

University of Rajshahi

Rajshahi-6205

Bangladesh.

**RUCL Institutional Repository**

**<http://rulrepository.ru.ac.bd>**

---

Institute of Biological Sciences (IBSc)

MPhil Thesis

---

2020-06

# Factors affecting Dental caries in Rajshahi Region: It's extent, Consequences June 2020 and Prevention

Parvin, Farhana

University of Rajshahi

---

<http://rulrepository.ru.ac.bd/handle/123456789/1021>

*Copyright to the University of Rajshahi. All rights reserved. Downloaded from RUCL Institutional Repository.*

**FACTORS AFFECTING DENTAL CARIES IN RAJSHAHI  
REGION: IT'S EXTENT, CONSEQUENCES  
AND PREVENTION**

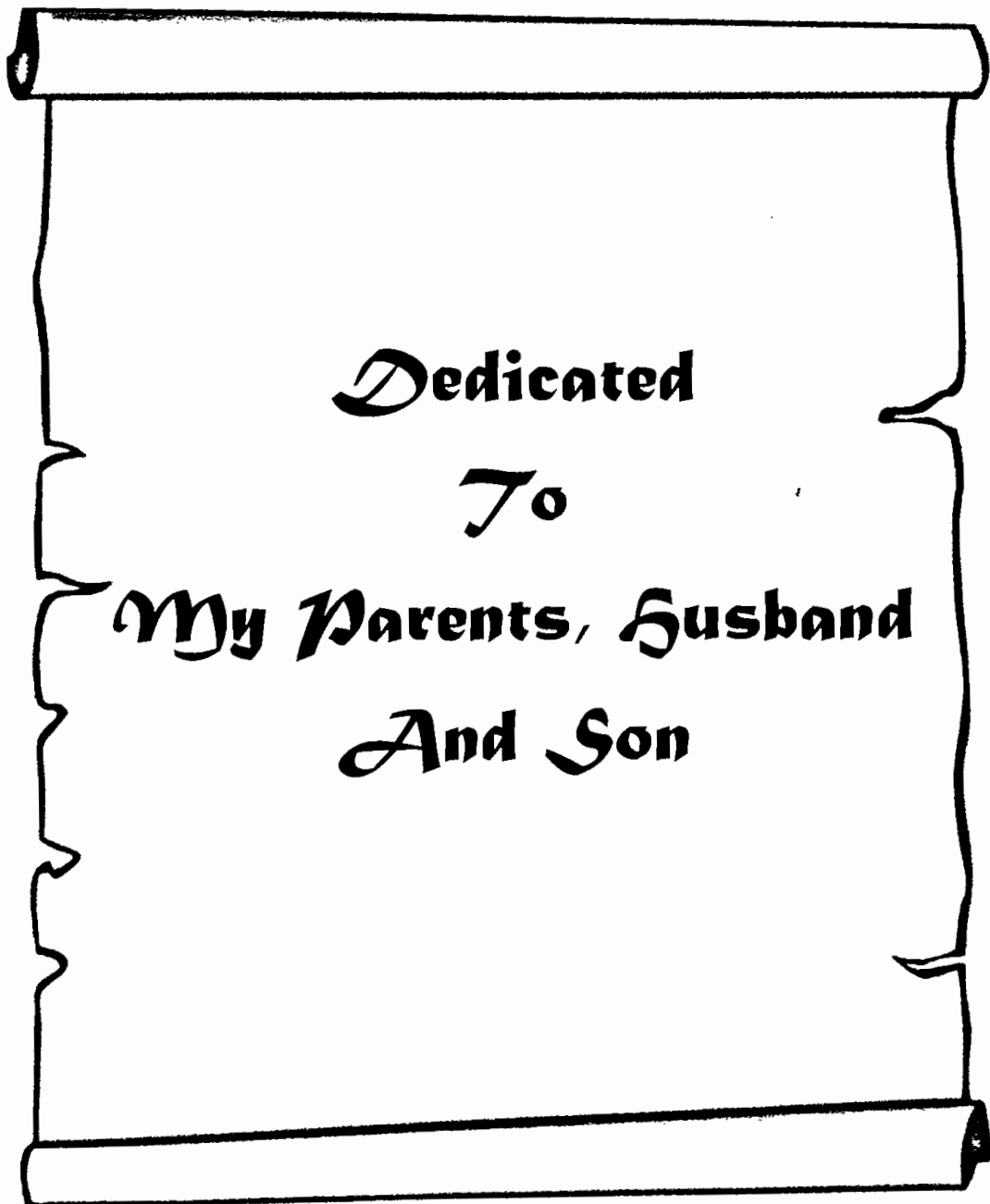


**THESIS SUBMITTED FOR THE DEGREE  
OF  
MASTER OF PHILOSOPHY  
IN THE  
INSTITUTE OF BIOLOGICAL SCIENCES  
UNIVERSITY OF RAJSHAHI  
BANGLADESH**

**BY  
FARHANA PARVIN**

**ENTOMOLOGY AND INSECT  
BIOTECHNOLOGY LABORATORY  
INSTITUTE OF BIOLOGICAL SCIENCES  
UNIVERSITY OF RAJSHAHI  
RAJSHAHI-6205, BANGLADESH**

**JUNE 2020**



***Dedicated***

***To***

***My Parents, Husband***

***And Son***



**Dedicated**

**To**

**My Parents, Husband**

**And Son**

## DECLARATION

I hereby declare that the research work embodied in this thesis entitled “**FACTORS AFFECTING DENTAL CARIES IN RAJSHAHI REGION: IT’S EXTENT, CONSEQUENCES AND PREVENTION**” has been carried out by me for the degree of Master of Philosophy under the guidance of **Professor Dr. Wahedul Islam**, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh.

I also declare that the result presented in this dissertation is my own investigation and any part of this thesis work has not submitted to elsewhere for any degree/diploma or for similar purpose.

June 2020



**Farhana Parvin**

M. Phil Fellow

Session: 2015-2016

Roll No: M 519

Institute of Biological Sciences

University of Rajshahi

Rajshahi-6205

Bangladesh.



**UNIVERSITY OF RAJSHAHI**  
**RAJSHAHI, BANGLADESH**

**CERTIFICATE**

This is to certify that **Farhana Parvin** worked under our supervision as M.Phil Fellow, Session: 2015-2016 Roll No: M 519 Institute of Biological Sciences, University of Rajshahi, Bangladesh. It is our great pleasure to forward her thesis entitled **“FACTORS AFFECTING DENTAL CARIES IN RAJSHAHI REGION: IT’S EXTENT, CONSEQUENCES AND PREVENTION”** which is a record of exclusive research carried out at Institute of Biological Sciences, University of Rajshahi. This work is original and has not been submitted so far in part or in full, for the award of any degree or diploma by any other institute in home or abroad. It is mentioned that **Farhana Parvin** has fulfilled all the requirements for submission of the thesis for the award of the degree of **Master of Philosophy** in Institute of Biological Sciences.

I wish success in her life.

**Supervisor**

**Dr. Wahedul Islam**

Professor

Institute of Biological Sciences

University of Rajshahi

Rajshahi-6205, Bangladesh

**Co-Supervisor**

**Dr. Shah Alam**

Professor

Department of Microbiology

Rajshahi Medical Collage

Rajshahi, Bangladesh

## ACKNOWLEDGMENTS

By the name of almighty **ALLAH** most precious and merciful who give me the courage and patience to complete this thesis work.

First of all I am extremely grateful and thankful to my honorable, Supervisor **Professor Dr. Md. Wahedul Islam**, Institute of Biological Sciences, University of Rajshahi, Bangladesh, for his regular intellectual and careful supervision, scientific knowledge and approach, valuable advice and encouragement during the period of my research work, which made it easy to prepare my research paper is worth mentioning.

I am thankful to **Professor Dr. Shah Alam**, Department of Microbiology, Rajshahi Medical Collage hospital, Rajshahi; for his support and proper guidance.

I am also gratitude to Md. Badrul Islam Principal Scientific Officer BCSIR Rajshahi, for providing me the laboratory supports during my study.

Finally, I am sincerely grateful to all my dental caries patients for their kind cooperation.

**The Author**



## ABSTRACT

Dental caries or cavity could also be a serious oral pathological state in most industrial countries, poignant 60-90% of school going kids and additionally the overwhelming majority of adults. Oral health standing of vulnerable people in developing countries tends to be lower priority than various health issues. Consequently few studies square measure done on the oral health standing of the agricultural people in developing countries and explore the possibility indicator of cavity tooth decay but no relationship could also be established between drinkable and caries. Throughout this study it had been aimed to prevent cavity by salt and Ca that's available with drinkable. This study was drained upazilla level in rajshahi region. The dust-covered coliform gift within the drinkable in addition a region of the study. Typically this may be usually a cross sectional descriptive type of study.

About 540 respondents of 9 upazillas in Rajshahi region were designated and knowledge was collected by a predesigned datasheet. 60 samples of water were collected from the respondents in every upazilla. The information the knowledge the knowledge concerning caries patients was taken within the data sheet. Sample analysis was wiped out 2 classes. One was chemical analysis and alternative was microbiological analysis of the water. The study represent that the Fluoride content in water that was collected from the respondents of various upazillas expressed in table 1 to 9. The F worth was found 16.126. The many result was found in Tanore, Puthia, Upazilla. Extremely important was discovered in Bagha, Bagmara, Charghat, Durgapar Upazilla. Whereas non-significant result was found in acid, Godagari, Mohonpur Upazilla. The metallic element content in water expressed in table 10-18. The, F worth was 41.151. The amount of great was totally different in



every upazilla. In Godagari Upazilla the result found is critical. In Paba, Bagha, Charghat, Durgapur, Mohonpur and Tanore the result were extremely important. The Fluoride concentration in water samples collected from totally different upazillas ranges from 0.1231 to 2.3254 ppm. Mohanpur Upazilla contains the best quantity of Fluoride and Godagari Upazilla contains rock bottom quantity of Fluoride. The vary between total of sq. and mean sq. is highest in Mohanpur. The metallic element concentration in water sample collected from totally different upazilla ranges from 27. 94 to 71 gm l-1. Tanore Upazilla contains the best quantity of metallic element and Charghat Upazilla contains rock bottom quantity of metallic element. The vary between total of sq. and mean sq. is highest in Godagari Upazilla. The microbiological analysis that represent the faecal coliforms contamination of water.

The present study establish that intensive drinking of those microorganism contaminated water affected teeth and deteriorated the dental health condition also as general health within the study space.

# CONTENTS

Title	Page No.
Declaration.....	I
Certificate .....	II
Acknowledgements.....	III
Abstract.....	IV-V
Contents .....	VI-IX
List of Tables .....	X-XI
List of Figures.....	XII-XIV
List of Abbreviations .....	XV-XVI
<b>Chapter I: Introduction .....</b>	<b>1-21</b>
1.1 Background.....	1
1.2 Classification of dental caries.....	3
1.3 Risk factors .....	6
1.4 Bacteria .....	8
1.5 Pathophysiology of dental caries.....	9
1.6 Signs and symptoms .....	10
1.7 Consequences of dental caries.....	12
1.8 Diagnosis of dental caries.....	13

1.9 Treatment of dental caries .....	15
1.10 Drinking water.....	18
1.10.1 Drinking water and oral health.....	18
1.11 Types of water source in upazilla level of Rajshahi region .....	18
1.12 Groundwater.....	19
1.13 Wells and boreholes .....	19
1.14 Shallow wells.....	20
1.15 Surface waters.....	21
<b>Chapter II: Review of Literature.....</b>	<b>22-33</b>
2.1 Fluoride in drinking water.....	23
2.2 Benefit of fluoride in drinking water.....	26
2.3 Mechanism of action of Fluoride in reducing cavity .....	27
2.3.1 Pre-eruptive effects .....	28
2.3.2 Post-eruptive result.....	28
2.4 Calcium in drinking water .....	28
2.5 Microbiological study of drinking water.....	31
2.5.1 Factors affecting fecal coliforms contamination.....	33
<b>Chapter III: Materials and Methods.....</b>	<b>34-53</b>
3.1 Study protocol .....	34
3.2 Objectives .....	35
3.2.1 General objective.....	35
3.2.2 Specific objectives.....	35

3.3 Material and methods .....	35
3.4 Place of study.....	36
3.5 Selection criteria .....	37
3.5.1 Inclusion criteria.....	37
3.5.2 Exclusion criteria.....	37
3.6 Data collection.....	37
3.7 Questionnaire.....	37
3.8 Collection of sample.....	43
3.8.1 Collection of sample from tap .....	43
3.8.2 Collection of sample from a tube well .....	44
3.8.3 Collection of sample from an open well.....	44
3.9 Method and methodology.....	45
3.9.1 SPADNS method.....	45
3.9.2 Calcium (Ca <sup>2+</sup> ) [Titration with EDTA] .....	47
3.10 Calculation.....	49
3.11 Microbiological analysis .....	49
3.11.1 Membrane filtration method .....	49
3.11.2 Required .....	50
3.11.3 Preparation of culture media .....	51
3.11.4 Method .....	52

<b>Chapter IV: Results .....</b>	<b>54-102</b>
4.1 Fluoride results .....	61
4.2 Calcium results .....	81
4.3 Microbiological analysis .....	101
<b>Chapter V: Discussion, Conclusion and Recommendation .....</b>	<b>103-108</b>
5.1 Discussion.....	103
5.2 Conclusion .....	107
5.3 Recommendation.....	108
<b>References .....</b>	<b>109-120</b>

## LIST OF TABLES

Table No.	Title	Page No.
Table 1:	Percent infection with dental caries (in different gender) among Patients studied. ....	54
Table 2:	Percent occurrence of different type of caries .....	55
Table 3:	Percent occurrence of different type of caries in male (♂) and female (♀).....	56
Table 4:	Water source of dental caries patient.....	57
Table 5:	Occurrence of dental caries in different age group .....	58
Table 6:	Distribution of respondents according to their Oral Hygiene practice.....	59
Table 7:	Distribution of the frequency of visit of the respondents to their Dentist for regular care.....	60
Table 8:	Fluoride content in drinking water collected from patient of Paba Upazilla. ....	61
Table 9:	Fluoride content in drinking water collected from patient of Bagha Upazilla. ....	63
Table 10:	Fluoride content in drinking water collected from patient of Bagmara Upazilla. ....	65
Table 11:	Fluoride content in drinking water collected from patient of Charghat Upazilla. ....	67
Table 12:	Fluoride content in drinking water collected from patient of Durgapur Upazilla. ....	69
Table 13:	Fluoride content in drinking water collected from patient of Godagari Upazilla. ....	71

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
Table 14:	Fluoride content in drinking water collected from patient of Mohanpur Upazilla. ....	73
Table 15:	Fluoride content in drinking water collected from patient of Puthia Upazilla. ....	75
Table 16:	Fluoride content in drinking water collected from patient of Tanore Upazilla. ....	77
Table 17:	Calcium content in drinking water collected from patient of Paba Upazilla.....	81
Table 18:	Calcium content in drinking water collected from patient of Bagha Upazilla. ....	83
Table 19:	Calcium content in drinking water collected from patient of Bagmara Upazilla. ....	85
Table 20:	Calcium content in drinking water collected from patient of Charghat Upazilla. ....	87
Table 21:	Calcium content in drinking water collected from patient of Durgapur Upazilla. ....	89
Table 22:	Calcium content in drinking water collected from patient of Godagari Upazilla. ....	91
Table 23:	Calcium content in drinking water collected from patient of Mohanpur Upazilla. ....	93
Table 24:	Calcium content in drinking water collected from patient of Puthia Upazilla. ....	95
Table 25:	Calcium content in drinking water collected from patient of Tanore Upazilla. ....	97
Table 26:	Microbiological analysis of drinking water collected from patient of different Upazilla.....	101



## LIST OF FIGURES

Figure No.	Title	Page No.
Figure 1.1:	Classification of caries.....	4
Figure 1.2:	Pathophysiology of dental caries .....	10
Figure 1.3:	Radiological image and different types of caries .....	11
Figure 1.4:	The tip of a dental explorer, which is used for caries diagnosis ....	14
Figure 1.5:	Diagram of a hand-dug well .....	20
Figure 3.1:	Drinking water collected from patient.....	45
Figure 4.1:	Percent infection with dental caries (in different gender) among Patients studied .....	54
Figure 4.2:	Percent occurrence of different type of caries .....	55
Figure 4.3:	Percent occurrence of different type of caries in male (♂) and female (♀) .....	56
Figure 4.4:	Water source of dental caries patient.....	57
Figure 4.5:	Occurrence of dental caries in different age group.....	58
Figure 4.6:	Distribution of respondents according to their Oral Hygiene practice .....	59
Figure 4.7:	Distribution of the frequency of visit of the respondents to their Dentist for regular care.....	60
Figure 4.8:	Fluoride content in drinking water collected from patient of Paba Upazilla.....	62

<b>Figure No.</b>	<b>Title</b>	<b>Page No.</b>
Figure 4.9:	Fluoride content in drinking water collected from patient of Bagha Upazilla. ....	64
Figure 4.10:	Fluoride content in drinking water collected from patient of Bagmara Upazilla. ....	66
Figure 4.11:	Fluoride content in drinking water collected from patient of Chorghat Upazilla. ....	68
Figure 4.12:	Fluoride content in drinking water collected from patient of Durgapur Upazilla. ....	70
Figure 4.13:	Fluoride content in drinking water collected from patient of Godagari Upazilla. ....	72
Figure 4.14:	Fluoride content in drinking water collected from patient of Mohanpur Upazilla. ....	74
Figure 4.15:	Fluoride content in drinking water collected from patient of Puthia Upazilla. ....	76
Figure 4.16:	Fluoride content in drinking water collected from patient of Tanore Upazilla. ....	78
Figure 4.17:	Maximum and minimum level of fluoride in different Upazila in Rajshahi region. ....	79
Figure 4.18:	Sum of square and Mean Square of fluoride in different Upazila in Rajshahi region. ....	80
Figure 4.19:	Calcium content in drinking water collected from patient of Paba Upazilla. ....	82

