.

In Bangladesh prevalence of class I malocclusion was 55.22%, class II was 33.33% (class II div 1 was 28.85% and class I div 2 was 4.48%) class III was 8.46%, open bite 0.99% and other nonspecific case 2% who were attending at Dhaka Dental College and Hospital in the department of Orthodontics<sup>14</sup>. Class II div-1 malocclusion was 32.8%, class-III malocclusion was 13.3% and class II div-2 malocclusion was 7.5%. According to Ahmed N, class I malocclusion was 45.84%, class II was 39.88% (Class II, div-1 was 32.74% and class II div-2 was 7.14%), class III patient was 14.28% who were attending at Dhaka Dental College and Hospital in the department of orthodontics<sup>15</sup>. Another dissertation work did at Dhaka Dental College and Hospital among the patient who are attending for the treatment during the period of 2002-2005. The prevalence of class III malocclusion was 31.66% and of them 11.66% was females and 20% was males. The results of this study markedly differ from other studies in respect of prevalence of class III malocclusion.

### Conclusion

Class I was the most frequently seen malocclusion, whereas Class II division 2 was the least common. The class III group was aware of their problem earlier as the lowest mean age was in the class III groups. Female subject were more than male among total subject and also in all malocclusion group. Female subject were more than male in all age groups.

**Disclosure**

All the authors declared no competing interest.

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# Crime Scene Visit Perspective Bangladesh: An Essence Before Autopsy

Ahmad Sadek<sup>1\*</sup> Mustafa Sumon Al Rashid<sup>2</sup> A M Ashraful Anam<sup>3</sup> Nashid Tabassum Khan<sup>4</sup>

## Abstract

*Crime scene is one of the most crucial aspects of an investigation and that the scene of the crime is where good forensic science begins. The article begins by demonstrating that high quality and useful evidence leading to accurate and fair criminal justice outcomes can only occur if the scene is processed effectively and professionally. In Bangladesh forensic doctors do autopsy on the basics of police inquest and other documentary papers. Unfortunately in majority cases they do not visit crime scene. So valuable clues are left. It's not only doctor's fault but also police doesn't give facilities or arrangement for doctors. In some cases doctor fall in difficult situation to finalize opinion due to insufficient information in inquest report which greatly hamper justice system. Bangladesh hasn't spectated any development of forensic law and the number of experts are almost non existent up until now. When the world is using sophisticated methodologies to trace the criminals, Bangladesh is still using some archaic and inexpedient mechanisms. Misdirected DNA tests by, overwhelmingly criticized two finger test, disoriented fingerprints are the most debated and questionable exponents in Bangladeshi criminal justice administration system. My review article focuses on current practice of using criminal investigation in Bangladesh and crime scene visit importance on our forensic view. This paper analyzes the major obstacles, limitations and way forward to use forensic science are also the major contents.*

## Key words

Autopsy; Crime scene; Investigation; Forensic expert.

## Introduction

Forensic science itself begins at the crime scene. The objects of study for forensic science are physical and chemical traces left at the crime scene; for example, hairs, fingerprints, glass chips. It argues that the crime scene is one of the most crucial aspects of an investigation and that the scene of the crime is where good forensic science begins. The article begins by demonstrating that high quality and useful evidence leading to accurate and fair criminal justice outcomes can only occur if the scene is

processed effectively and professionally. Traces themselves only become evidence if they are collected, analyzed and reported effectively. Importantly, this initial stage of the forensic process is both a scientific and a social process (As is true for the whole forensic process). In other words, forensic evidence is not simply 'found' at a crime scene, it is socially constructed. The social construction of forensic evidence is undertaken by numerous people with various types of expertise throughout the process<sup>1</sup>. It needs no mention that with the advent of different types of crimes in both number and peculiarity, the on growing demand by public to solve a medicolegal case immediately, often creates a chaos at society that spreads to every corner by prompt interest of media. This is an out coming problem for maintenance of law and order and being responsible citizen dealing with such cases, doctors are at a greater risk to be challenged. Nowadays, several crime incidences happened where forensic experts played important role<sup>2</sup>. In absence of a separate investigating agency in Bangladesh, the Investigating Officers who belong to the police force are at times lagging behind the professionalism in wrapping up investigation of crimes for a plethora of reasons. Though separate judicial magistracy started its journey about eight years ago, delayed, defective and biased investigation of crimes is one of the major stumbling blocks