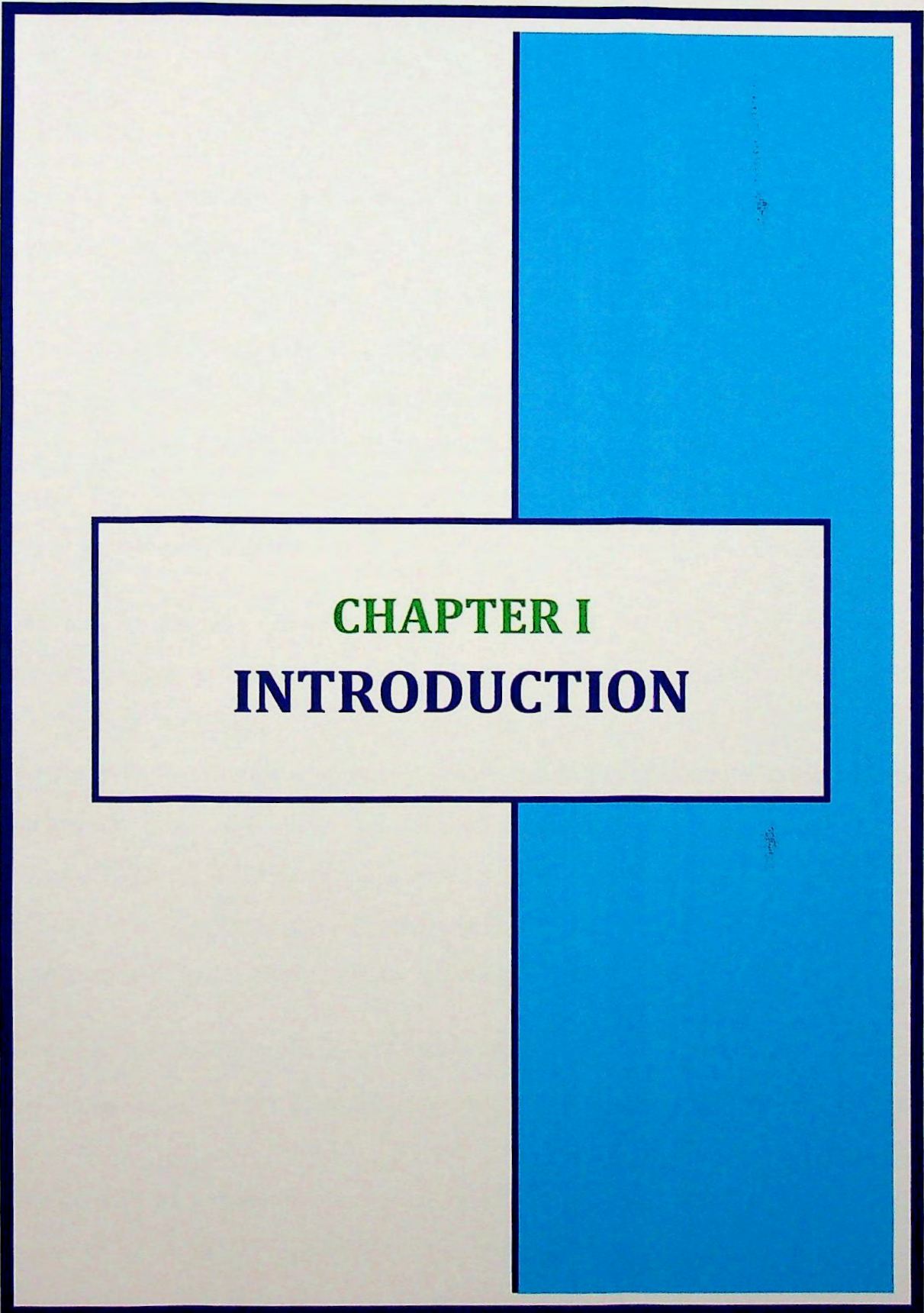
<b>Figure No.</b>	<b>Title</b>	<b>Page No.</b>
Figure 4.20:	Calcium content in drinking water collected from patient of Bagha Upazilla. ....	84
Figure 4.21:	Calcium content in drinking water collected from patient of Bagmara Upazilla. ....	86
Figure 4.22:	Calcium content in drinking water collected from patient of Charghat Upazilla. ....	88
Figure 4.23:	Calcium content in drinking water collected from patient of Durgapur Upazilla. ....	90
Figure 4.24:	Calcium content in drinking water collected from patient of Godagari Upazilla. ....	92
Figure 4.25:	Calcium content in drinking water collected from patient of Mohanpur Upazilla. ....	94
Figure 4.26:	Calcium content in drinking water collected from patient of Puthia Upazilla. ....	96
Figure 4.27:	Calcium content in drinking water collected from patient of Tanore Upazilla. ....	98
Figure 4.28:	Maximum and minimum level of Calcium in different Upazila in Rajshahi region. ....	99
Figure 4.29:	Sum of square and Mean Square of Calcium in different Upazila in Rajshahi region. ....	100
Figure 4.30:	Microbiological analysis of drinking water collected from patient of different Upazilla. ....	102

## LIST OF ABBREVIATIONS

The following abbreviations have been used through the text:

ARCPOH	:	Australian Research Centre for population oral Health.
CaCO <sub>3</sub>	:	Calcium Carbonate.
DDW	:	Distilled deionized water.
DHHS	:	Department of Health and Human Services.
ECC	:	Early childhood caries.
EDTA	:	Ethyl dihydro tetra acetic acid.
EPA	:	Environmental protection agency.
FAO	:	Food and agricultural organization.
g	:	Gram.
g/ml	:	Gram / millilitre.
GDWQ	:	guidelines for drinking water quality.
GERD	:	Gastro esophageal reflux disease.
HCl	:	Hydrochloric acid
hrs	:	Hours.
IARC	:	International agency for research on cancer.
IPCS	:	International programme on chemical safety.
MBC	:	Minimum bactericidal concentration.
MF	:	Membrane filter.

mg	:	Milligrams
mg/ml	:	Milligrams per millilitre.
min	:	Minutes
ml	:	Millitre.
NaOH	:	Sodium hydroxide.
PPM	:	Parts per million.
RR	:	Reelative risk.
S. mutans	:	Streptococcus matans.
UHC	:	Upazilla health complex.
USA	:	Unitade States of America.
WHO	:	World Health organization.



**CHAPTER I**  
**INTRODUCTION**

# INTRODUCTION

## 1.1 Background

Dental Caries is an irreversible microbial unwellness of the calcified tissues of the teeth and characterised by demineralization of inorganic portion and destruction of organic substance of the tooth, which frequently ends up in cavitation's. Tooth decay or tooth decays may be a major oral ill health in most industrial countries, touching 60-90% of college youngsters and also the overwhelming majority of adults. (Cawson RA, 2002) it's the foremost common oral unwellness. Some dental caries are negletable however some is also painful. This pain suggests that sensitivity of teeth initially.

The cavities are also variety of various colors from yellow to black. Colours are also determinative factors in severity of tooth decay. Symptoms could embrace pain and problem with intake. Sensitivity of tooth is that the initial symptom. It is the baleful symptom for development of tooth decay. Complications could embrace inflammation of the tissue round the tooth, tooth loss and infection or symptom formation (David R Ferguson, 2012). Not solely kids however additionally adults are affected in cavity. Throughout tooth decay tooth become sensitive. This is often the continuation of materialization (Saravan *et al.*, 2008).

The early manifestation of the decay method could be a tiny paten of dematerialized (softened) enamel of the tooth surface, usually hidden from sight within the fissure (grooves) of teeth or in between the teeth. This is often referred to as hidden decay. The destruction spreads into the softer, sensitive a part of the tooth below the dentin (Desh JK *et al.*, 2002). The weakened enamel then collapses to make a cavity and therefore the tooth is increasingly destroyed. Decay can even attacks the roots of



teeth that become exposed by gum recession. This point dentin is incredibly sensitive to hot and funky food. This is often a lot of common in older adults. As a result of they suffer from numerous alternative general diseases like diabetes high blood pressure etc. (Shouric KL, 1941). Our mouths square measure filled with microorganism. Most of them square measure cariogenic microorganism square measure traditional flora (Antia Fe, 1962). Many differing types live to tell the tale our teeth, gums, tongue and alternative places in our mouths. Some microorganism square measure useful. They're referred to as traditional flora. However some may be harmful like those who play a task within the decay method. Throughout the day, a tug of war takes place within our mouths. On one team square measure dental plaque—sticky, colorless film of bacteria—plus foods and drinks that contain sugar or starch (such as milk, bread, cookies, candy, soda, juice, and lots of others). This is often a war between germs and body protecting system that achieved by numerous nutrient. The war between this 2 team usually means that causes and therefore the interference of unhealthy method. Throughout intake or drinking one thing that contains sugar or starch, the microorganisms use them to provide acids. These acids begin to eat away the tooth's arduous outer surface, or enamel. It's the responsible team. On the opposite team square measure the minerals gift in spit (Calcium and phosphate) associated Fluoride from dentifrice, water, and alternative sources. This team is that the preventive team that is helps enamel repair itself by substitution minerals lost throughout an “acid attack.” Our teeth bear this process of losing minerals and restoration minerals all day long (Margherita Fontana, 2018).

## **1.2 Classification of dental caries**

### **1. In line with the placement, dental caries will be**

- a) Primary dental caries
- b) Secondary dental caries

### **2. In line with the quantity of tooth involvement dental caries will be**

- a) Inchoate dental caries
- b) Advanced dental caries

### **3. In line with the speed of progression dental caries will be**

- a) Acute dental caries
- b) Chronic dental caries
- c) In remission dental caries

### **4. In line with anatomical web site**

- a) Crown dental caries
- b) Root dental caries

### **5. In line with status of lesion**

- a) Initial dental caries
- b) Repeated dental caries

### **6. In line with pathway of dental caries unfold**

- a) Forward dental caries
- b) Backward dental caries



**(a) Occlusal caries**



**(b) Cervical caries**



**(c) Proximal caries**



**(d) Proximal caries**



**(e) Proximal caries**



**(f) Proximal caries**

**Figure 1.1: Classification of caries**

**7. Per tissue involvement**

- a) Initial cavity
- b) Superficial cavity
- c) Moderate cavity
- d) Deep cavity
- e) Deep difficult cavity

**8. Per chronology**

- a) Infancy cavity
- b) Adolescent cavity
- c) Adult cavity

**9. Infancy cavity.**

- a) Mild
- b) Moderate
- c) Severe
  - I. Kind I computer code delicate to moderate
  - II. Kind two computer code moderate to severe.
  - III. Kind three computer code severe.

(Dr. Shabeel PN, 2009)

### 1.3 Risk factors

Everyone United Nations agency has teeth is in danger of obtaining cavities, however the subsequent factors will increase risk:

- **Location of Tooth:** Tooth location is that the main issue of decay. Decay most frequently happens in your back teeth (molars and premolars). These teeth have numerous grooves, pits and fissures, multiple pores that may collect food particles. As a result, they are tougher to stay clean. It's simple to scrub anterior teeth than posterior teeth owing to their position.
- **Types of foods and drinks:** Some Foods and Drinks square measure liable for cavity. Foods that hooked up to the teeth for an extended time — like milk, ice cream, honey, sugar, soda, edible fruit, cake, cookies, candy and mints, dry cereal, and chips — square measure additional. This sort of sticky food possible to cause decay than foods that square measure simply washed away by spittle (Laudenbach JM and Simon Z 2014).
- **Snacks and sip honeyed drinks:** this sort of food and drinks also are supply of cavity. Once snack or sip honeyed drinks were taken. It's offer mouth microorganism additional fuel to provide acids that attack the teeth and wear them down. And sipping soda or alternative acidic drinks throughout the day helps produce a continuous acid bathtub over the teeth (Sukhabogi J *et al.*, 2014).
- **Bottle feeding:** throughout bed time babies square measure given hour bottles full of milk, formula, juice or alternative sugar-containing liquids, these beverages stay on their teeth for hours whereas they sleep, feeding decay-causing microorganism. This harm is commonly referred to as baby bottle dental caries (Karunakaran R *et al.*, 2014).

- **Inadequate brushing:** It's the foremost vital issue for childhood tooth decay. As a result of if teeth aren't clean properly before long when ingestion and drinking, plaque forms quickly and therefore the initial stages of decay will begin.
- **Inadequate fluoride:** It's a typical ingredient in dentifrice and mouth rinses. Fluoride may be a present mineral that helps in tooth decay interference. It will even reverse the earliest stages of tooth injury. Attributable to its advantages for teeth, Fluoride is accessorial to several public water provides. It is also a typical ingredient in dentifrice and mouth rinses (Shah N *et al.*, 2007).
- **Age:** Cavities are common each in kids and teenagers. Older adults are also at higher risk. Over time, teeth will traumatize and gums might recede, creating teeth a lot of prone to root decay (Schwendick *et al.*, 2015).
- **Systemic diseases:** several general diseases are the reason for decay because of lack of secretion, that helps forestall decay by laundry away food and plaque from our teeth. Older adults might use a lot of medication that reduces secretion flow, increasing the chance of decay. Substances found in secretion additionally facilitate counter the acid created by microorganism. Sure medications, some medical conditions, radiation to move or neck, or sure therapy medicine will increase risk of cavities by reducing secretion production (Silk H, 2014).
- **Partial dental plate and wrong fillings or dental devices:** Over the years, dental fillings will weaken, begin to interrupt down or develop rough edges. This permits plaque to create up a lot of simply and makes it more durable to get rid of. Dental devices will stop fitting well, permitting decay to start beneath them.



- **GERD:** symptom or gastro gastroesophageal reflux unwellness (GERD) will cause abdomen acid to flow into our mouth (reflux), eroding the enamel of teeth and inflicting important tooth injury. This exposes additional of the dentin to attack by microorganism, making decay (Lukaes Junior and Langaespada LL, 2006).
- **Eating disorders:** eating disorder and bulimia will result in important tooth erosion and cavities. Abdomen acid from recurrent puking (purging) washes over the teeth and begins dissolving the enamel. Consumption disorders can also interfere with spit production (Mulu W *et al.*, 2014).

#### 1.4 Bacteria

The most common bacteria associated with dental cavities are the mutans streptococci, most prominently *Streptococcus mutans* and *Streptococcus sobrinus*, and lactobacilli. Cariogenic bacteria are present in dental plaque, but they are usually in too low concentrations to cause problems unless there is an imbalance. This is driven by some cause such as frequent sugar intake or inadequate tooth brushing. Adequate oral hygiene maintenance is the procedure of biofilm removal. If this is left untreated, the disease can lead to pain, tooth loss and infection.

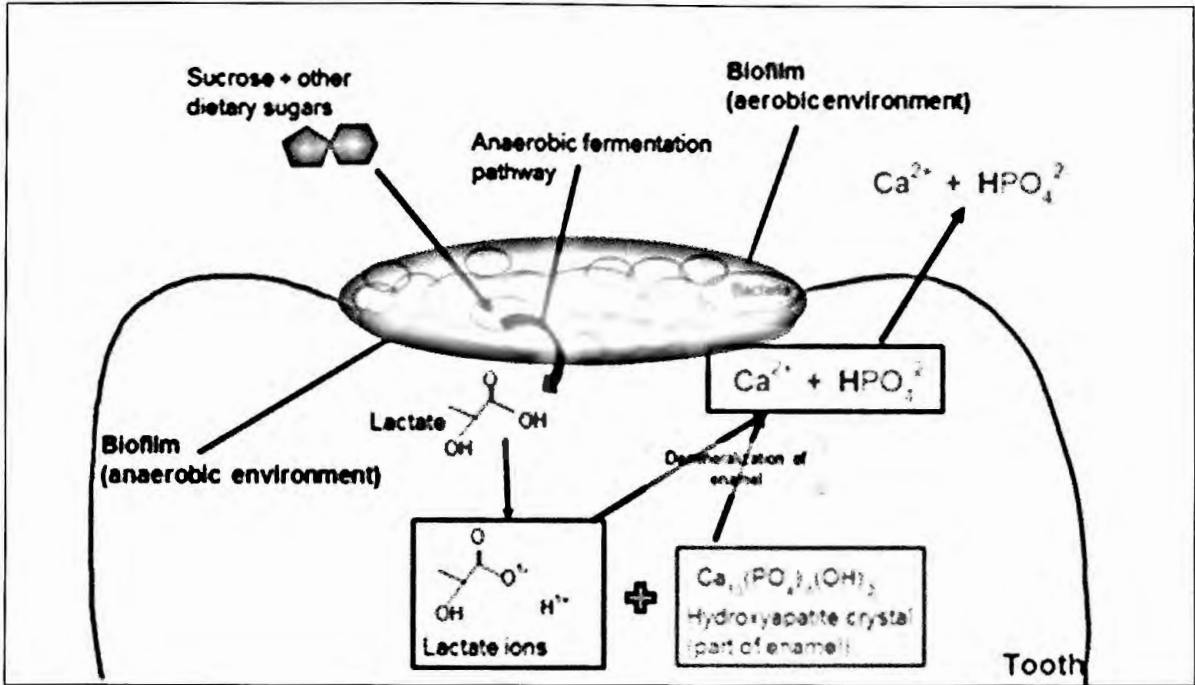
The mouth contains a wide variety of oral bacteria, but only a few specific species of bacteria are believed to cause dental caries *Streptococcus mutans* and *Lactobacillus* species among them. *Streptococcus mutans* are gram-positive bacteria which constitute biofilms on the surface of teeth. All the bacteria are not risky. Those organisms can produce high levels of lactic acid following fermentation of dietary sugars and are resistant to the adverse effects of low pH. This is the essential properties for cariogenic bacteria (Madigan MT and Martinko JM, 2006).



As the cemented of root surfaces is more easily demineralized than enamel surfaces, a wider variety of bacteria can cause root caries. So root caries is more usually cause than any other caries. The causative organisms for root caries are *Lactobacillus acidophilus*, *Antinomies spp.*, *Nocardia spp.*, and *Streptococcus mutans lactobacillus*. Bacteria collect around the teeth and gums in a sticky, creamy-coloured mass called plaque, which serves as a biofilm. Some sites collect plaque more commonly than others, for example, sites which have low rate of salivary flow (molar fissures). Posterior teeth have groove on the occlusal surfaces provide microscopic retention sites for plaque bacteria, as do the interproximal sites on those teeth. Plaque may also collect above or below the gingiva, where it is referred to as supra- or sub-gingival plaque and calculous, respectively (Hardie JM, 1982).

### 1.5 Pathophysiology of dental caries

Microbiological remnant attaches to the surface of tooth and they form a biofilm which white in colours. When the oxygen being used up then the oral environment is become anaerobic. So the biofilm grows an anaerobic environment. The sucrose and other dietary sugars are the food for the microbe. Microbe attaches to tooth surface and create a biofilm. As the biofilm grows an anaerobic environment forms from the oxygen being used up. Microbes use sucrose and other dietary sugars as a food source. The dietary sugars go through anaerobic fermentation pathways producing lactate. The lactate is excreted from the cell onto the tooth enamel then ionizes. The lactate ions demineralize the hydroxyapatite crystals causing the tooth to be degraded (Holloway *et al.*, 1983).



**Figure 1.2:** Pathophysiology of dental caries

The progression of pit and fissure tooth decay resembles 2 triangles with their bases meeting on the junction of enamel and dentin.

Teeth square measures bathed in spittle and have a coating of bacterium on them (biofilm) that frequently forms. The minerals within the onerous tissues of the teeth (enamel, dentin and cementum) square measure perpetually undergoing processes of demineralization and remineralisation. Tooth decay results once the demineralization rate is quicker than the remineralisation and there's internet mineral loss.

## 1.6 Signs and symptoms

Person experiencing caries may not be aware of the disease. The earliest sign of a new carious lesion is the appearance of a chalky white spot on the surface of the tooth, which is called the demineralization of enamel. This is referred to as a white spot lesion, an incipient carious lesion. In this time patient suffering from sensitivity. This is small size caries this can be termed as micro-cavity. As the

lesion continues to demineralize, it can turn brown but will eventually turn into a cavitation. Before the cavity forms, the process is reversible, but once a cavity forms, the lost tooth structure cannot be regenerated. A person experiencing caries may not be aware of the disease. A lesion that appears dark brown and shiny suggests dental caries were once present but the demineralization process has stopped, leaving a stain (Silverstone LM, 1983).

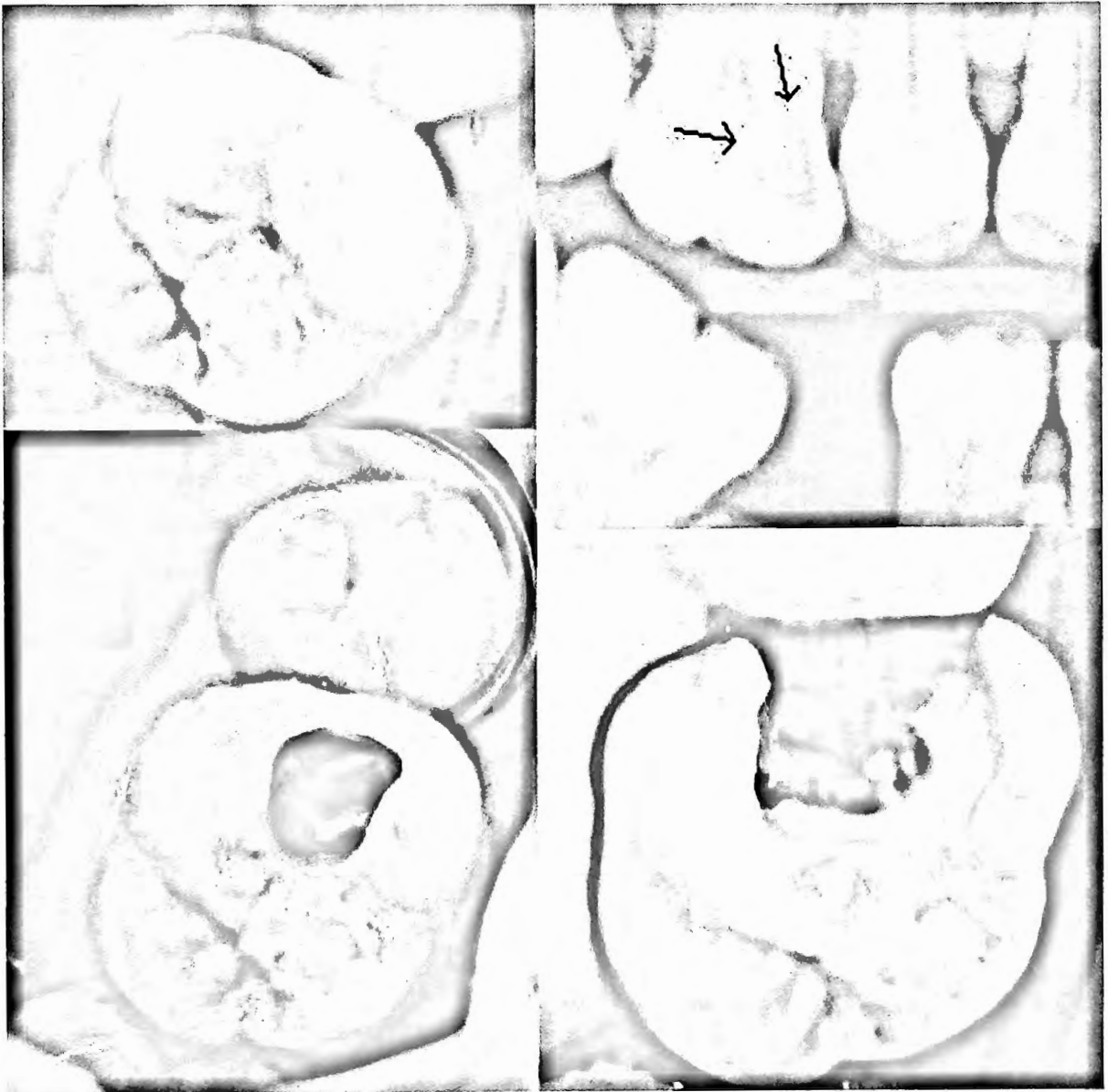


Figure 1.3: Radiological image and different types of caries.

As the enamel and dentin area unit destroyed, the cavity becomes additional noticeable. There's sensitive throughout taking hot or cold food. The affected areas of the tooth modification color and become soft to the bit. Once the decay passes through enamel, the dentinal tubules, that have passages to the nerve of the tooth, become exposed, leading to pain which will be transient, quickly worsening with exposure to heat, cold, or sweet foods and drinks. During this time the tooth is extremely sensitive. Robinson C *et al.*, (2000). A tooth weakened by intensive internal decay will generally suddenly fracture beneath traditional mastication forces. Once the decay has progressed enough to permit the microorganism to overwhelm the pulp tissue within the center of the tooth, a odontalgia may result and also the pain can become additional constant. Once this condition is sustained the death of the pulp tissue and infection area unit common consequences. The tooth cannot be sensitive to hot or cold, however are often terribly tender pressures cavity may also cause dangerous breath and foul tastes because of necrosed pulp. once a time in extremely progressed cases, Associate in Nursing infection will unfold from the tooth to the encircling soft tissues. Complications like sinus cavernous occlusion and Ludwig angina are often grievous condition. These area units the fatal cosequences of cavity (De oliveria KMH *et al.*, 2017).

### **1.7 Consequences of dental caries**

Dental caries are therefore common that therefore folks don't take them seriously. Even just in case youngsters of youngsters of kids dental caries or childhood cavity folks assume that it does not matter if children get cavities in their baby teeth. However, cavities and caries will have serious and lasting complications, even for kids and adult (Bali RK *et al.*, 2004).

**Complications of cavities could include:**

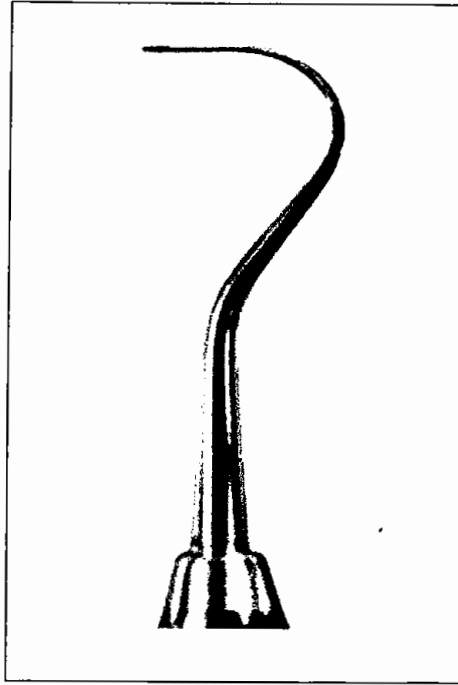
- Pain
- Tooth symptom
- Swelling or pus around a tooth
- Damage or broken teeth
- Chewing issues
- Positioning shifts of teeth once tooth loss

**When cavities and decay become severe:**

- Pain that interferes with daily living
- Weight loss or nutrition issues from painful or troublesome consumption or change of state
- Tooth loss, which can have an effect on your look, further as your confidence and vanity
- In rare cases, a tooth symptom — a pocket of pus that is caused by microorganism infection — which may result in a lot of serious or maybe grave infections, (Wong A *et al.*, 2013).

**1.8 Diagnosis of dental caries**

The presentation of caries is highly variable. It differs from person to person. But, the risk factors and stages of development are similar. At first it may appear as a small chalky area, which may eventually develop into a large cavitation. Sometimes caries may be visible in naked eye. Other methods of detection such as radiological image used for less visible areas of teeth and to judge the extent of destruction. Bitewing x-ray is now used for detection of interproximal decay (Yewe-Dyer M, 1993).



**Figure 1.4:** The tip of a dental explorer, which is used for caries diagnosis.

Primary diagnosis involves inspection of all visible tooth surfaces using a good light source, dental mirror and explorer. These are the principal instruments for caries diagnosis. It's called the clinical diagnosis. Dental radiographs may show dental caries before it is otherwise visible, in particular caries between the teeth. Large areas of dental caries are often apparent to the naked eye, but smaller lesions can be difficult to identify. Because they are invisible (Dhara V *et al.*, 2007). Visual and tactile inspection along with radiographs are employed frequently among dentists, in particular to diagnose pit and fissure caries. Uncavitated caries or hidden caries is often diagnosed by blowing air across the suspect surface. Some dental specialists have cautioned against the utilization of dental explorers to search out decay, as a result of they're not cavitated however the pressure from the dental person may cause a cavity. Since the unhealthy method is reversible before a cavity is gift, it's going to be potential to arrest decay with Fluoride and metal which may remineralize the tooth surface. However once a cavity is gift, a restoration are required to interchange the lost tooth structure (Fejerskov O *et al.*, 2008).

Caries in the pit and fissure may be difficult to detect. Bacteria can penetrate the enamel to reach dentin, but then the outer surface may remineralize, especially if fluoride is present from different source such as paste water mouth rinses. These caries, sometimes referred to as "hidden or invisible caries", will still be visible on X-ray radiographs, but visual examination of the tooth would show the enamel intact or minimally perforated (Bourgeois D *et al.*,1998).

### 1.9 Treatment of dental caries

Treatment of caries depends upon the patient's complains.

Cariou lesion	Inactive lesion		No treatment
	Active lesion	Non-cavitated lesion	Non-operative treatment
		Cavitated lesion	Operative treatment
Existing filling	No defect		No replacement
	Defective filling	Ditching, overhang	No replacement
		Fracture or food impaction	Repair or replacement of filling
	Inactive lesion		No treatment
	Active lesion	Non-cavitated lesion	Non-operative treatment
		Cavitated lesion	Repair or replacement of filling

Beltran-anguilar ED *et al.*,(2005)

**Prevention:**

It is said that prevention is better than cure. The main emphasis of preventive dentistry is on selecting priorities for the expenditure of efforts and resources for primary preventive services. The services are presented in the following outline.

**I. Primary preventive services provided within the community**

- A. Community water fluoridization.
- B. Faculty water fluoridization.
- C. Fluoride supplements programs.
- D. Fluoride mouth rinse programs.
- E. Faculty sealing material programs.

**II. Primary preventive services provided by the dental skilled**

- A. Skilled topical Fluoride applications.
- B. Pit and Fissure sealants.
- C. Diet substance.
- D. Plaque management programs.
- E. Caries activity tests.

**III. Primary preventive services provided by the individual.**

- A. Fluoride dentifrices.
- B. Self-applied topical Fluoride merchandise.
- C. Oral hygiene practices (Dr. Tirthankar Debnath, 2002).



### Dental caries: Individual, community, and dental professional preventive dentistry services

Levels of prevention	Primary		Secondary	Tertiary	
	Health promotion	Specific protection	Early diagnosis and prompt treatment	Disability limitation	Rehabilitation
Services provided by the individual	Diet planning; demand for preventive five services; periodic visits to the dental office	Appropriate use of fluoride ingestion of sufficient fluoridated water Appropriate use of fluoride prescriptions. Use of a fluoride dentifrice. Oral hygiene practices	Self-examination and referral; use of dental services	Use of dental services	Use of dental services
Services provided by the community	Dental health education programs; promotion of research efforts; lobby efforts	Community or school water fluoridation; school fluoride mouth rinse program; school fluoride tablet program; school sealant program	Periodic screening and referral; provision of dental services	Provision of dental services	Provision of dental services
Services provided by the dental professional	patient education; plaque control program; diet counseling; recall reinforcement; dental caries activity tests	Topical application of fluoride; fluoride supplement/ rinse prescription; pit and fissure sealants	Complete exam; prompt treatment of incipient lesions; preventive resin restorations; simple restorative dentistry; pulp capping	Complex restorative dentistry; pulpotomy; root canal therapy; extractions	Removable and fixed prosthodontics; minor tooth movement; implants

(Bhaskar Dj *et al.*, 2011 and Dr. Tirthankar Debnath2002).

## 1.10 Drinking water

Drinking water additionally referred to as potable water is water that's safe to drink or to use for food preparation. The quantity of beverage needed to take care of healthiness varies and depends on physical activity level, age, health connected problems, and environmental conditions community water systems acquire water from 2 sources surface water and water (Featherstone JDB2004). Surface water is water that collects on the bottom or in an exceedingly stream, river, lake, reservoir or ocean. In line with EPA, sixty eight of community water system users received their water from a surface (Almedon AM *et al.*, 2009).

### 1.10.1 Drinking water and oral health

The benefits of water are wide noted, particularly once it involves our bodies. The us is one in all the few counties that adds Fluoride to its water. Fluorides is "natural cavity fighter" and supply several edges to our teeth (Barrard WR and Wordstrom DK1982). Fluoride mixes with enamel in developing teeth and helps forestall caries. It conjointly works with spittle to stop plaque. It is a very little noted proven fact that caries thrives on dry mouths spittle truly defunds caries, particularly the minerals in spittle like Ca and phosphate. Water helps forestall against dry month by making a lot of spittle to stay caries away (Damle SE and Patel AR, 1994).

## 1.11 Types of water source in upazilla level of Rajshahi region

There are a unit 2 main supply of water spring water and surface water. "Water source" are often accustomed mean each the origin of the water and additionally the place wherever folks get their water (Piped offer to family faucet, water purpose, well etc.). Water sources are often classified as protected or unprotected. Protected supply area unit lined by stone work, concrete or different materials that forestall

the entry of physical, chemical and biological contaminants. Unprotected sources are unit those with no barrier or different structure to guard the water from contamination. All surface water sources like Lakes Rivers and streams or poorly created wells, are unit samples of unprotected sources (USEPA, 1982). Water from unprotected sources can't be thought-about safe to drink unless it's been treated surface water is at all-time low of the laden and piped water into the family is at the highest. There are unit members of improved and unimproved sources between these 2. Different improved sources are unit public faucets or shorebird, tube wells or boreholes, improved mamma wells, improved springs, fresh water assortment. Unimproved sources are unit unimproved mamma well, cart with little tank/drum, drinking water. Surface drink sources are unit stream, dam, lake, pond, stream, canal, irrigation channels (Fitzgerald J *et al.*, 2000).

### **1.12 Groundwater**

Groundwater was outlined as water that's found underground inside rocks. Its presence depends totally on the kind of rock. Leaky rocks have small areas between the solid rock particles that enable rock particles that enable water and different squids to labor under and to be control inside the rock structure. The layers of rock that hold groundwater is taken from and also the kinds of leaky rock it's saw are vital factors are vital through are vital factors that have an effect on its quality. Groundwater, significantly from deep sources, my give water of fine microbiological quality (Usepa 1985).

### **1.13 Wells and boreholes**

Wells and boreholes is delineate by their depth, or by the approach they're made. They'll additionally use differing types of pump at the surface to lift the water.

### 1.14 Shallow wells

Shallow wells and boreholes typically have a depth of but 30m, though they'll be the maximum amount as 60m deep. Wells are often excavated by hand if the soil isn't too onerous or the groundwater level is high droop mammary gland wells have a comparatively giant diameter because they need to be wide enough for an individual to be able to stand within and dig (NTP, 1990).

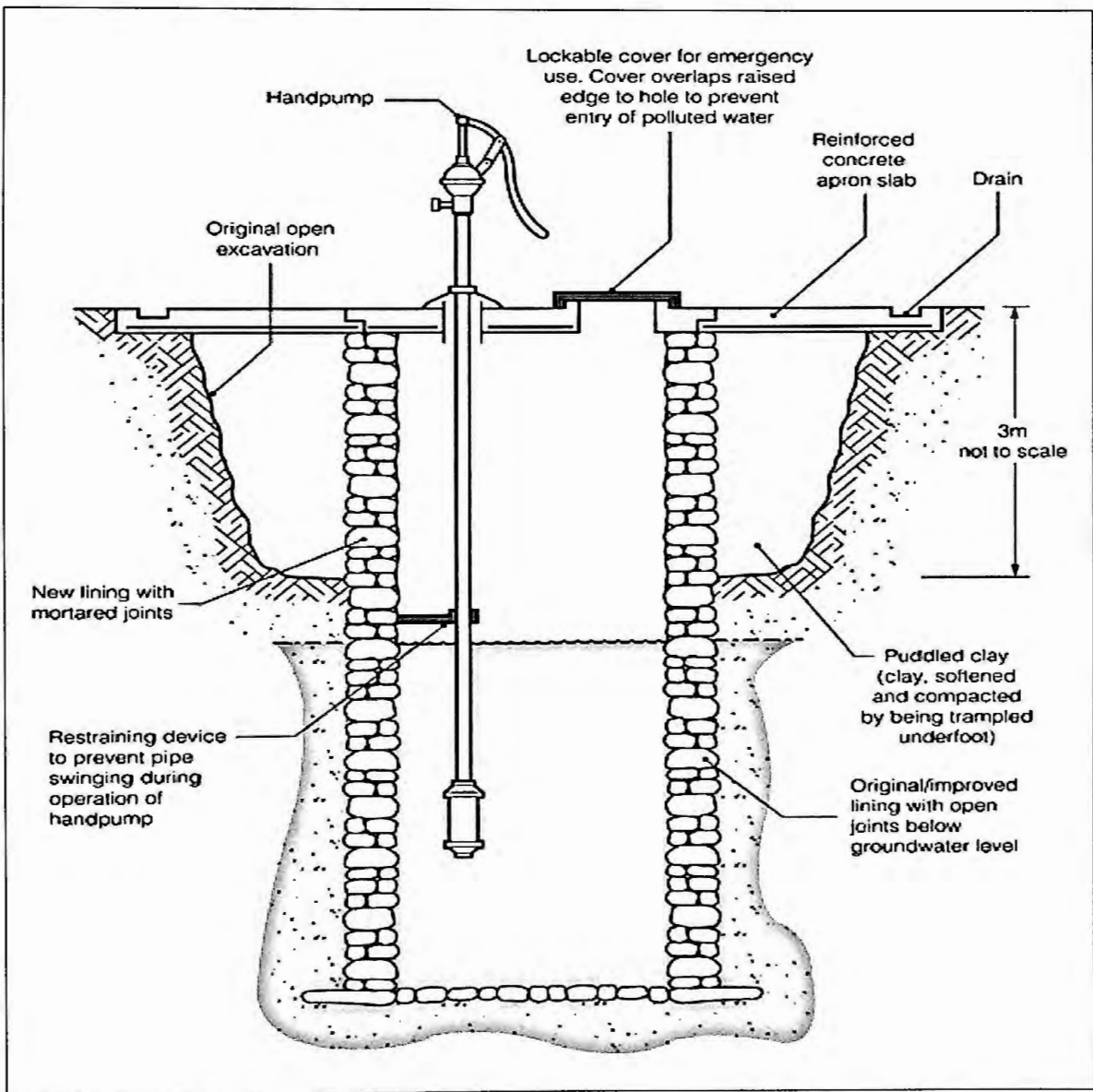


Figure 1.5: Diagram of a hand-dug well.

### **1.15 Surface waters**

The quality and amount of surface water varies from one place to a different and over time, because of factors like earth science, climate and close land use. The variable quality of surface water means that it's to be treated to form it safe for domestic consumption (Oludairo O and Aiyedun J, 2016). There square measure many completely different ways of water treatment at giant and tiny scale. The amount of water in rivers and lakes clearly varies with precipitation and there are often wide fluctuations at completely different times of year. To confirm year spherical provide, dams are often created to make reserve from that water are often extracted before treatment (Pip E, 2000).

**CHAPTER II**  
**REVIEW OF LITERATURE**

## REVIEW OF LITERATURE

Water fluoridization is that the controlled adjustment of Fluoride to a public water system to scale back dental caries. Fluoridated water contains Fluoride at tier that's effective for preventing cavities; this may occur naturally or by adding Fluoride (Bagramian RA *et al.*, 2009). It prevents dental caries by a minimum of 25% in each kids and adults just by drinkable (Chen EJ *et al.*, 1988). The North American nation in 2015 lowered the counseled quantity of Fluoride in drinkable provides, from up to 1.2 miligrams per liter' chiefly to chop down on potential harm to teeth. North American nation additionally defines 0.7 miligrams per litre because the optimum level of fluoridization (Choubisa SI *et al.*, 1997). The U.S centers for unwellness management and bar maintains that community water fluoridization is safe and in 1999 even topped it one among the 10 greats Public health achievements of the 20<sup>th</sup> century. In 2000, German researchers reportable that dental caries fell in cities wherever Fluoride ceased to be further to the water. The department of Health and Human Services (DHHS) sets the optimum level of Fluoride for preventing dental caries at 0.7 ppm or 0.7 miligrams (mg) in each litre of water. In 2015, it absolutely was maintained to the lower limit. Water authorities add Fluoride to the municipal water system, as a result of studies have shown that adding it in square measure as wherever Fluoride levels within the water are law will scale back the prevalence of dental caries within the native population. In 2017 a report was revealed suggesting that exposure to Fluoride before birth may lead to poorer psychological feature out comes within the future (Dean HT, 1942). The U.S National Academy of Sciences Institute of medication has counseled and adequate intake of Fluoride from all Sources as 0.05 mg\lkg weight in a very day outlined because the estimate in take that has been shown to scale back the prevalence of

decay maximally in a very population while not inflicting unwanted aspect effects together with moderate dental pathology. The U.S Environmental Protection Agency sets a most stuff level of 4.0 mg/litre to shield against skeletal pathology, and a Secondary worth of two mg/litre to shield against moderate to severe dental pathology (Daida Y *et al.*, 2006). The WHO's drinkable quality Guideline worth for Fluoride is 1.5 mg/litre. United Nations agency firms that in setting national standards for Fluoride it's notably necessary to contemplate Climate conditions, volumes of water intake of Fluoride from alternative souses (e.g food and air) (Jolly SS *et al.*, 1968).WHO additionally noted that in areas with high national Fluoride levels the rule of thumb worth could be tough to attain in some circumstances with the restricted technology grailable.