1. Associate Professor of Forensic Medicine & Toxicology Army Medical College Chittagong, Chattogram.
2. Assistant Professor of Forensic Medicine & Toxicology Aichi Medical College, Dhaka.
3. Associate Professor of Forensic Medicine & Toxicology Dhaka Community Medical College, Dhaka.
4. Associate Professor of Forensic Medicine & Toxicology Z H Sikder Womens Medical College, Dhaka.

\*Correspondence to :

**Dr. Ahmad Sadek**

Cell: 01881 204982

Email : dr.sadek555@gmail.com

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that haunt our crippling criminal justice system. Colonial rules, too much reliance on confession of the accused rather than evidence oriented way of investigation and heavy workloads of law enforcing agency are major causes for lackadaisical investigation. The central goal of a criminal investigation is to identify, gather and preserve evidence<sup>3</sup>. Criminal investigation is a search for witnesses and evidence to support the charge in court by proving beyond any reasonable doubt that the crime was committed by the accused. To be more precise, a criminal investigation is an applied science that involves the study of facts, used to identify, locate and prove the guilt of a criminal. It necessarily encompasses searching, interviews, interrogations, evidence collection and preservation and various methods of investigation. Our apex court has rightly observed that law enjoins upon a police officer the duty of investigation into a crime. In discharge of the said statutory duty he has to embark upon a quest for the discovery of truth<sup>4</sup>. Criminal investigators now commonly employ many modern scientific techniques known collectively as forensic science. A criminal investigation is an undertaking that seeks, collects, and gathers evidence for a case or specific purpose. Criminal investigators undertake several scientific techniques in order to find the necessary evidence for a case. However, such avenue still remains largely unexplored in Bangladesh<sup>3</sup>.

### Search Strategy

The google scholar database were queried for relevant articles to the topic of Crime Scene Visit Perspective Bangladesh. A literature search was performed in PubMed using the search word "Appraisal of Criminal Investigation", "Critical Issues in Criminal Justice" and "Importance of Autopsy and Crime Scene". The search term following key words used in various combination : Crime scene; Autopsy; Forensic exper.

### Discussion

#### What is Investigation?

According to Code of Criminal Procedure, 'investigation includes all the proceedings under the Code for the collection of evidence conducted by a police officer or by any person (other than a Magistrate) who is authorised by Magistrate<sup>5</sup>. Any step thus taken by a police officer or a person authorised by a Magistrate towards collection of evidence in regard to an offence falls within the ambit of investigation<sup>6</sup>. An investigation follows the commission of such an offence and does not precede it<sup>7</sup>. There is clear distinction between investigation and inquiry. Inquiry may be conducted by any person while investigation is always done by the members of the law enforcing agency.

#### Who Can Investigate?

Generally, a police officer not below the rank of Sub Inspector (SI) is the investigation officer of a cognisable offence. Police has the statutory right to investigate into a cognisable offence whether a report is made to that effect or not and if reported, irrespective of the authority of the reporter<sup>8</sup>.

#### Investigation Steps

The Supreme Court of Bangladesh summarized the following steps which are included in the criminal investigation: <sup>9</sup>

- Proceeding to the spot
- Ascertainment of facts and circumstances of the case
- Discovery and arrest of suspected offender (s)
- Collection of evidence relating to commission of the offence alleged which may require examination of various persons including the accused and the reduction of their statements into writing if the officer thinks fit
- The search of places or seizure of things considered necessary for the investigation and to be produced at the trial.
- Formation of opinion as to whether on the materials collected there is a case to place the accused before a court for trial and if so, taking necessary steps for the same by filing of a police report under section 173.
- Making a Case Diary (CD) containing the record of facts ascertained by the officer during investigation and action he has taken showing the time and date against every action he has taken.