## 2.1 Fluoride in drinking water

Fluoride may be a common component that doesn't occur within the elemental state in natural thanks to its high reactivity. It reacts to alternative component terribly straightforward. It happens for regarding 0.3g/kg of the world is crust and exists within the variety of fluorides in an exceedingly variety of minerals of that mineral, cyolite and fluoro mineral area unit the foremost common. This is often the first state of Fluoride (Janseen PJCM *et al.*, 1988). The number of the Fluoride particle is – one (Liang C *et al.*, 1997). Levels of daily exposure to Fluoride rely primarily on the geographical region. Daily intakes of Fluoride vary wide in line with the assorted sources of exposure values starting from 0.46 to 3.6 - 5.4 mg/day are according in many steadier, IPCS (1984) be considerably higher, IPCS (2002). As an example in some countries in China wherever coal features high Fluoride content and is burned in homes with poor ventilation, the common daily intake of Fluoride ranged from 0.3 to 2.3 mg via air and from 1.8 to 8.9 mg via food. They need some downside (Cao SR *et al.*, 1992 and Sloff W *et al.*, 1988). In areas with



comparatively high Fluoride concentration in groundwater, water becomes progressively vital as a supply of Fluoride, there concentration is extremely high (Fuhong R and Shuqin J, 1988). Many medical specialty Studies of attainable adverse effects of future consumption of Fluoride via water.

These studies clearly establish that Fluoride via water are administrated (Ingle metallic element *et al.*, 2014). These Studies clearly establish that Fluoride primarily produces effects on skeletal tissues (bones and teeth). Low concentrations give protection against tooth decay, particularly in youngsters. Professional cluster on vitamins and minarals (2003). The Pre and post eruptive protecting result of Fluoride (involving the incorporation of Fluoride into the matrix of the tooth throughout its formation, the event of shallower tooth grooves, that one consequently less susceptible to decay and surface contact with enamel increase with concentration up to regarding a pair of 2 mg of Fluoride per/litre of water, the minimum concentration of Fluoride in water needed to supply it's or so 0.5mg/litre. Thus vary of safety is 0.5-2 mg/litre (FAO/WHO, 2001). Fluoride may also have Associate in Nursing advance result on enamel and will produce to delicate dental pathology (Prevalence: 12% - 33%) at water concentrations between 0.9 and 1.2 mg/litre (Dean HT, 1942).The period of greatest condition is at the time of mineralization of the secondary higher central tooth at regarding 22-26 months aged. This has been confirmed in various resultant studies, together with a recent massive Scale survey administrated in China (Chen EJ *et al.*, 1988).Which showed that, with water containing 1 mg of Fluoride per/litre, dental pathology was detestable in 46% of the population examined (Anomy M, 1990).

The extent of exposure from food wasn't clear in these Studies. Normally dental pathology doesn't occur in temperate areas at concentrations below 1.5-2 mg of

Fluoride per/litre of water. In hotter areas, thanks to bigger amounts of water consumed, dental pathology will occur at lower concentration within the water (IPCS, 2002; US independent agency, 1985; CaO SR and Li *et al.*, 1992). It's attainable that in areas wherever Fluoride in take via alternative routes aside from water (e.g, air, food) is elevated, dental pathology can develop at concentration in water below 1.5 mg/litre (CaO SR *et al.*, 1992). The results of many medical specialty Studies on the attainable adverse effects of Fluoride in water on physiological state outcome indicate that there no apparent relationship between the rates of slowness on innate malformation and also the consumption of fluoridated water (IPCS, 1984 & 2002; USA independent agency, 1985 and Janssen PJCM *et al.*,1988). cavity happens once bacterium within the mouth (Plaque) break down sugars in food and manufacture damaging acid that dissolve the laborious enamel surface of teeth (Hiremath A *et al.*,2016). The great news is that there are units proved ways to stop cavities. As an example adding Fluoride to a community's water is area unit of the simplest and most price effective strategies of protective youngsters and adults from cavity (Homa Amini, 2015). The U.S centers for sickness management and bar lists water fluoridization in concert of "10 nice Public health achievements of the 20<sup>th</sup> century." In the primary population level study of its kind within the world, researchers have found that fluoridated water is preventing cavity for all adults notwithstanding age and considerably for folks that have had exposure to Fluoride for many of their lives. Once Fluoride levels in water area unit at optimum levels it helps to guard teeth against cavities. This is often why such a large amount of community water systems add further Fluoride a method referred to as "fluoridation" (Ando M *et al.*,1998).

A study conducted by the Australian analysis center for population oral Health (ARCPOH) at the university of Adelaide's college of dental medicine, the study

adds to the established proof that Fluoride in water has dental health edges for youngsters (Liu JW, 1987). Previous studies had incontestable fluoridated water provided dental edges to youngsters, however researchers have shown for the primary time that it conjointly helps stop cavity in adults (Elena Elisseeva, 2013).

Those folks that have had longer exposure to Fluoride in water clearly can have the bigger profit academic Roberts Thomson (2013).

## 2.2 Benefit of fluoride in drinking water

- 1. Prevents decay:** Water addition is that the best thanks to forestall one in all the foremost common childhood diseases tooth decay. Associate calculable 51 million college hours and 164 million work hour's square measure lost every year because of dental connected malady (Joshi N *et al.*, 2013). Community water addition is thus effective at preventing decay that the centers for sickness management and bar named it one in all 10 nice public health achievements of the 20<sup>th</sup> century (Mahesh KP *et al.*, 2005).
- 2. Protection against cavities:** Studies show that Fluoride in community water Systems prevents a minimum of 25% of decay in kids and adults .fluoride from different sources, like Fluoride tooth paste, mouth rinses, pill are obtainable (Liang C *et al.*, 1997).
- 3. Safe and effective:** 04-70 years, the simplest obtainable scientific proof systematically indicator that community water addition is safe and effective (NPT, 1990). it's been supported by various U.S. Surgeons General and over one hundred health organization acknowledge the health advantages of water addition for preventing dental decay, as well as the centers for sickness management and bar, the American medical Association, the World Health Organization, the American Dental Association and therefore the American Academy of medicine (Li Y, 2001).

4. **Cash saver:** The typical life time value per person to fluoridize water is a smaller amount than the price of 1 dental filling (Tewari A and Chawla HS, 2002). In each massive city 20 Bangladeshi monetary units endowed in water addition saves 3000 taka in dental treatment prices. The price of dental treatment goes out of hands of individual's day by day (Marsh metal *et al.*, 2015).
  
5. **Nature element:** Fluoride is of course gift in groundwater and therefore the oceans water addition is that the adjustments of Fluoride to a counseled level for preventing decay. It's just like strengthening salt with iodine milk with vitamin D, fruit crush with Ca and bread with pteroylglutamic acid (Moures JK, 1990). Fluoride is offered in nature however isn't usable. The foremost dramatic reduction in prevalence of cavity with addition has been found among those that have drunk the fluoridated water all this lives. However all the foremost studies conjointly show that some reduction happens once fluoridated water is eaten for under a part of the amount of tooth calcification. Addition conjointly reduces caries within the teeth and therefore the helpful effects are shown to be womb-to-tomb instead of just to delay the onset of caries (Murry JJ, 1986).

### 2.3 Mechanism of action of Fluoride in reducing cavity

Enamel deposition and calcification of Fluoride in blood → Enamel maturation  
Fluoride in tissue fluid.

The concentration of Fluoride in enamel ↑ with ↑ Fluoride content of facility towards the surface of enamel (Murry JJ *et al.*, 1986).

### 2.3.1 Pre-eruptive effects

**Enamel shaped within the presence of Fluoride has:**

- Improved crystallinity and ↑ crystal size, and so and so Solubility.
- A lot of rounded cusps and fissure pattern however result little.
- Termination of general Fluoride ends up in ends up in in dental caries, so pre-eruptive effects should be restricted (Ralphr *et al.*,1971).

### 2.3.2 Post-eruptive result

**Newly erupted teeth derive the foremost benefit:**

- Inhibits demineralization and promotes remineralization of early dental caries. Fluoride enhances the degree and speed of remineralization and renders the remineralized enamel a lot of resistance to succeeding attack.
- Decrease acid production in plaque. A drop by pH ends up in ends up in associate ionic Fluoride that augments this action.
- An ↑ concentration of Fluoride in plaque inhibits the synthesis of animate thing saccharide. It's been recommended that Fluoride affects investment and plaque formation however this can be uncorroborated (Kidd EAM, 2004).

## 2.4 Calcium in drinking water

Calcium in both ionised and non-ionised form is arguably one of the most important body components such as teeth and bones. Teeth, the essential structures for the initial processing of food are made up of dental enamel and dentine, whose hardness and fitness for purpose is again due to the crystals of calcium phosphate of which they are composed (David R. Ferguson *et al.*,1999).

The retention of the teeth within the jaws depends upon the anchoring of dentistry fibers within the firm calcified tissues of bone and solid body substance (Geol R *et al.*, 2015). Dietary intake of metallic element is usually around 1.0g/day (25mmol/ day). It's derived from milk and farm merchandise and alternative sources are comparatively minor inorganic phosphate could be a supporting substance and it Causes bone and tooth growth in conjunction with aliment (Sjors H and Gunnarsson U, 2002).The metallic element concentration of water varies from one to 1-135mg/l across the USA and North American country. Most Surface waters were found to possess comparatively low metallic element concentrations with a median of 21.8 mg/l. pure water contain a negligible metallic element concentration. On the premise of the findings of the World Health organization meeting of consultants command in Rome, European country in 2003 to debate nutrients in water.

The present cluster targeted its thought on Ca that next to Fluoride proof of health edges related to skinny presence in beverage is strongest (Bell RA *et al.*, 2002). Water security and safely is of important concern in arid and dry regions all told over the World. Potable water accessibility and provide is restricted because of unsteady environmental condition condition and environmental pollution that longer the complete sameness of should water sources (Abok Elisha *et al.*, 2018). Many of us struggle to get access to safe water. Clean and treated water to every house is also the norm in Europe and North America. Over ninety 99% of total body Ca is found in bones and teeth, wherever it functions as a key structure part (Masironi *et al.*, 1976). The remaining body Ca functions in metabolism, serving as a proof for important physiological processes, together with tube contraction, coagulation shortening and nerve transmission. Inadequate intakes of Ca are related to inflated risks of pathology, lithiasis (Kidney Stones), body part cancer, high blood pressure and stroke, arteria illness, endocrine resistance and fat further as bone and tooth deformity. Most of those disorders have treatments however no cures. as a result of

lack of compelling proof for the role of calcium Ca atomic number 20 metallic part metal as one tributary element in reference to these discuses, estimates of Ca demand are created on the premise of bone health outcomes, with the goal of optimizing bone mineral density. Ca is exclusive among nutrients, within the body's reserve is additionally purposeful (Akhter P *et al.*, 2004).

- 1. Fracture of bone:** It's the hidden killer of bone. Pathology could be a condition of skeletal fragility characterised by low bone mass and by micro-architectural determination of bone tissue, with a resultant increase in risk of fracture. It results in multiple fracture of bone. Ca is that the largest constituent of bone comprising 32% by weight (Charlton KE *et al.*, 2005). an outsized body of primary proof from irregular controlled frails shows that increasing Ca intake, particularly in people who have had routinely low Ca intakes, will increase bone mass throughout growth and reduces bone loss and fracture risk late in life. Pathology is one in every of the foremost rife aged connected illness (Galan P *et al.*, 2002).
- 2. Nephrolithiasis:** The link between Ca intakes and therefore the incidence of renal calculus depends on whether or not Ca is consumed with food of singly. Ca that reaches the lower tiny intestines truly defend against renal calculus by binding ethanedioic acid (Morr S *et al.*, 2006). Ca eaten from water along with food would have an equivalent impact. Medicine proof is robust that dietary Ca reduces the incidence of urinary organ stones (Li Y, 2001). In distinction, the results of an outsized irregular trial recommend AN inflated risk of urinary organ stones go along with Ca supplements, presumably as a result of the Ca wasn't eaten with food or the supplements were taken by people who exceeded the higher level of 2500 mg/day (Yang CY, 1998).

3. **High pressure and Stroke:** High pressure (hypertension) could be a risk issue for many illnesses. It will happens mealt illness, obesity, drug resistance etc. it's a vital ill health particularly in developed countries and developing countries. Individuals in developing countries square measure suffer a lot of (Charlton *et al.*, 2005). Though high blood pressure is multi factorial in origin. Adequate Ca intake has been related to down risk of elevated pressure in some, however not all told studies. A transparent mechanism has not been known. Farm products over Ca are related to reduce pressure in prospective studies and with reduced risk of stroke in prospective Studies (Reid IR *et al.*, 2002). It is a brand new era of treatment of high blood pressure and stroke.
4. **Endocrine resistance:** endocrine resistance is related to kind 2 diabetes, the prevalence of that is escalating with the chance in fat worldwide (Karssebaum American state *et al.*, 2017). Dietary Ca is also involved within the etiology of endocrine resistance through the fluctuations in Ca control hormones in states of Ca insufficiency and deficiency. This is often a district of active research; therefore it's premature to use such a clinical outcome because the basis for account recommendations for dietary intake of Ca.

## 2.5 Microbiological study of drinking water

Water microbiology is the concerned with the study of all biological aspects of the micro-organisms (bacteria, viruses, fungi, parasites and protozoa) that exist in water.

Bacteriological water analysis could be a technique of analyzing water to estimate the numbers of bacterium gift and if required to seek out what kind of bacterium they're (Gangil R *et al.*, 2013). It's a microbiological analytical procedure that uses



samples of water and from these samples determines the concentration of bacterium. It's then potential to draw interferences regarding the quality of the concentration of bacterium (Almedon AM *et al.*, 2009). Microbiological water analysis in the main is especially is principally supported the construct of feculent indicator bacterium gift in water are mainly feculent coliform.

The presence of feculent coliform bacterium in adequate environments indicator that the water has been contaminated with the feculent material of man and different animals. Feculent coliform bacteria indicate the presence of sewerage contamination of a waterway and therefore the potential presence of unhealthful organisms (APHA, 1992). Feculent coliform is then potential to draw interferences regarding the quality of the water to be used from this concentration. Microbiological water analysis is especially supported the construct of feculent indicator bacterium (Balogun SA *et al.*, 2014).

The main bacterium gift in human and animal faeces (focusing on their behavior within their hosts and in the environment). Routing basic microbiological analysis of water ought to be dole out by assaying the presence of *E. coli* by culture ways.

**Fecal coliform:** Facial indicators are a gaggle of organism that demonstrates the efficaciousness of a method. A feculent coliform could be a facultative anaerobic, rod shaped, gram –negative, non-sporulation microorganism. Coliform bacterium usually originates within the intestines of heat –blooded animal (Anony M, 1990).

These are those organisms that indicate the presence of feculent contamination. There are completely different enteric micro-organisms that are proverbial to infect humans (Olajire AA and Imeokparia metallic element, 2001). Enteric microorganisms are excreted within the faces of infected people or animals and will

directly or indirectly contaminate water used for human consumption. On the opposite hand acceptable medical care practices by water utilities, drastically cut back the incidence of water bone malady and maintain the standard of water (Boubeta A *et al.*, 2011 and Radhakrisna *et al.*, 2003).

### 2.5.1 Factors affecting fecal coliforms contamination:

Fecal coliforms are mostly available in human waste, so the bacterium are found and goes down the drains of business and can enter stream through leaky sanitary sewer connection.

- **Waste of animal:** unclean coliform is discharged within the wastes product by animals. This could be a significant drawback in geographic area.
- **Sedimentation load:** terribly high quantity of sediment are usually associated with high concentration of moribific bacterium.
- **Higher temperature:** bacterium grows quicker at higher temperatures than lower temperature.
- **Nutrient:** Nutrient at higher rate will increase the expansion rate of bacteria (Mr. Brain Oram, 2020).

**CHAPTER III**  
**MATERIALS AND METHODS**

# MATERIALS AND METHODS

## 3.1 Study protocol

Dental caries could be a damaging unwellness of the onerous tissues of the teeth and there's sensible proof that it's initiated by acid created by the fermentation of macromolecule substrates by bacterium in bacterial plaque. The role of other factors, such as chelation and bacterial enzyme action, is somewhat controversial, but they are probably of little significance in the etiology of enamel caries. A number of methods have been shown to be at least partly effective in preventing dental caries. The most effective method, at least on a community basis, is water fluoridation. Other methods involve diet, oral hygiene, restorative dentistry, or the topical application of tissue sealants or preventive agent of the teeth. Nutritional influences are greatest during tooth development. Apart from fluoride and possibly other trace elements such as calcium, strontium, molybdenum, and others. Deficiency of this nutrient cause delay in tooth eruption and may lead to slightly increased susceptibility to caries studies of caries free recruits have shown that a high proportion of the come from geographic areas with drinking water containing relatively high level of nutrients fluoride, calcium, Strontium, molybdenum, lithium and others. There is evidence that the method of cooking or preparation of vegetables has a major effect on the consumption of trace elements.

## **3.2 Objectives**

### **3.2.1 General objective**

1. To detect the different factors in dental caries Patient.

### **3.2.2 Specific objectives**

1. To measure Fluoride content in drink employed by patient decay of various Upazilla of Rajshahi Region.
2. To measure calcium content in drinking water used by patient of dental caries of different Upazilla of Rajshahi Region.
3. To assess microbiological analysis of drinking water of different Upazilla of Rajshahi Region.

## **3.3 Material and methods**

**Type of study:** It is a cross sectional descriptive type of study. Here no control group and no comparison are made.

**Study Population:** Diagnosed case of dental caries, age between 3 to 60 years of old, attending Rajshahi Medical College and different Upazilla health complex of Rajshahi region.

**Sample size:** The sample size of this Study was 119.

**Determination of sample size:** The sample size was determined by using the following formula:

$$\text{Sample size, } n = (z^2 pq/d^2)$$

Now, for the present study,

$$z = 1.96 \text{ at } 95\% \text{ of CI}$$

$$p = 0.86 \text{ (prevalence of dental caries } 86\%) \text{ SS Rahman } et \text{ al., } 2010 \\ \text{(Bangladesh Medical Journal } 2010, 43; 1-2)$$

$$q=0.09 \quad d = 0.05$$

$$n = \{(1.96)^2 \times (0.86)(0.09)\} / (0.05)^2 \\ = (3.8416 \times 0.86 \times 0.09) / 0.0025 \\ = 118.9359 \text{ so total sample size is } 119.$$

**Sampling technique:** Purposive sampling technique was followed to recruit subjects by exploitation the inclusion criteria.

### 3.4 Place of study

Laboratory tests were performed in Asian nation Council of Scientific and Industrial analysis (BCSIR) Laboratories, Rajshahi and Central research laboratory, Rajshahi University. Microbiological checks were conducted in microbiological Laboratory in Rajshahi Medical faculty.

**Study Period:** As directed by Institute of bioscience (IBSc).

**Sample:** Drinking water.

**Selection of Patient:** Patient with decay in their Permanent and deciduous teeth as a result of decay are common in each kids and adults. Throughout my analysis amount, 540 patients (male, feminine and children) were examined and enclosed within the study. Male were a 180, feminine were 220 and kids were 140 which aged in between 3-60 years recent.

### 3.5 Selection criteria

#### 3.5.1 Inclusion criteria

- Patient came in the outdoor in Rajshahi Medical College and different Upozilla health complex of Rajshahi region.

#### 3.5.2 Exclusion criteria

- Patient refuse to participate in the study.
- Patient have apical lesion in the affected tooth.

### 3.6 Data collection

Data were being obtained with the help of questionnaire after getting informed and consent from the patient/guardian. All relevant information and laboratory findings were recorded in a predesigned datasheet.

### 3.7 Questionnaire

Serial No: <input type="text"/>	Date:...../...../.....
Interviewer's Name: Dr.....	
Interviewer's Designation: .....	
Interviewer's Institution: .....	
	.....
Name and Address of Patient Attending Hospital / Clinic / Health Care Center:	
.....	