After recording the police case the officer-in-charge may himself/herself investigate the case or instructs a police officer not the below rank of Sub Inspector (SI) to investigate the same<sup>10-11</sup>. In practice, an investigating officer inspects the place of occurrence, prepares the sketch map along with the index of the spot, records the statements of the witnesses who are supposed to be acquainted with the facts and circumstance of the occurrence seizes the seized articles (Alamat) and thus prepares the seizure lists in presence of witnesses arrests or tries to apprehend the accused and suspects and forwards them to the nearest Magistrate within 24(Twenty four) hours of their arrest detains and interrogates them in his custody, prays for detention in his custody (Remand) produces the accused or victim before the Magistrate to have

his confession/ statement recorded sometimes conducts the inquest of the deceased victim conducts Test Identification Parade (TIP) sends the deceased for autopsy, collects medical certificates and other expert reports, maintains diary of proceedings of investigation and finally submits the police report<sup>12-19</sup>.

### **Reports of Experts**

Ante mortem, post mortem, chemical examination, DNA, Viscera, handwriting or fingerprint reports by the experts is an important step by which the investigating officer may ascertain the truth or otherwise of the allegations brought against the accused.

### **Inquest**

When the officer-in-charge of a police station or other authorized officer receives an information that a person has committed suicide or has been killed by another or by animal or by accident or has died under circumstances raising reasonable suspicion that someone has committed an offence and none is accused in the information, the officer starts an Unnatural Death (UD) case and after giving information to the nearest Executive Magistrate regarding the unnatural death proceeds to the place where the body is lying. He is required to hold an investigation, draws up a report of the apparent cause of the death describing the nature and marks of injuries found on the dead body stating what weapons or instruments were used to inflict such injuries<sup>20</sup>.

### **Autopsy**

Autopsy or post mortem examination is done by a forensic expert according to the direction of a Civil Surgeon. At least three doctors will append their signatures in the report. Concerned doctor will collect the relevant part of the body of the deceased for viscera examination. The investigating officer may seize the wearing apparels of the deceased for DNA test. Post mortem report is prepared in triplicate in BP Form<sup>21</sup>.

### **Exhume**

According to Code, an Executive Magistrate may cause the body of the deceased to be exhumed and examined for ascertaining the cause of his death<sup>22</sup>. However, in a case of serious nature concerned Judicial Magistrate should give the directions to the District Magistrate for taking necessary arrangements and further order thereto<sup>23</sup>.

### **Delay in Collecting Expert Report**

Criminal investigation is the process of determining the events that happened before, during and after a crime was committed. Multiple law enforcement officials are involved in the investigation including the investigating officers, forensic experts and laboratory analysts. The primary goal in a criminal investigation is to find evidence to bring the criminal to justice. There is also delay in collecting medical certificates and other expert reports which ultimately procrastinate the proceeding.

### **Less-Explored Forensic Science**

Criminal investigators now commonly employ many modern scientific techniques known collectively as forensic science. A criminal investigation is an undertaking that seeks, collects, and gathers evidence for a case or specific purpose. Criminal investigators undertake several scientific techniques in order to find the necessary evidence for a case. However, such avenue still remains largely unexplored in Bangladesh. Lack of modern equipment: Police are quite handicapped in undertaking effective investigation for want of modern gadgets such as cameras, audio-visual surveillance equipment. Forensic science laboratories are scarce. Recourse should be had to scientific study of crimes and involvement of the offenders by resorting to techniques of forensic science, rather than too much reliance on lackadaisical recording of oral testimony by the investigators<sup>24</sup>.

### **Lack of Efficient Officer**

Paucity of efficient and committed officers haunt our investigating agency. There is dearth of forensic and cyber experts in Police force. The investigation of crime is a highly technical specialized art requiring a lot of patience, expertise, training and clarity about legal position of the specific offences and subject-matter of investigation and socio-economic factors. It obviously requires specialisation and professionalism of a type not yet fully perceived by our agencies<sup>25</sup>.

### **Crime Scene Visit is An Essential Tool for Investigation**

Death scene investigation may include a combination of the following types of incidents and examinations:

- i) Accidental deaths, which include a multitude of circumstances, including misadventure
- ii) Suicidal deaths, which include a multitude of circumstances

- iii) Homicidal deaths, which include a multitude of circumstances
- iv) Sudden deaths, with or without suspicious circumstances
- v) Difficult victim identification, which includes mummification and putrefaction
- vi) Disaster victim identification dealing with multiple casualties.

“Every contact leaves a trace”, this statement of Dr. Edmond Locard ponders the significance of the “forensic evidence” in a very articulate manner. Following this notion the science of collecting and analysing physical evidence has come a long way into the modern technology. The accuracy and conclusiveness of forensic evidence sometimes makes the criminal investigation errorless and fair to a great extent. Finger print, foot print, DNA testing, blood spatter analysis etc are the recent development in criminal science that really narrows down the scope of the investigation and make the life of police officers a little bit easier and also helping the Court to bring the absolute justice. For example, a simple blood spatter pattern can unravel positioning of the victim, the depth of the cut, the murder weapon even sometimes the height of the killer.

In developed country like UK, USA etc when a crime scene investigator (CSI) arrives at a crime scene, he doesn't just jump in and start recovering evidence. The goal of the scene recognition stage is to gain an understanding of what this particular investigation will entail and develop a systematic approach to finding and collecting evidence. At this point, the CSI is only using his eyes, ears, nose, some paper and a pen. The first step is to define the extent of the crime scene. If the crime is a homicide, and there is a single victim who was killed in his home, the crime scene might be the house and the immediate vicinity outside. Does it also include any cars in the driveway? Is there a blood trail down the street? If so, the crime scene might be the entire neighborhood.



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Securing the crime scene-and any other areas that might later turn out to be part of the crime scene is crucial. A CSI really only gets one chance to perform a thorough, untainted search furniture will be moved, rain will wash away evidence, detectives will touch things in subsequent searches, and evidence will be corrupted. and any other areas that might later turn out to be part of the crime scene is crucial. A CSI really only gets one chance to perform a thorough, untainted search furniture will be moved, rain will wash away evidence, detectives will touch things in subsequent searches, and evidence will be corrupted. Usually, the first police officers on the scene secure the core area the most obvious parts of the crime scene where most of the evidence is concentrated. When the CSI arrives, he will block off an area larger than the core crime scene because it's easier to decrease the size of a crime scene than to increase it press vans and onlookers may be crunching through the area the CSI later determines is part of the crime scene. Securing the scene involves creating a physical barrier using crime scene tape or other obstacles like police officers, police cars, and removing all unnecessary personnel from the scene. A CSI might establish a "safe area" just beyond the crime scene where investigators can rest and discuss issues without worrying about destroying evidence<sup>26</sup>. The main purpose of collecting and processing any evidence is to produce in the Court of law for the purpose of ensuring conviction. In recent times, our criminal justice system has done some significant works based on the forensic evidences. For example, in a very recent case where Oishee Rahman was proven as guilty, as the DNA of Mahfuzur and his wife matched the samples taken from Oishee's bloodstained clothes and ornaments<sup>27</sup>. While Police had taken Oishee into remand they had to face strong criticisms as the defence tried to