*Consent Form*

My name is ..... I am informed that a study is conducting on factors affecting dental caries under the institute of Biological Sciences of Rajshahi University.

I have understood the importance of the research works. It is assured that all the information. I provided will be kept secretly, no name is needed to mention in the research paper and no mental or physical stress will be applied. Under the circumstances I, being fully aware of agree to contribute on voluntary basis.

1. Patient's Signature :..... Date: .....

Patient's Name: .....

Patient's Address: .....

.....

Patient's Mobile No. ....

Husband / Father / Mother Mobile No.....

2. Interviewer's Signature: ..... Date: .....

Digital (TMJ, Major Salivary glands, lymph nodes, any other)-  
.....  
.....

Intraoral Examination (I.O.E): Oral malodour.....

Fraeunm- Labial.....Buccal ..... Lingual.....

Occlusion: CI-I.....CI-II div.....CI-III.....

Calculus: Subgingival: ..... Supragingival.....



Caries:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Tooth Hypersensitivity:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Attrition: ..... Abrasion: ..... Erosion: .....

Overhanged Restorations:

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Prostodontics appliance/s: .....

Orthodontic appliance/s: .....

Investigation/s: .....

.....

**Differential Diagnosis:**

1) .....

2) .....

3) .....

Diagnosis:.....

### Patient Examination

**Chief complain/s:**

- 1. ....
- 2. ....
- 3. ....

**History of Present Illness:**

- 1. ....
- 2. ....
- 3. ....

**Past Dental History:**.....

**Medical History:** Rheumatic fever, Hospitalization, Hypertension, Diabetes, Hep. B/C, Jaundice, Bleeding tendencies, Allergy to drugs, Convulsions, Thyroid, Asthma, other.....

**Somoking:**..... pck/yr Smokeless tobacco:..... Pan/Betel unt:.....

**Tooth Brushing:** Tool.....Frequency.....Time.....

**Technique**.....**Remarks**.....

**Vital signs:** Pulse...../min. Resp. rate.....br./min Temp.....°F B.P.....mm/Hg

**Extraoral Examination (E.O.E):**

**Visual** (appearance, facial symmetry, lip competency, scar, swelling, discoloration, any other).....

.....  
.....

**Food Habit**

Vegetarian

Non-vegetarian

**a) Drink fluoride containing water**

Yes

No

**b) Use of Fluoride containing salt during cooking**

Yes

No

**c) Have water fluoridation**

Yes

No

**Amount of Diet Taken per Day:** Vegetables:.....g, Fruits:.....g,

Fish:.....g, Milk:.....mL, Fast Food: .....g, Water: .....ml,

**Information about OHE:**

**1. Procedure of maintaining OH:**

a) By toothpaste and tooth brush

b) By tooth powder and toothbrush

c) Other's

**2. Toothpaste/Toothpowder:**

a) Containing Fluoride

b) Having no fluoride

c) Other's

**3. Regular visit to Dentist:**

- a) 6 months interval
- b) 1 year interval
- c) During any problem.

**Sample Collected from Patient:**

- Drinking Water

**Examination of Chemical Parameter**

Fluoride Content in Drinking water

Calcium content in drinking water

**Examination of Biochemical Parameter**

Fecal coliform in Drinking Water

### **3.8 Collection of sample**

At first dental caries is diagnosed by radiograph. Then patient was asked to bring drinking water in a bottle from the permanent resident or patient lived there since childhood. The bottle was labelled with patient name, ID number and transferred to the laboratory.

#### **3.8.1 Collection of sample from tap (Monica Cheesbrough, 2012)**

1. All external fittings (anti-splash nozzle or rubber tube) were far away from the faucet.
2. The surface nozzle was clean within the faucet.
3. The faucet was turned up full, and allowed the water to run to waste for 1 minute. This permits time for the nozzle of the faucet to be flushed and any stagnant water within the service pipe to discharge.
4. The faucet was sterilized by victimisation the flame of a blowtorch or gas torch, or by igniting a bit of cotton soaked in denatured alcohol and holding it with a try of pair of tongs about to the nozzle till the entire faucet is unbearably hot to the bit.
5. The faucet was cooled by running the water to waste for a number of seconds.
6. The sample bottle was stuffed from a delicate flow of water, and replaces the cap of the bottle.
7. The bottle was numbered by victimisation water-proof marker or grease pencil, variety the bottle with the sample code variety.

**3.8.2 Collection of sample from a tube well (Monica Cheesbrough, 2012)**

1. Continuously operating the hand pump for 5 minutes.
2. Heating the mouth of the pump, preferably by means of a blow lamp or gas torch and pump several gallons of water to waste.
3. Aseptically collecting a sample of water by allowing the water from the pump to flow directly into the sterile bottle. Carefully replace the bottle cap and cover.
4. The bottle was labeled with the sample code number.

**3.8.3 Collection of sample from an open well (Monica Cheesbrough, 2012)**

In the well is one from that water will be raised solely by means that of a bucket and cord use a weighted bottle to gather the sample as follows:

1. The sterile sample bottle was tied on to a weighted length of rope or robust string. Employing a stone or significant piece of metal as a weight, and also the bottle was hooked up simply higher than the burden.
2. Aseptically the faucet was removed the cap from the bottle, and lowering the bottle into the well to a depth of concerning 1 meter.
3. Once no additional air bubbles rising to the surface raise the bottle out of the well and punctiliously replace the cap.
4. The bottle was tagged with the sample code range.



**Figure 3.1:** Drinking water collected from patient

### 3.9 Method and methodology

**Laboratory produce:** Chemical measuring of Fluoride in potable employed by patient caries of various upozilla of Rajshahi region.

#### 3.9.1 SPADNS method: (According to WHO guideline)

The SPADNS (trisodium 2-parasulfophenylazo-1,8-dihydroxy-3,6-naphthalene disulfonate or 4,5 —dihydroxy-3-paraphenylazo-2,7-naphthalenedisulfonic acid trisodium salt) methodology of determination of Fluoride in potable could be a straightforward and a fast technique with high accuracy. It is applied on to most water samples while not previous pre-treatment, and isn't terribly sensitive to the opposite ions that square measure sometimes found in potable water. The chemical agent utilized in this methodology i.e. SPADNS -  $ZrOCl_2$ , could be a red coloured

complicated, that changes color once it reacts with Fluoride. The amendment in concentration of SPADNS  $ZrOCl_2$  causes a change within the transmitted light weight that is detected by the measuring instrument. The reaction between Fluoride and SPADNS chemical agent is fast and thence the samples is tested at intervals ten minutes once adding them to the chemical agent. SPADNS reacts with zirconyl chloride to administer a wine - red coloured complicated that more reacts with Fluoride to administer a replacement complicated. Because the quantity of Fluoride will increase, the colour made becomes more and more lighter.

The reaction rate between Fluoride and metal ions is influenced greatly by the acidity of aped therefore SOH the reaction mixture. If the proportion of acid within the chemical agent is enlarged, the reaction is created nearly instant.

#### Reagents:

1. **Normal F: resolution (100 ppm):** In an exceedingly 1mL beaker, take 0.221g of anhydrous NaF. Dissolve it with  $H_2O$  and dilute it up to mark.
2. **SPADNS solution:** Dissolve 0.958g of SPADNS in  $H_2O$  and dilute to 500cc.
3. **Zirconyl-acid reagent:** Dissolve 0.133g of zirconyl chloride octahydrate ( $ZrOCl_2 \cdot 8H_2O$ ) in regarding 25cc  $H_2O$ . Add 350cc of conc. HCl and dilute to 500cc with water.
4. **Acid zirconyl-SPADNS chemical agent:** combine equal volumes of SPADNS resolution and zirconyl-acid reagent.
5. **Metallic element arsenite solution:** Dissolve 0.5g of  $NaAsO_2$  in water and dilute to a 100cc.



**Procedure:**

1. Take 50.0cc of H<sub>2</sub>O with a 25cc measuring instrument into a 250cc, round shape flask.
2. Add one drop of NaAsO<sub>2</sub> resolution (for presence of excess Cl<sup>-</sup>).
3. Add 10cc of Acid zirconyl-SPADNS chemical agent and shake well.
4. Pour the answer into cell of photometer and live absorbance at 570nm. 0 it.
5. Now, take 10.0cc of the sample and add 40cc of H<sub>2</sub>O.
6. Perform steps (2) and (3).

**3.9.2 Calcium (Ca<sub>2</sub><sup>+</sup>) [Titration with EDTA] (Irene W and Kimaru *et al.*, 2018)**

**Theory:** In aqueous solution disodium salt of EDTA (ethylenediaminetetraacetic acid), represented as Na<sub>2</sub>H<sub>2</sub>Y (whose active ion is H<sub>2</sub>Y<sup>2-</sup>), reacts with several metal ions in 1:1 ratio as follows:  $M^{n+} + H_2Y^{2-} = MH_2Y^{n-4} + 2 H^+$  (M = Zn, Ca, Mg, etc.)

If water contains both Ca<sup>2+</sup> and Mg<sup>2+</sup> it combines first with Ca<sup>2+</sup>. So, Ca<sup>2+</sup> can be determined directly with EDTA when the pH is made sufficiently high (pH 12 - 13) that the Mg<sup>2+</sup> is - largely precipitated as Mg(OH)<sub>2</sub> and an indicator is used that combines with Ca<sup>2+</sup> only.

From the above reaction, it is clear that 1 mole of metal ion reacts with 1 mole of Na<sub>2</sub>H<sub>2</sub>Y (equivalent to EDTA). For calcium,

**1 mL 0.01 M EDTA solution  $\equiv$  0.40078 mg of Ca<sup>2+</sup> ion**

**Reagents:**

1. **NaOH Solution (10%, w/v):** Dissolve 10.0g of NaOH pellets with distilled deionized water (DDW) to make a volume of 100 mL.
2. **Indicator *Murexide* (ammonium purpurate) Solution:** Dissolve 0.075g of the ground dye in 50mL of DDW (better absolute ethylene glycol). The aqueous solution is not very stable and use within 1 day. [Alternatively, mix 0.5g of murexide with 100g of  $K_2SO_4$ , and grind well.]
3. **Standard EDTA Solution (0.01 ND):** Dry EDTA salt at 80°C. Dissolve 3.723 g of  $Na_2EDTA$  in DDW and dilute to 1mL. If do not dissolve completely, add a few drops of NaOH solution. Better to standardize by titrating with standard  $Ca^{2+}$  solution.
4. **Standard  $Ca^{2+}$  Solution (Calibration purpose):** To 1.249g of  $CaCO_3$  (Previously dried at 180°C for 1 h), add 50 mL of DDW. Dissolve the suspension by adding 10.0mL of cone. HCl drop wise and diluted to 1000mL with the water. The prepared stock Ca solution have a concentration of 500  $mg L^{-1}$ . Prepare other solutions by dilution.

**Procedure:**

1. Take 5.0 mL of water sample with a pipette into a 250 mL conical flask. Add 45.0 mL of DDW using a measuring cylinder.
2. Add 5.0 mL, or so 10% NaOH solution. Check pH of the sample and make it 12-13.
3. Add 5 drops of murexide indicator solution in the flask.

4. Titrate immediately with Standard EDTA solution. (The indicator is unstable at this pH). At end point pink color will be changed to purple or light blue.
5. Perform a blank titration.

### 3.10 Calculation

a mL of 0.01M EDTA is required for Sample

And b mL of 0.01M EDTA is required for Blank.

So, net 0.01 M EDTA required = (a - b) mL x mL (suppose)

5.0 mL sample contains  $(x \times 0.40078)$  mg of  $\text{Ca}^{2+}$  ion

$\therefore$  1000 mL Sample contains  $(x \times 0.40078 \times 200)$  mg of  $\text{Ca}^{2+}$  ion = y mg of  $\text{Ca}^{2+}$  ion (suppose)

Hence,  $[\text{Ca}^{2+}] = y \text{ mg L}^{-1}$

### 3.11 Microbiological analysis (Dr. Graeme K Pearce, 2005)

Bacteriological testing of drinking water by membrane filtration method.

#### 3.11.1 Membrane filtration method

Membrane filtration could be a methodology of separating particles in liquid solutions or gas mixture. This is a wide used technique for process the waste water treatment. A membrane could be a skinny layer of semi permeable membrane that separates substance once a thrust is applied across the membrane .Membrane processes are more and more used for removal of microorganism, micro-organism, particulates and natural organic material ,which can impart color, tastes and odors to water and react to create medical care byproduct.

**3.11.2 Required (Nisha Rijal, 2019)**

1. Brooder or water bathtub.
2. Membrane filtration equipment complete with vacuum supply and suction flask. The membrane filters ought to have uniform consistency of planned size (generally 0.45  $\mu\text{m}$ )
3. Autoclave for sterilizing ready culture media.
4. Boiling pan or bathtub.
5. Racks for bottle of ready culture media and dilution water.
6. Distilling equipment with storage capability for a minimum of 5 litres of water.
7. Hot air sterilizer for sterilizing pipettes and glass or metal petri dishes.
8. Measuring instrument for checking standardization of brooder or water bathtub.
9. Measuring instrument cans for sterilizing pipettes.
10. Boxes for petri dishes to be used in hot air autoclave
11. 74 millimetre diameter steriled polysaccharide pads.
12. Reusable pipettes, glass, capability 1 metric capacity unit and 10 metric capacity units.
13. Reusable bottles for culture media.

14. Mensuration cylinders, capability 100 mL and 250 mL.
15. Flasks for preparation of culture media.
16. Blunt edge extractor.
17. 50-60 mm diameter sterilized petri dishes.
18. Lauryl salt broth. 2.5 metric capacity units for every water sample.

### 3.11.3 Preparation of culture media

#### Lauryl sulphate broth

Lauryl sulphate broth recommended for detection and enumeration of coliform bacteria in water, waste, dairy product and other food sample.

Ingredient	Gram/L
Tryphosa	20.00
Lactose	5.00
Sodium Chloride	5.00
Dipotassium Hydrogen Phosphate	2.75
Potassium dihydrogen Phosphate	2.75
Sodium Lauryl sulphate	0.10

$P^H$  (25°C)  $6.7 \pm 0.2$

**Directions:** 35.60 grams suspended in 1000 ml distilled water. Heating the media to dissolve it completely. Distributing into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculating of 1 ml or less, using single strength medium. For inoculating of 10 mL or less, using single strength medium. For inoculating of 10 mL or more, double strength or proportionate.

#### **3.11.4 Method (Acharya Tankeshwar, 2010)**

1. Collected sample was taken and dilution was made if necessary.
2. Previously made louryl sulphate broth was dispensed into a sterile petri dish and the absorbent pad was evenly saturated.
3. The forceps was placed on flame and the membrane was removed from the sterile package.
4. The membrane filter was placed into the funnel assembly.
5. The pouring lip of the sample container was flamed and the sample was poured into the funnel.
6. The vacuum was turned on and the sample was allowed to draw completely through the filter.
7. The forceps was flamed and the membrane filter was removed from the funnel.
8. The membrane filter was placed into the prepared petri dish.

9. The petri dish was incubated at the proper temperature and for the appropriate time period.
10. The colonies were counted under 10-15X magnification.
11. The colonies were confirmed and the result was reported.

**Calculation:**

$$\text{No. of CFU per 100 ml} = \frac{\text{No. of colonies on the membrane}}{\text{Volume filtered}} \times 100$$

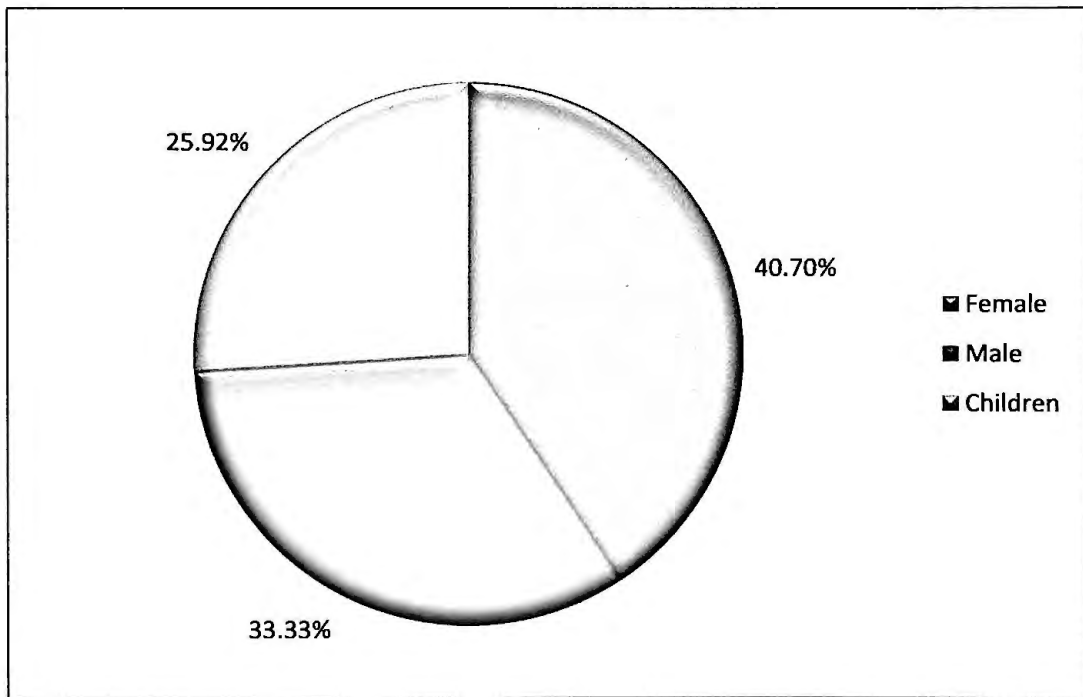
**CHAPTER IV**  
**RESULTS**



## RESULTS

**Table 1:** Percent infection with dental caries (in different gender) among Patients studied.

Female	Male	Children
220 (40.7%)	180 (33.33%)	140 (25.92%)



**Figure 4.1:** Percent infection with dental caries (in different gender) among Patients studied.

Table 1 & Figure 4.1 describes that 40.7% male patient, 33.33% female and 25.92% respondents were affected by dental caries in different upazilla in Rajshahi region among 540 respondent.

**Table 2:** Percent occurrence of different type of caries.

Root caries	Recurrent caries	Smooth surface caries	Occlusal pits and fissure caries
5.76%	0.96%	12.50%	80.76%

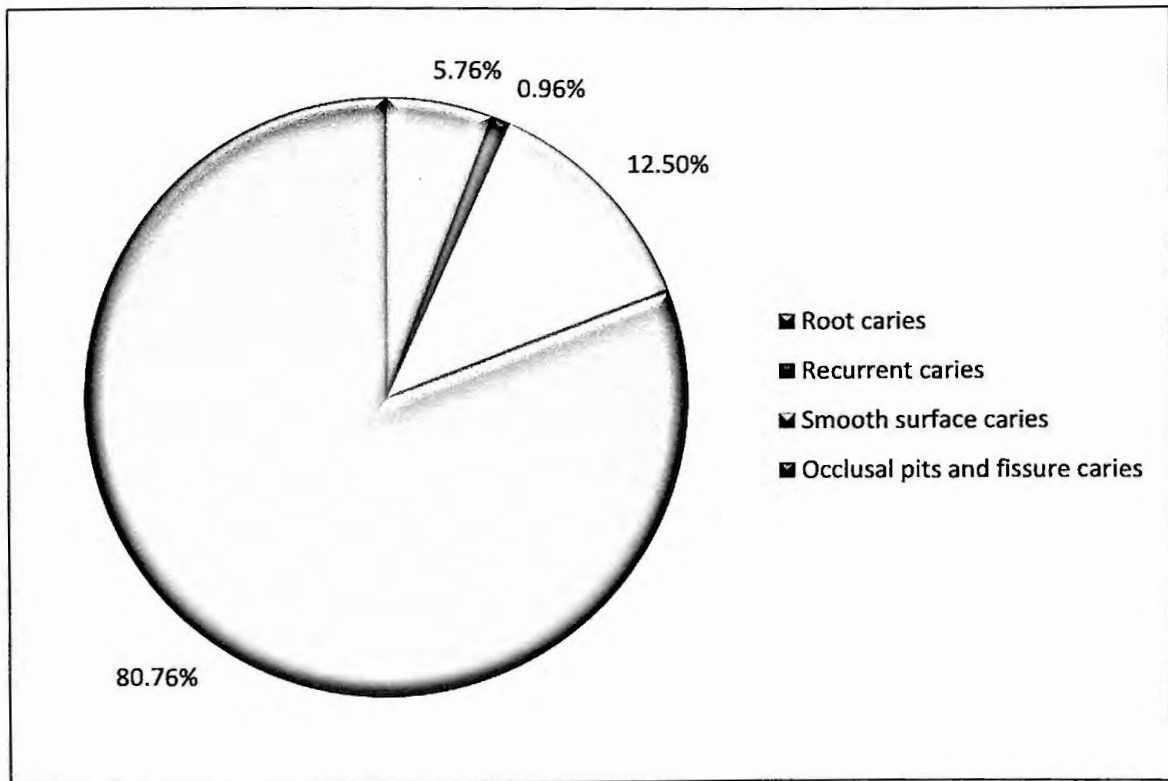
**Figure 4.2:** Percent occurrence of different type of caries.