establish that Oishee was a minor based on the school documents. However, following an order by the Court, an examination was subsequently conducted to determine Oishee's actual age. After that, Oishee was sent to Dhaka Medical College and Hospital for physical examination where doctors concluded that she was nearly 19 years old then, based on which the Court passed the judgment of death penalty against her<sup>28</sup>. Another notable work done by the forensic department is – in cases of the identification of terrorists who were dead in Gulshan attack and Kalyanpur, Narayanganj and Mirpur raids. Police collected DNA samples from the bodies of nine militants killed in a special anti-terror operation in Kalyanpur on July 26, 2016 where eight of them were identified after their fingerprints matched up with those stored in the Election Commission database<sup>29</sup>. To confirm the identities scientifically, police collected DNA samples from seven families. There was another remarkable case cited as the Bangladesh Jatiyo Mahila Ainjibi Samity v. Bangladesh, Writ Petition No. 5359 of 2006, the Court found that, the result of Sibling DNA Test shows that all the seven children are unlikely to be related to each other whom were claimed as children of the Former Deputy Inspector General of Police (DIG) of Bangladesh and his wife Mrs. Anwara Rahman<sup>27</sup>. They actually procured those children to traffic them out of country.

As we can see, that we have laws and other technological advancement to apply forensic science in our justice system, however, a question arises, then why many of the cases are still pending because of lack of proper forensic report. In this regard two sensational cases can be referred such as "Sohagi Jahan Tonu murder case" and "journalist couple Sagar Sarowar and his wife Meherun Runi double murder case"<sup>30</sup>. In the first case, two autopsies have been done to help the investigators since the first one drew huge criticism as it on April 4, 2016 said that Tonu was not raped<sup>31</sup>. The second and last post-mortem report, published on June 12, revealed the news of sperm of three people being found in the corpse<sup>32</sup>. In the second double-murder case, several difficulties arose when no viscera test was conducted during the first autopsy. Moreover, many people entered the crime scene which also contaminated the samples that were later discovered in the labs. After that, a Court order was issued for the second autopsy two and a half months after their murder. However, the bodies were severely decomposed. The forensic experts said that it would have been easier, if the test had been done during the first autopsy.

After analysing these two cases, it can be seen that there are still some technical lacking in collecting physical evidences. It is important to mention that physical evidence is also vital evidence in solving criminal cases. In every criminal case the confession of the accused is considered as evidence before the Court. As per the procedure of taking a confession according to the Evidence Act 1872, every confession must be taken in the presence of a Magistrate<sup>33</sup>. Therefore, similarly forensic test and report should be prepared in the presence of a Magistrate so that no contamination, forged report making can happen. Since, if the first investigation goes wrong then it becomes more difficult to find out evidences as in later investigations as bodies started decomposed within few days. It can be said that, we have well established laws and system for collecting forensic evidences and using them before the Court as evidence. However, the society or victim should first help themselves by doing the forensic test at the right time especially in the case of sexual assault. Forensic evidence provides precision in proving the connection of a person to the crime, either it proves someone is guilty or it proves their innocence. The use of forensic evidence is a very efficient method, utilising it properly will only contribute to the development of the criminal justice system. As an end note, it can be iterated that with the contemporary global changes and progresses, the overseas strategies of the forensic science is being developed not only around the world but also in Bangladesh in the field of criminal investigation. This flow of development should be continued both theoretically and in practice<sup>34</sup>. Many investigators save themselves from tedious task of collection of tiny and minute clues. Investigating officers heavily lean towards oral evidence, instead of concentrating on scientific and circumstantial evidence<sup>35</sup>. Further, inherited colonial mentality, too much reliance on confession of the accused rather than evidence oriented way of investigation, discourteous attitude towards public etc. are major causes for lack of confidence in police force which in turn, undermines the process of criminal justice<sup>36</sup>.

### Conclusion

The medico legal expert should visit the death scene before the autopsy if it is possible. Although, death investigation differs in different countries, there is always a crime scene investigation team. If the medico legal expert does not have the opportunity to visit the death scene himself, he would check the documents (Notes, sketches, photographs, etc) which crime

scene investigation team prepared. Many medico legal deaths may be resolved by death scene investigation. A medico legal expert should never forget: If the death scene investigation is not performed before the autopsy, that autopsy will be an imperfect autopsy.

The forensic medicine expert should visit the death scene before the autopsy if it is possible. In some cases, it is important to distinguish accidental manner from suicidal or homicidal ones. For example, if a ligature mark is present on the neck, this is usually suicide or homicide. Children have an increased risk for injury or death from accidents for a variety of reasons compared to adults. Accidental asphyxia can occur in childhood as a result of variety situations. So its necessary take action health ministry to give scope forensic expert along with law enforce agency otherwise barely autopsy doesn't find in all cases cause and manner of death.

#### Disclosure

All the authors declared no competing interest.

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# Regenerative Endodontic Procedures in the Management of Non-Vital Immature Permanent Tooth with Periapical Periodontitis : Case Reports

Md. Abu Saeed Ibn Harun<sup>1\*</sup> Foysal Sirazee<sup>2</sup> Shakhawat Tabrej<sup>3</sup> Asif Sobhan<sup>4</sup> Najmul Alam<sup>5</sup>

## Abstract

A growing body of evidence is demonstrating the possibility for regeneration of tissues within the pulp space and continued root development in teeth with necrotic pulps and open apices. The purpose of this case reports is to present Regenerative Endodontic Procedures (REPs) in the management of immature non vital permanent teeth with periapical periodontitis. Two Patients age 10 and 25-year boys whose involved 11 and 12 number tooth with the history of trauma developed pulpal necrosis and symptomatic apical periodontitis. After preparing an access cavity, its necrotic pulp was removed. The canal was irrigated with 1.5% Sodium Hypochlorite (NaOCl) solution and dried with paper points. A triple antibiotic mixed with distilled water was packed in the canal and left for 14 days. In second visit removed the antibiotic mixture, bleeding was induced by XP endo finisher, allowed to clot at Cemento Enalmal Junction (CEJ). White mineral trioxide aggregate (RetroMTA) was placed directly over the blood clot. Three days later, the tooth was double-sealed with permanent filling materials. Clinical examination 5 and 1/2 months later revealed no sensitivity to percussion or palpation tests. Radiographic examination of this tooth showed resolution of the periapical lesion, further root development, and continued apical closure. After 2 years case 1 was healed and apical constrictor is formed. REPs are the best option for the management of immature non-vital permanent teeth with open apex.

## Key words

Endodontic regenerative procedures; Immature tooth; Open apex; MTA.

## Introduction

Immature teeth with pulp necrosis and apical periodontitis have been a challenge for endodontic treatment because of the thin root wall and open apex. These cases are usually caused by trauma, caries or a developmental malformation, such as dens evaginates or dens invaginatus resulting in pulp necrosis and arrested root development<sup>1,2</sup>. Apexification is a conventional treatment modality for these cases in which either calcium hydroxide paste is used to induce an apical barrier or Mineral Trioxide Aggregate

(MTA) is placed as an apical barrier in order to achieve closure of the apex. Although the success rate of apexification has been reported to be between 74% and 100%, it may result in abnormal root morphology such as the formation of calcified tissue inside the root canal, and long term calcium hydroxide placement may weaken the dentin and induce root fracture<sup>3,4</sup>.

Regenerative Endodontic Procedures (REPs) provides a new treatment modality for the immature non vital tooth with periapical periodontitis. In the 1960s Dr. Nygaard-Ostby first raised the concept of tissue regeneration in side the root canal<sup>5</sup>. In 2004, Dr. Banchs and Trope introduced a modified clinical regenerative endodontic protocol that involved minimal instrumentation, copious irrigation, and placement of antibiotic paste as intracanal medicament followed by inducing bleeding and the formation of a blood clot inside the root canal. In 2016, the American Association of Endodontists (AAE) proposed a standard protocol for regenerative endodontic procedures<sup>6,7</sup>.