Table 2 & Figure 4.2 represent that among the respondents in different upazillas 5.76% were suffering from root caries, 0.96% were suffering from recurrent caries, 12.50% patient was diagnosed as smooth surface caries and 80.76% was occlusal pits and fissure caries.

**Table 3:** Percent occurrence of different type of caries in male (♂) and female (♀).

Female (♂)				Male (♀)			
RC	RK	SC	OFC	RC	RK	SC	OFC
45 (20.5%)	40 (18.18%)	40 (18.18%)	95 (43.18%)	35 (19.44%)	52 (28.88%)	47 (26.11%)	46 (25.55%)

**RC:** Root caries; **RK:** Recurrent caries; **SC:** Smooth surface caries; **OFC:** Occlusal pits and fissure caries.

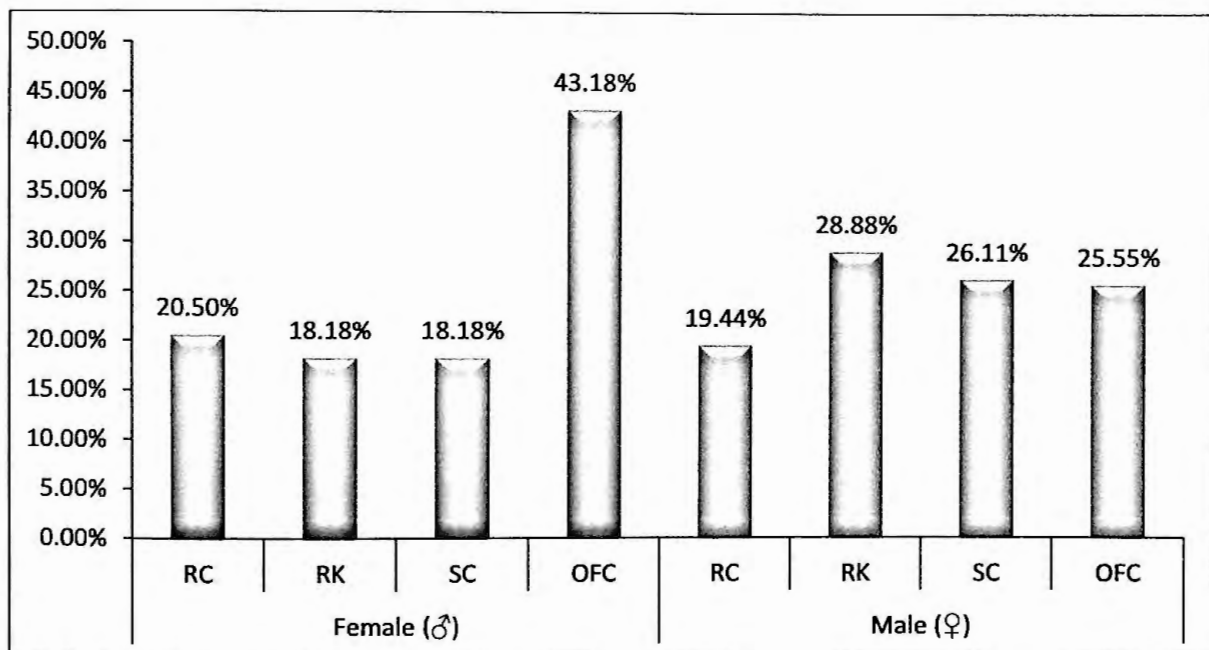
**Figure 4.3:** Percent occurrence of different type of caries in male (♂) and female (♀).

Table 3 & Figure 4.3 describe that among 540 patient of different upazillas there were 180 patient (male) which were root caries about 45(20.5%) in case female it was 35(19.44%). Patient (female) have recurrent caries which was 40(18.8%) but in same case the male 52(28.8%), the smooth surface caries in male and female 47(26.11%) and 40(18.18%). The occlusal pits and fissure caries in case of male it was 46(25.55%) and incase of female it was 95(43.18%).

**Table 4:** Water source of dental caries patient

Shallow well	Well	Surface water
252 (46.67%)	112 (20.74%)	32 (5.18%)

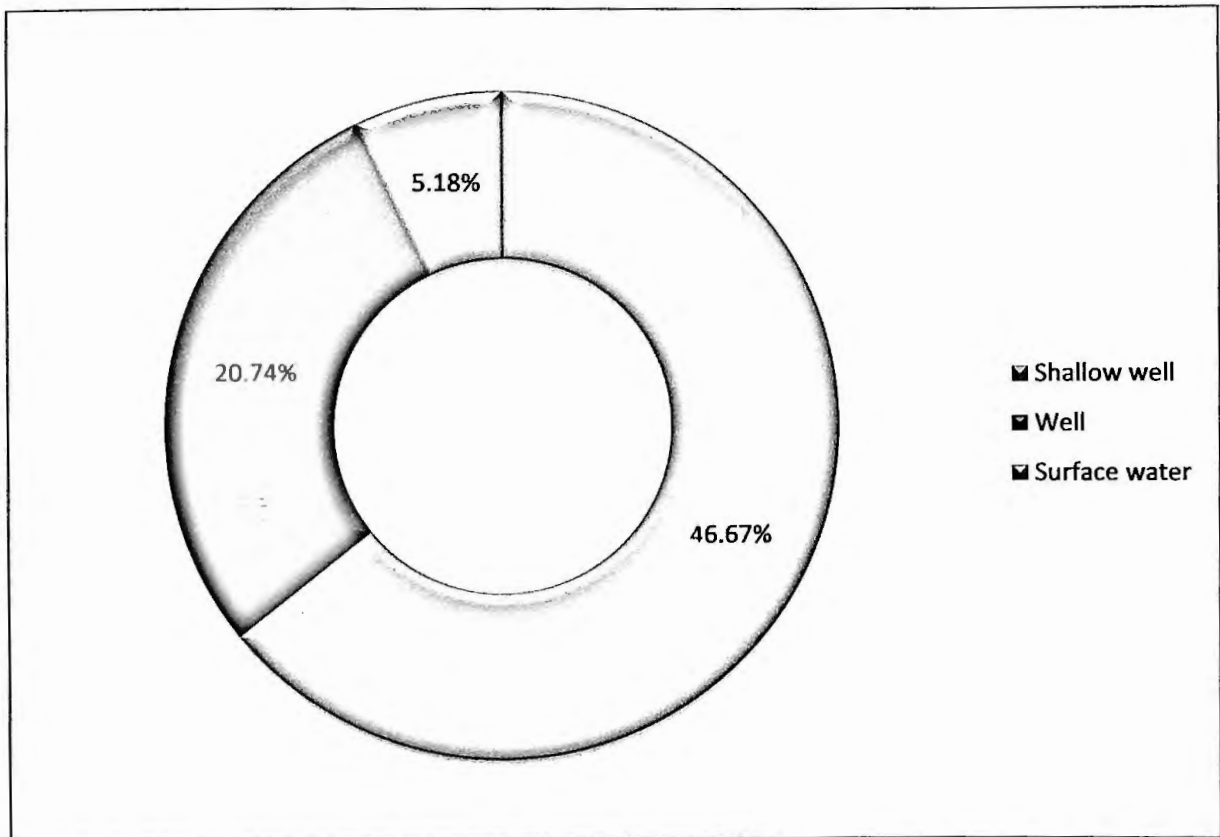
**Figure 4.4:** Water source of dental caries patient.

Table 4 & Figure 4.4 express about 46.67% of the 540 respondents use shallow well for drinking water. There were 252 patient in this category 112 respondents use well and 32 respondents used surface water. It was about 20.74% and 5.18% respectively.

**Table 5:** Occurrence of dental caries in different age group.

Age group (Years)	Number and Percentage of Patients
0-10	131(24.25%)
11-20	122(22.59%)
21-30	32(5.9%)
31-40	182(33.70%)
41-50	38(7.03%)
51-60	35(6.48%)

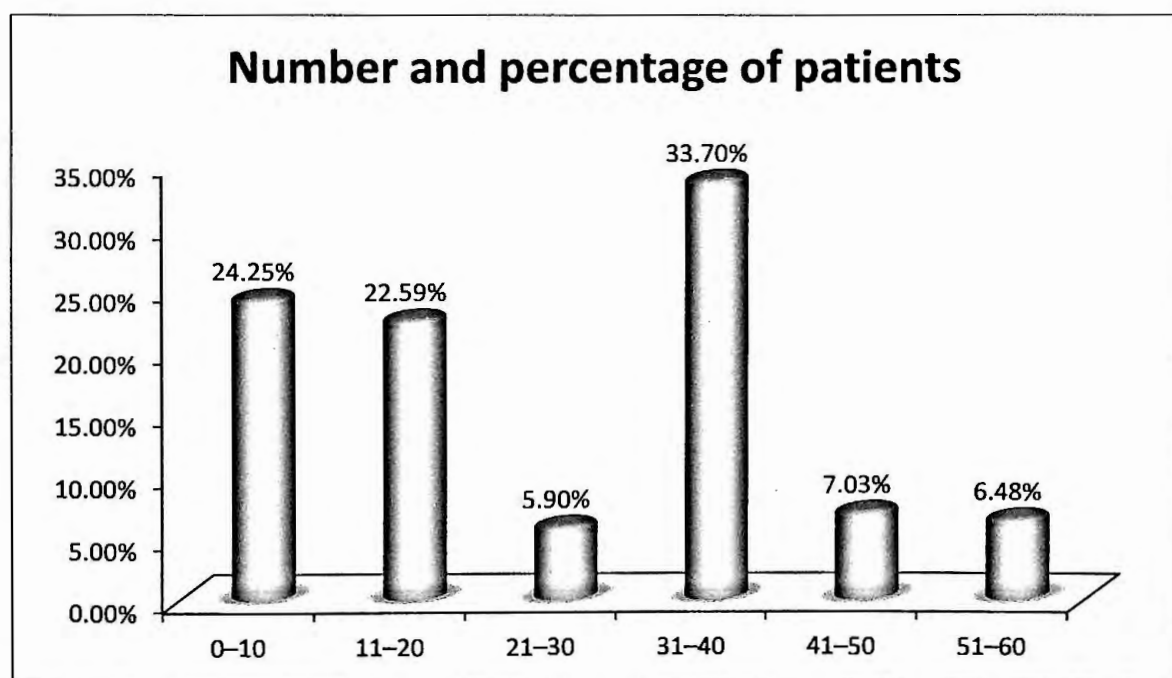
**Figure 4.5:** Occurrence of dental caries in different age group.

Table 5 & Figure 4.5 describe that 131 (24.25%) respondents were less than 10 years old, 122(22.59%) respondents were less than 20 years old, 32(5.9%) respondents were less than 30 years old, 182(33.70%) patient were under 40, 38(7.03%) patient were in between 41-50 years old and 35(6.48%) were in between 51-60old.

**Table 6:** Distribution of respondents according to their Oral Hygiene practice.

Oral hygiene practice	Frequency	Percentage (%)
Tooth brush	178	32.96
Neem stick	170	31.48
Finger	56	10.37
Others	156	25.22
<b>Total</b>	<b>540</b>	<b>100</b>

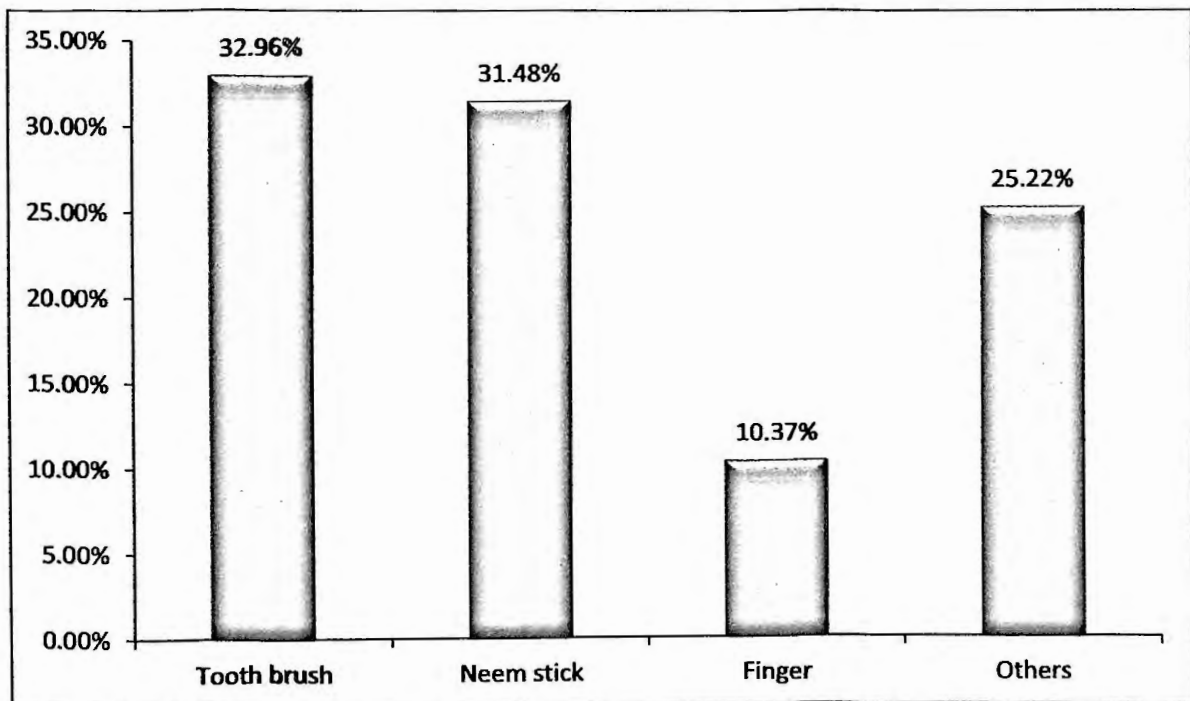
**Figure 4.6:** Distribution of respondents according to their Oral Hygiene practice.

Table 6 & Figure 4.6 describe that 178 (32.96%) respondents used tooth brush, 170 (31.48%) and 56 (10.37%) patient maintain their oral hygiene by the use of name stick and finger. About 156 respondents were applied others method.

**Table 7:** Distribution of the frequency of visit of the respondents to their Dentist for regular care.

Visit to dentist	Frequency	Percentage (%)
Not at all	175	32.40%
Some times when pain arises	125	23.14%
Yearly once a time	61	11.29%
Visited to village untrained doctor	135	26.88%
Every 6 Monthly	34	6.29%
<b>Total</b>	<b>540</b>	<b>100</b>

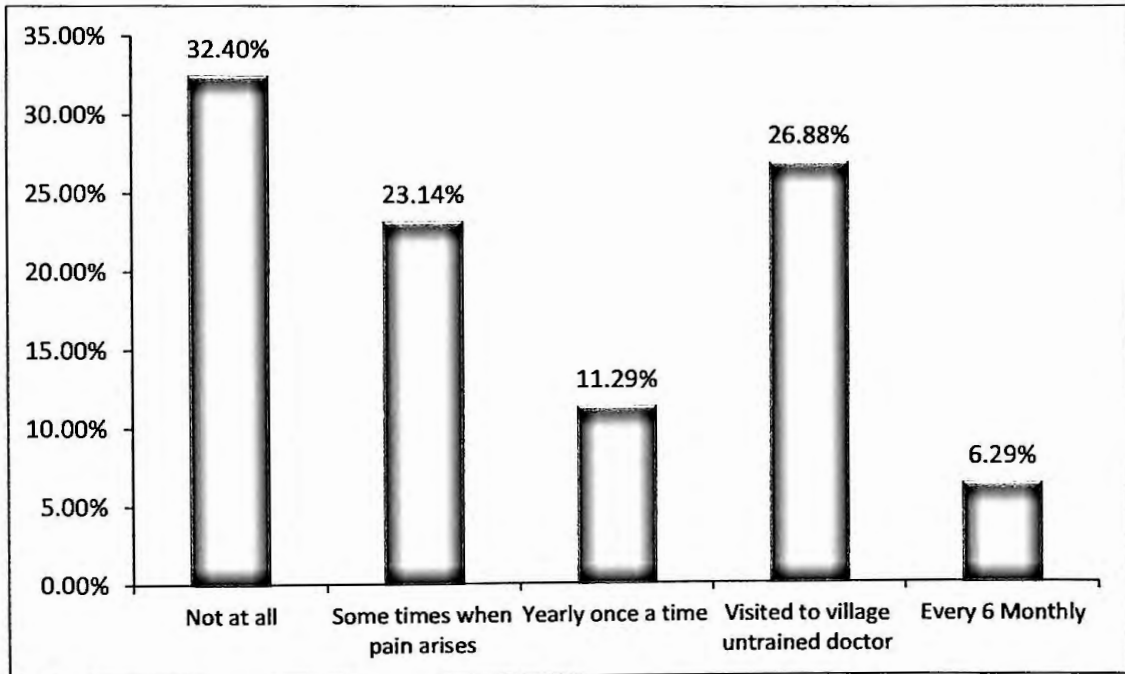
**Figure 4.7:** Distribution of the frequency of visit of the respondents to their Dentist for regular care.

Table 7 & Figure 4.7 express that the distribution of the frequency of visit of the respondents to their dentist regular care. Respondents visited their dentist sometimes when pain raised 125(23.14%). Respondents never visited to their dentist 175(32.40%) respondents visited to dental quaks, 135(26.88%). Respondents visited to dentist twice a year 34(6.29%). 61(11.29%) Respondents visited to their dentist once a year.

## 4.1 Fluoride results

Table 8: Fluoride content in drinking water collected from patient of Paba Upazilla.

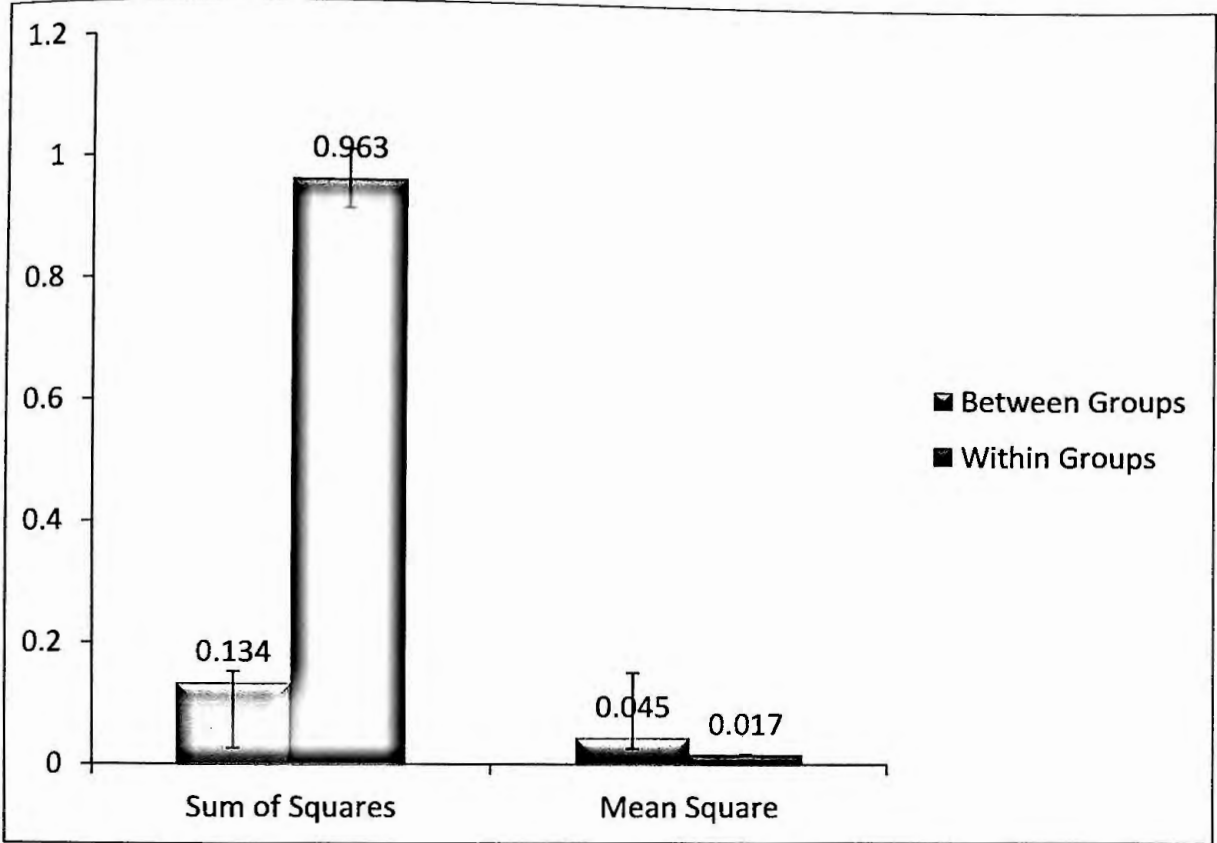
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
					A	15		
B	15	.442313	.1941137	.0501199	.334817	.549810	.2237	.7925
C	15	.371500	.1236852	.0319354	.303005	.439995	.2107	.5927
D	15	.351487	.1066695	.0275419	.292415	.410558	.2172	.5929
Total	60	.369343	.1363807	.0176067	.334112	.404574	.2107	.7925

A=3-20, B=21-40, C= 41-60, D = Above 60

## ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.134	3	.045	2.595	.061
Within Groups	.963	56	.017		
Total	1.097	59			





**Figure 4.8:** Fluoride content in drinking water collected from patient of Paba Upazilla.

Table 8 & Figure 4.8 illustrates among the 60 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). The sum of squares between groups is 0.134 and within groups is 0.963. The mean square between groups is 0.045 and within groups is 0.017. The ANOVA analysis of the result describe that level of significance is 0.061 which is non-significant. The F value 2.595.

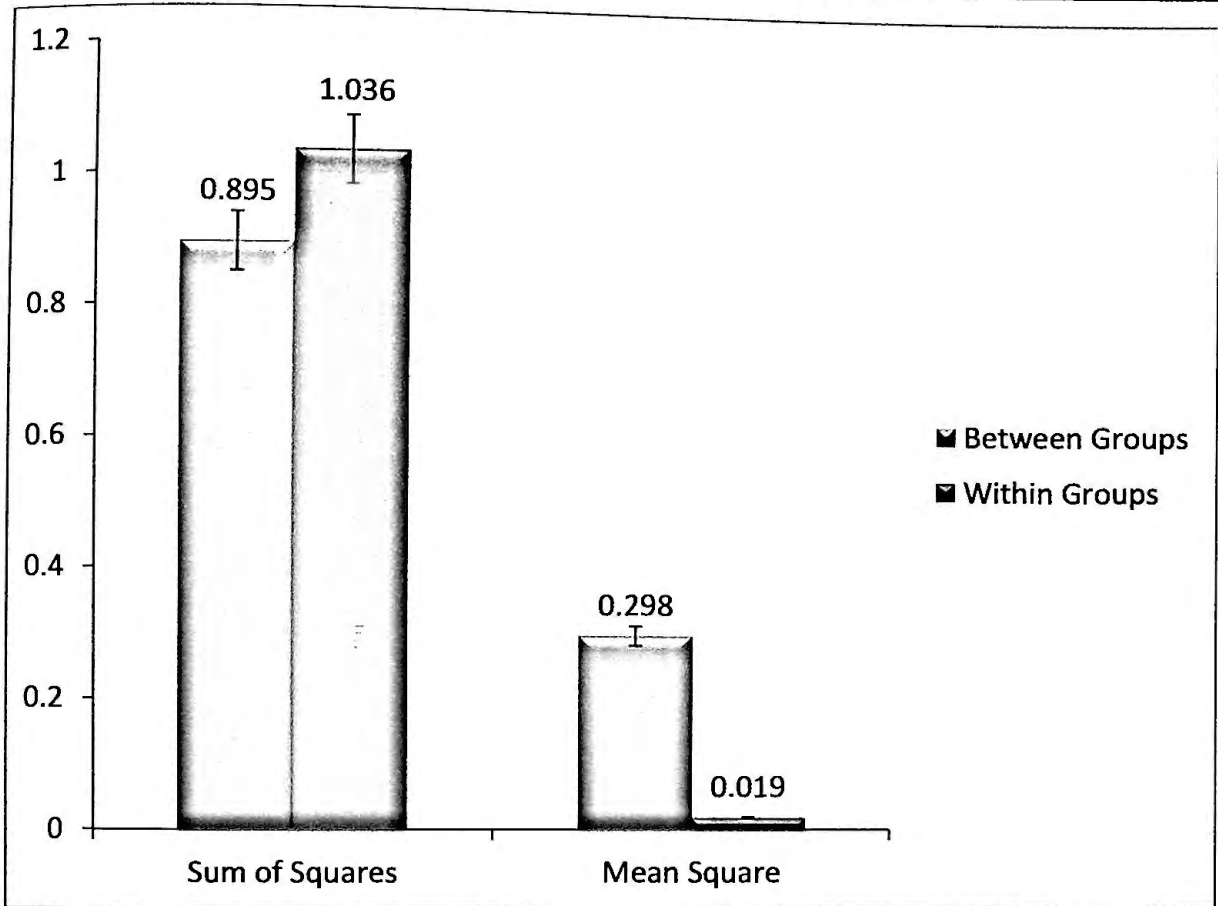
**Table 9:** Fluoride content in drinking water collected from patient of Bagha Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.471220	.1891513	.0488387	.366471	.575969	.2103	.7295
B	16	.634344	.1248038	.0312010	.567840	.700847	.4329	.8252
C	14	.304700	.0724875	.0193731	.262847	.346553	.2013	.4295
D	15	.391473	.1291518	.0333469	.319951	.462995	.2137	.5925
Total	60	.455928	.1809430	.0233596	.409186	.502671	.2013	.8252

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.895	3	.298	16.126	.000
Within Groups	1.036	56	.019		
Total	1.932	59			



**Figure 4.9:** Fluoride content in drinking water collected from patient of Bagha Upazilla.

Table 9 & Figure 4.9 illustrates among the 60 respondents, age were divided in groups 15 respondents were in A groups (3-20), 16 respondents were in B groups (21-40), 14 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). On ANOVA analysis of the result sum of squares and Mean square is 0.89 S (Between group), and 1.036 (Within group). The F value is 16.126 and level of significance is 0.000 which is very highly significant.

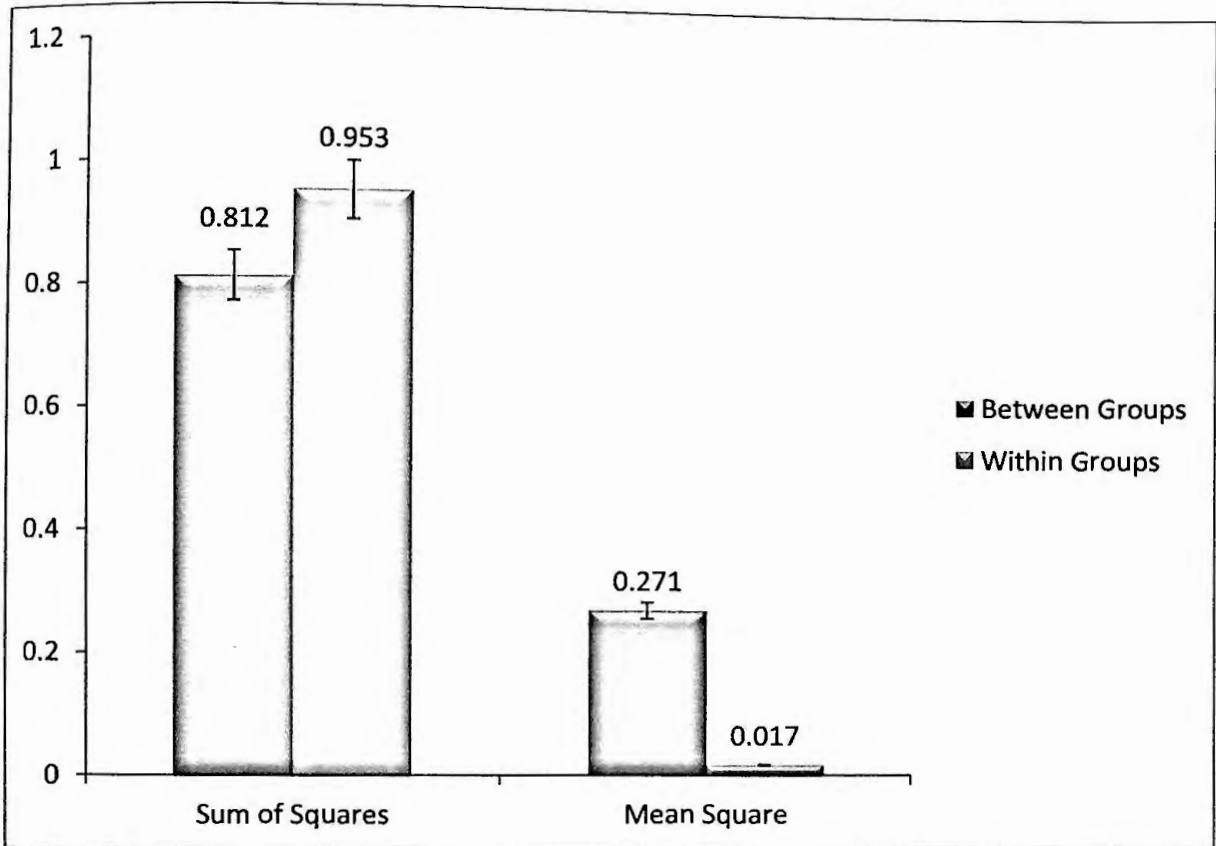
**Table 10:** Fluoride content in drinking water collected from patient of Bagmara Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.377507	.1155354	.0298311	.313525	.441488	.2105	.5929
B	15	.232206	.0205364	.0053025	.220833	.243579	.2129	.2987
C	15	.444387	.1317353	.0340139	.371434	.517339	.2497	.6955
D	15	.553347	.1922824	.0496471	.446864	.659829	.3201	.8275
Total	60	.401862	.1729770	.0223312	.357177	.446546	.2105	.8275

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.812	3	.271	15.899	.000
Within Groups	.953	56	.017		
Total	1.765	59			



**Figure 4.10:** Fluoride content in drinking water collected from patient of Bagmara Upazilla.

Table 10 & Figure 4.10 illustrates among the 60 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). After ANOVA analysis the sum of squares and Mean Square accordingly is 0.82 and 0.271 (Between groups) other were 0.953 and 0.015 (within group). The F value 15.899 and Level of significance was .000 which is very highly significant.

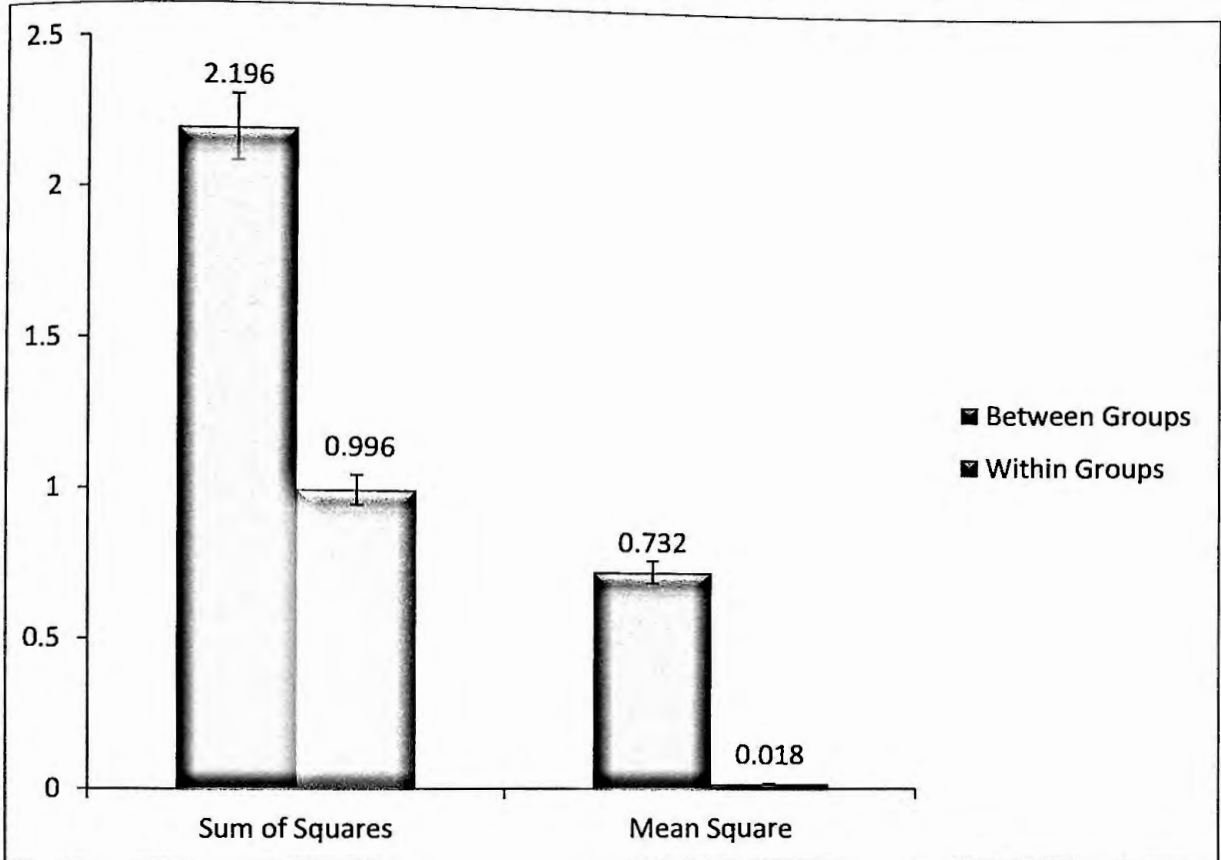
**Table 11:** Fluoride content in drinking water collected from patient of Charghat Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	17	.481182	.1530442	.0371187	.402494	.559870	.2137	.7292
B	15	.283800	.0550081	.0142030	.253338	.314262	.2201	.3925
C	13	.390554	.1250752	.0346896	.314972	.466136	.2301	.5929
D	15	.798627	.1672055	.0431723	.706031	.891222	.6201	1.2100
Total	60	.491562	.2326169	.0300307	.431470	.551653	.2137	1.2100

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.196	3	.732	41.151	.000
Within Groups	.996	56	.018		
Total	3.193	59			



**Figure 4.11:** Fluoride content in drinking water collected from patient of Charghat Upazilla.

Table 11 & Figure 4.11 illustrates among the 60 respondents, age were divided in groups 17 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 13 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 2.196 (Between groups) and 0.996 (within groups). Mean Square 0.732 (Between groups) and 0.018 (within groups). F value was 41.151 and signification was 0.000 which is very highly significant.

**Table 12:** Fluoride content in drinking water collected from patient of Durgapur Upazilla.

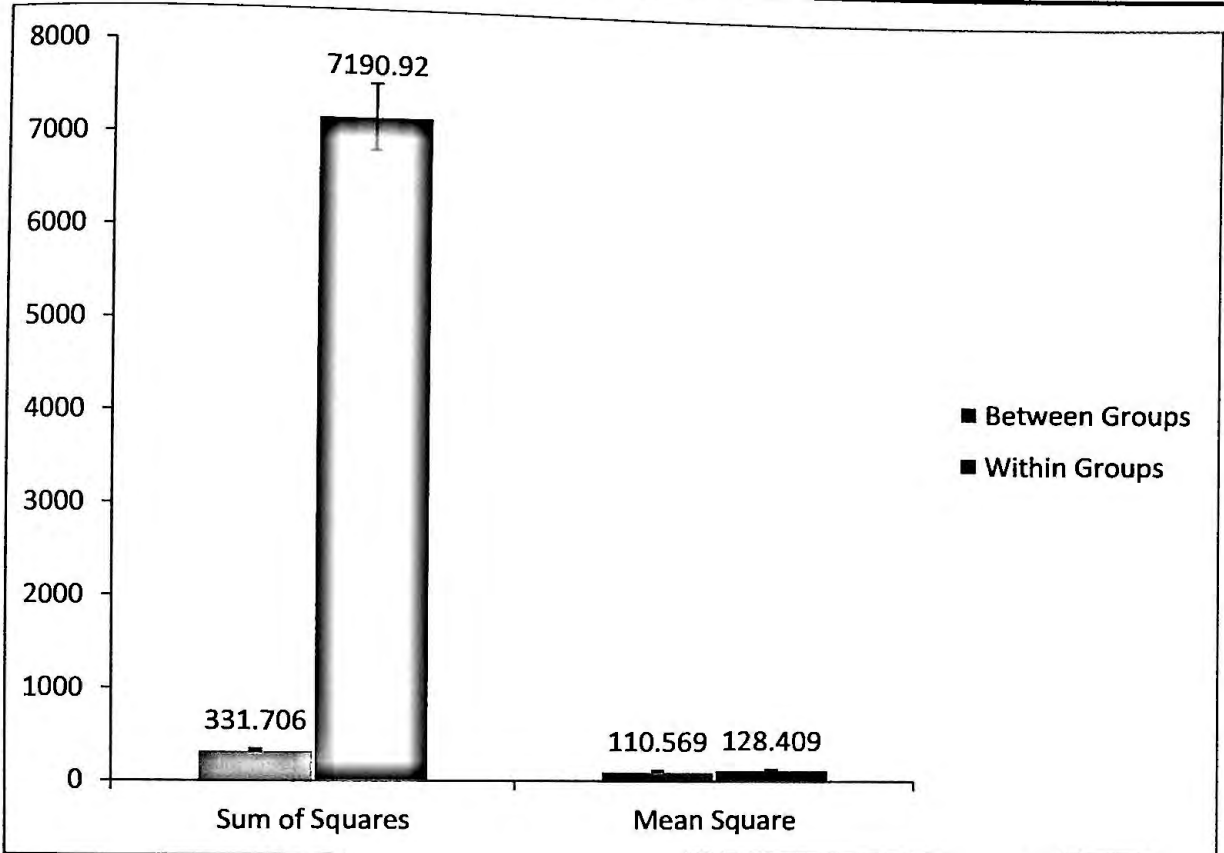
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.445226	.1764202	.0455515	.347528	.542924	.2104	.7392
B	15	.370947	.1066655	.0275409	.311877	.430016	.2103	.5929
C	15	.275893	.0582173	.0150317	.243654	.308133	.2013	.3972
D	16	.430944	.1450302	.0362576	.353663	.508225	.2329	.6929
Total	61	.381575	.1430018	.0183095	.344951	.418200	.2013	.7392

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.269	3	.090	5.335	.003
Within Groups	.958	57	.017		
Total	1.227	60			





**Figure 4.12:** Fluoride content in drinking water collected from patient of Durgapur Upazilla.

Table 12 & Figure 4.12 illustrates among the 61 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 16 respondents were in C groups (41-60), 16 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 0.269 (Between groups) and 0.958 (within groups). Mean Square 0.090 (Between groups) and 0.017 (within groups). F value was 5.335 and signification was 0.003 which indicate that very highly significant.