The primary goal of REPs is the elimination of symptoms and the evidence of bony healing. The secondary goal is increased root wall thickness and length (desirable but perhaps not essential). The tertiary goal is positive response to vitality testing (which, if achieved, could indicate more organized vital pulp tissue)<sup>7</sup>. The purpose of this report was to illustrate non vital immature teeth with periapical periodontitis managed by REPs.

1. Associate Professor of Conservative Dentistry and Endodontics Chattagram International Dental College, Chattogram.
2. Assistant Professor of Conservative Dentistry and Endodontics Chattagram International Dental College, Chattogram.
3. Senior Lecturer of Conservative Dentistry and Endodontics Chattagram International Dental College, Chattogram.
4. Lecturer of Conservative Dentistry and Endodontics Chattagram International Dental College, Chattogram.

\*Correspondence to :

Dr. Md. Abu Saeed Ibn Harun

Cell: 01711 15 75 86

Email : drharunpg7@icloud.com

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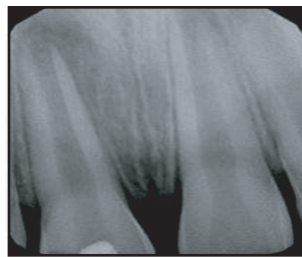
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**Case Report-1**

A healthy 10 years old boy with no relevant medical history, presented with pain of his upper anterior region for 3 months. The patient recalled, he had previous history of trauma before 6 month and also added that he attained a dentist. On clinical examination revealed periodontal abscess on involving tooth number 11 with crown fracture involving enamel, dentin and pulp chamber (Fig-1). This tooth was open apex with periradicular radiolucency in intraoral radiograph (Fig-2). A decision was made to managed this tooth with ERPs.



**Figure 1:** Periodontal abscess.



**Figure 2:** Radiolucency and open apex.

**Case Report-2**

A 25 years old medical student with no relevant of medical history presented with swelling with discharging pus from the upper anterior region. Patient had previous history of trauma when he was 8 years old. On clinical examination sinus tract was present (Fig-3) pus discharged through sinus tract. In periapical radiograph, the tooth was open apex with well defined radiolucency (Fig-4). A decision was made to treat the tooth with REPs.



**Figure 3 :** Intra oral sinus tract.



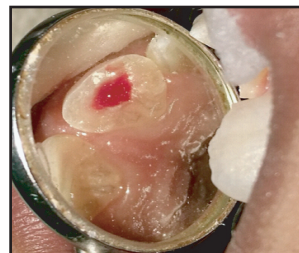
**Figure 4 :** Periapical radiolucency with open apex.

**Regenerative Endodontic Procedurs (REPs)**

After local anaesthesia with 2% lidocaine, the tooth was isolated with rubber dam and access was made with round bur. The canal was then irrigated with 20ml 1.5% sodium hypochlorite solution, irrigation solution was activated by ultrasonic agitation and removed from canal by negative pressure irrigation (Endovac system). 17% EDTA solution was used as final irrigation. The canal was dried with a paper point and

0.1mg/ml triple antibiotic paste was delivered into canal system with lentulo spiral in which ciprofloxacin, metronidazole and clindamycin hydrochloride were equally mixed with distilled water. The tooth was temporarily sealed with Caviton.

After 2 weeks, patients presented for the second visit. The symptom was relieved, asymptomatic teeth proceeded to next step of REPs. After local anaesthesia with 2% lidocaine (Without adrenaline) and rubber dam isolation, the teeth were reaccessed. The triple antibiotic paste was removed with normal saline and 20ml 17% EDTA was applied to the canal; after this, the canal was dried with paper points. Bleeding was induced by over instrumenting with a #25 XP endo finisher and allowed to reach 3-4mm below the Cemento-Enamel Junction (CEJ) to form blood clot. White MTA (Retro MTA) was placed over the blood clot followed sealed with self adhesive flowable composite, and packable composite.



**Figure 5 :** Induced bleeding from beyond apex.



**Figure 6:** MTA placed over clot.

**Follow-up Observation and Measurement**

Periapical radiographic image was taken before treatment. The patients were scheduled to follow up at 3,6,9 and 12 months after the completion of therapy. Clinical symptoms such as pain, swelling, sinus tract, mobility, and the occlusion relationship were recorded and aperiapical radiograph was taken every 3 months. In this report, treatment success was defined as the elimination of symptoms, the disappearance of apical radiolucency with an increase of root length or decrease of the apical foramen or both.

Case 1 had been shown that elimination of clinical sign and symptoms of periapical periodontitis (Fig-7). In periapical radiographed, the tooth was increased in length with apical constrictor, the apical radiolucent was disappeared after 24 months (Fig-8).

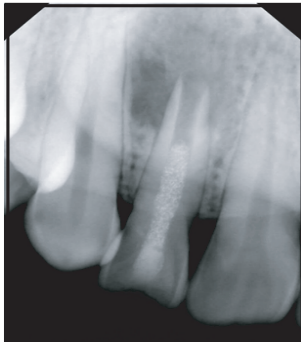


**Figure 7:** Healing of periodontium.



**Figure 8:** Increase the length of root with the apex.

Case 2 showed that elimination symptoms after 6 months follow up schedule. In periapical radiograph, the periapical radiolucency has been disappearing (Fig-9).



**Figure 9:** Disappearing the periapical radiolucency.

### Discussion

There is often deliberation on the definition of treatment 'success' in REPs case reports. It can be argued that resolution of pain, infection and periapical pathology in the absence of continued growth are considered 'successful' cases that was happened in case no 2 because it reflects functional measures of healing with tooth retention in the longer term<sup>8</sup>. However, the most desirable outcome of REPs is to have the the clinically significant continuation of root development that happens in case 1.

Documentation of side effects or late-stage effects (eg. undesirable discoloration, pulp canal obliteration, atypical root morphology development, and loss of vitality after apical closure) was sparse. Tooth discoloration after REPs was reported in 50% of studies. Aside from minocycline, bismuth oxide content in MTA has also been associated with coronal discoloration<sup>9</sup>. Additionally, materials show greater color changes after contact with blood which has implications in REPs because they are placed in contact with BC scaffold<sup>9</sup>. In presented cases, minocycline was replaced with clindamycin hydrochloride. Clindamycin modified triple antibiotic had remarkable antimicrobial effects, cell-friendly, and stain-free properties<sup>10</sup>.

It is clear from the available evidence at this time that the absence of intracanal infection is conducive to the regeneration of tissue within the root canals immature teeth. Newer technologies used in effective disinfection of the root canals before regenerative procedures with a reduced effect on periapical vital tissues should be explored. The use of negative pressure irrigation those used in presented cases has been shown to be efficacious in disinfecting immature teeth in animal model<sup>11</sup>.

17% EDTA was used as root canal conditioner in both cases. Because chemically released growth factors are important in regenerative procedures<sup>12</sup>. Transforming growth factor beta 1 (TGF- $\beta$ 1) induces cell proliferation, differentiation, and chemotaxis in different cell types. Hence, it is considered the key molecules for pulp regeneration<sup>13</sup>. The dentin matrix acts as a reservoir from which growth factors can be released on demand. The release of growth factors can also be induced during chemomechanical root canal preparation by removing the inorganic portion of the smear layer and conditioned the canal wall with 17% EDTA<sup>14</sup>.

### Conclusion

Based on clinical and radiographic observations, endodontic regenerative procedures of the present cases was successful because of the absence of signs and symptoms and the resolution of apical periodontitis as well as thickening of the canal walls and continued root development.

### Disclosure

All the authors declared no competing interest.

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