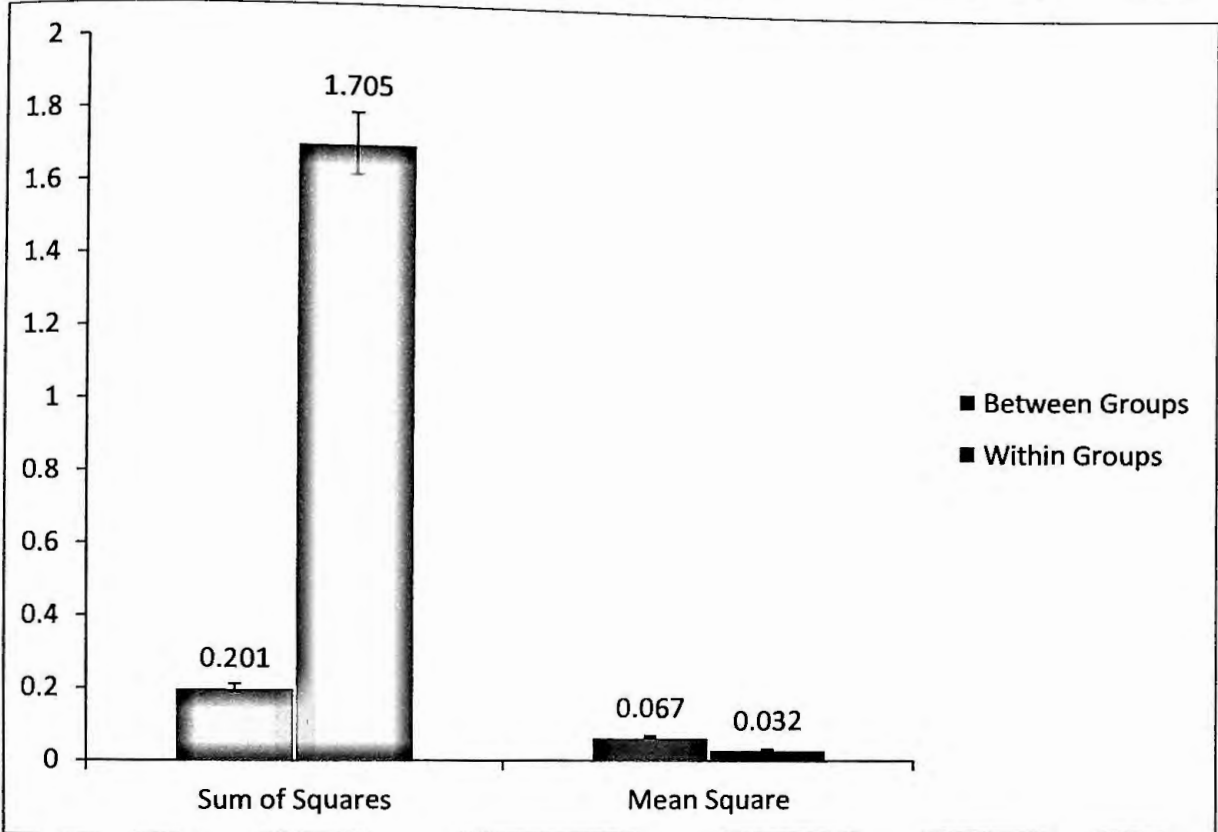
**Table 13:** Fluoride content in drinking water collected from patient of Godagari Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.278733	.0689492	.0178026	.240551	.316916	.1231	.3925
B	13	.351792	.1294260	.0358963	.273581	.430004	.2315	.5929
C	15	.442207	.2672209	.0689961	.294225	.590189	.2103	.9892
D	15	.358693	.1768436	.0456608	.260761	.456626	.2013	.7929
Total	58	.358066	.1828726	.0240123	.309982	.406149	.1231	.9892

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.201	3	.067	2.123	.108
Within Groups	1.705	54	.032		
Total	1.906	57			



**Figure 4.13:** Fluoride content in drinking water collected from patient of Godagari Upazilla.

Table 13 & Figure 4.13 illustrates among the 58 respondents, age were divided in groups 15 respondents were in A groups (3-20), 13 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares were 0.201 (Between groups) and 1.705 (within groups). Mean Square 0.067 (Between groups) and 0.032 (within groups). F value was 2.123 and signification is 0.108 which is non-significant.

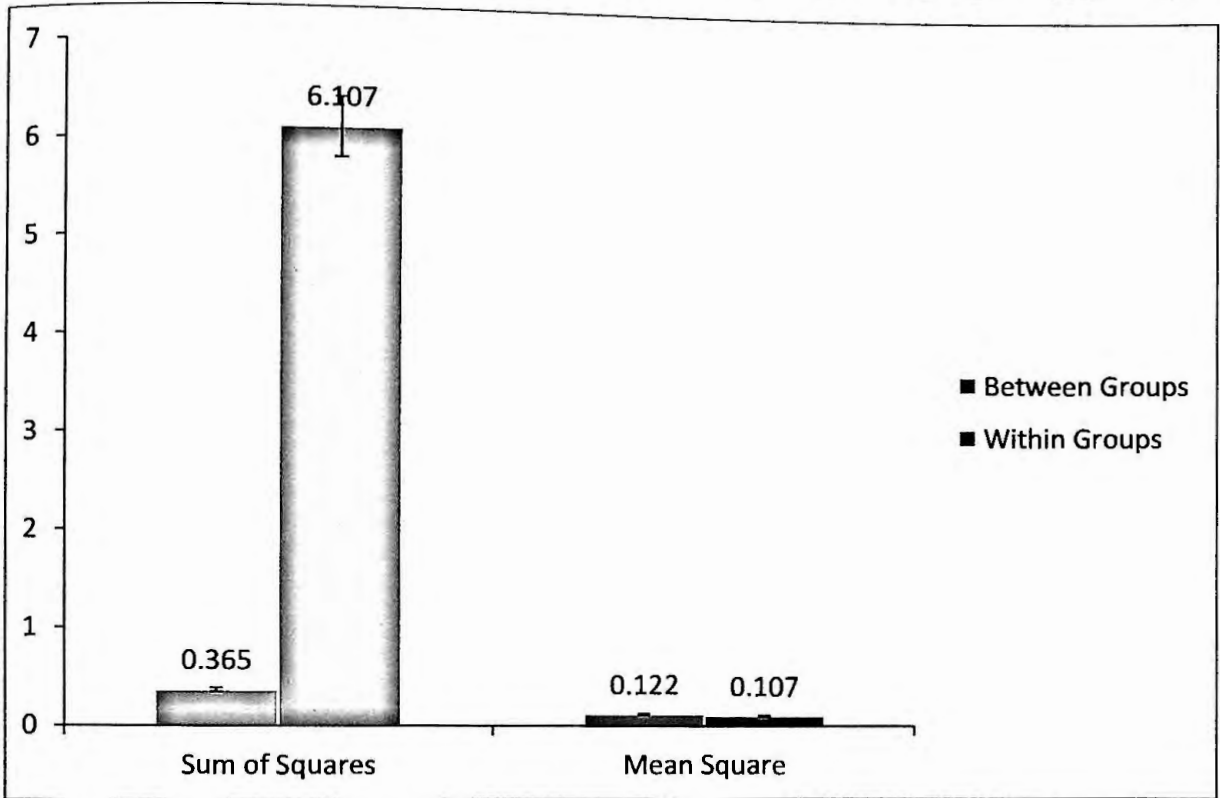
**Table 14:** Fluoride content in drinking water collected from patient of Mohanpur Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	16	.641781	.5638521	.1409630	.341326	.942237	.2107	2.3254
B	15	.426107	.1481384	.0382492	.344070	.508143	.2259	.6927
C	15	.556680	.2160091	.0557733	.437058	.676302	.2729	.8927
D	15	.536140	.1643093	.0424245	.445149	.627131	.2137	.7701
Total	61	.541843	.3284278	.0420509	.457728	.625957	.2107	2.3254

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.365	3	.122	1.134	.343
Within Groups	6.107	57	.107		
Total	6.472	60			



**Figure 4.14:** Fluoride content in drinking water collected from patient of Mohanpur Upazilla.

Table 14 & Figure 4.14 illustrates among the 61 respondents, age were divided in groups 16 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 0.365 (Between groups) and 6.107 (within groups). Mean Square 0.122 (Between groups) and 0.107 (within groups). F value was 1.134 and signification was 0.343 which is non-significant.

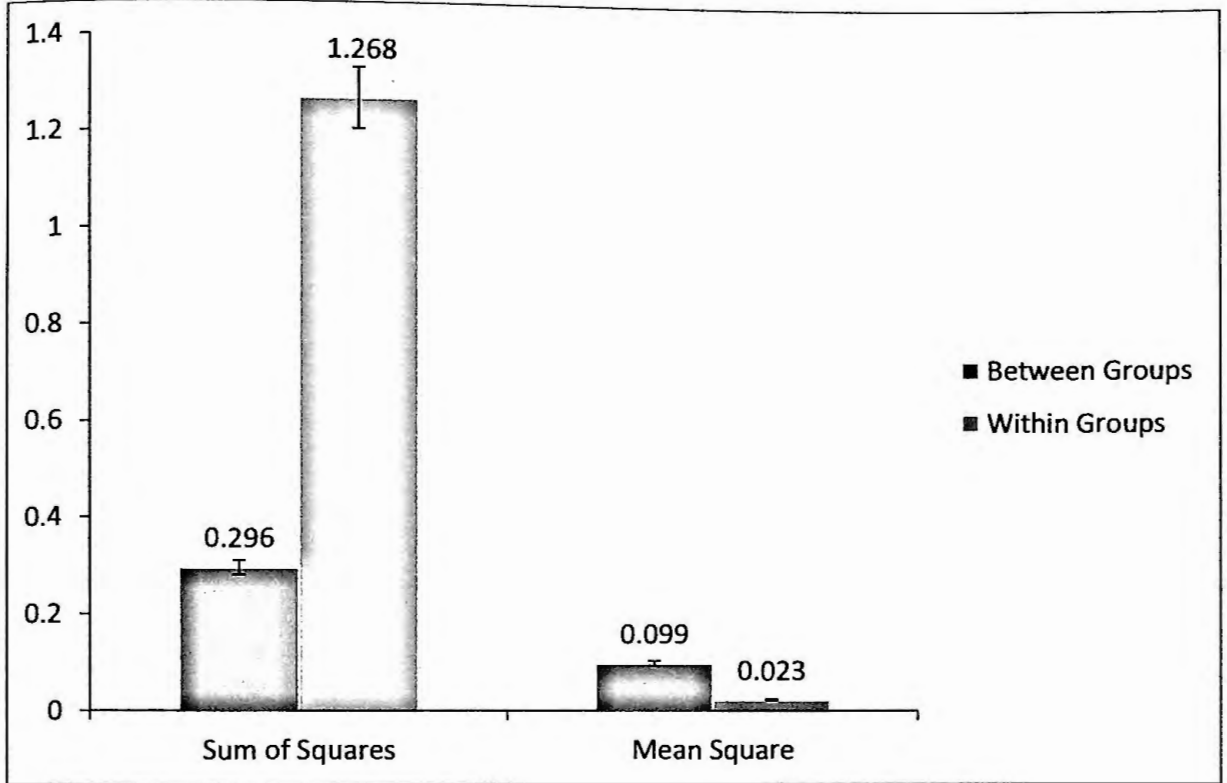
**Table 15:** Fluoride content in drinking water collected from patient of Puthia Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.378847	.1171051	.0302364	.313996	.443697	.2107	.5927
B	13	.295746	.0563656	.0156330	.261685	.329808	.2201	.3929
C	15	.392820	.1748583	.0451482	.295987	.489653	.2207	.7927
D	15	.499680	.2087551	.0539003	.384075	.615285	.2139	.8729
Total	58	.395084	.1656827	.0217552	.351520	.438649	.2107	.8729

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.296	3	.099	4.207	.010
Within Groups	1.268	54	.023		
Total	1.565	57			



**Figure 4.15:** Fluoride content in drinking water collected from patient of Puthia Upazilla.

Table 15 & Figure 4.15 illustrates among the 58 respondents, age were divided in groups 15 respondents were in A groups (3-20), 13 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 0.296 (Between groups) and 1.268 (within groups). Mean Square 0.099 (Between groups) and 0.0263 (within groups). F value 4.207 and signification 0.010 which is significant.

**Table 16:** Fluoride content in drinking water collected from patient of Tanore Upazilla.

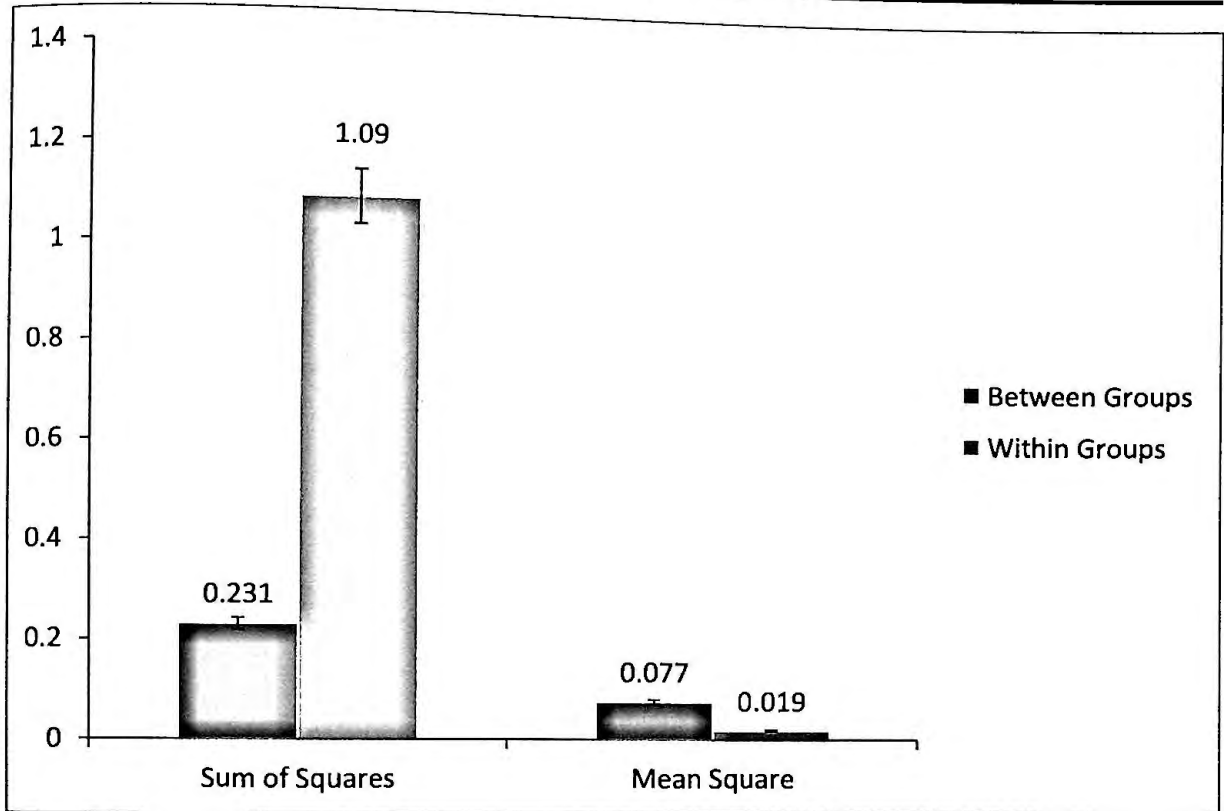
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	.403493	.1488702	.0384381	.321052	.485935	.2209	.6796
B	15	.470261	.1985109	.0512553	.360329	.580192	.2121	.7925
C	15	.299933	.0597229	.0154204	.266860	.333007	.2103	.3927
D	15	.361427	.1128160	.0291290	.298951	.423902	.2021	.5927
Total	60	383779	.1496349	.0193178	.345124	.422433	.2021	.7925

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

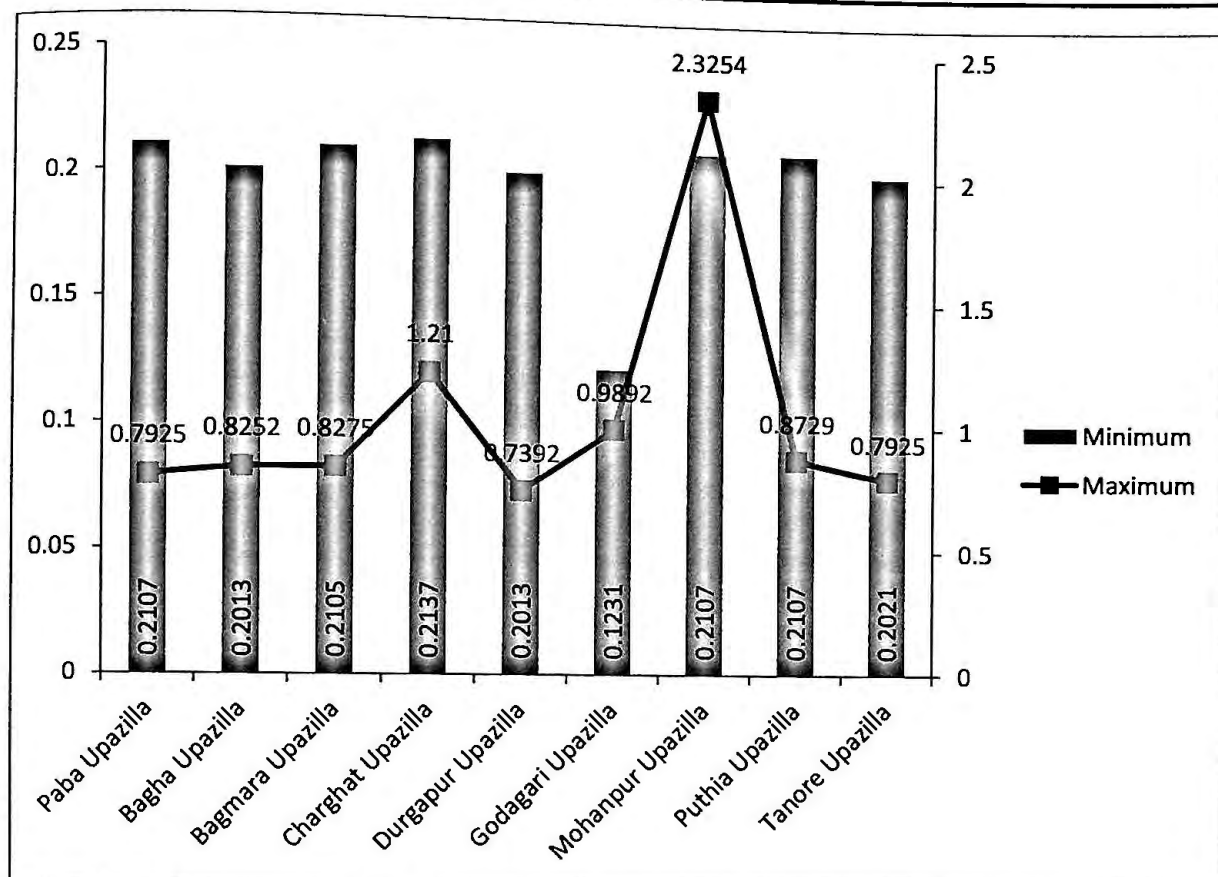
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.231	3	.077	3.955	.013
Within Groups	1.090	56	.019		
Total	1.321	59			





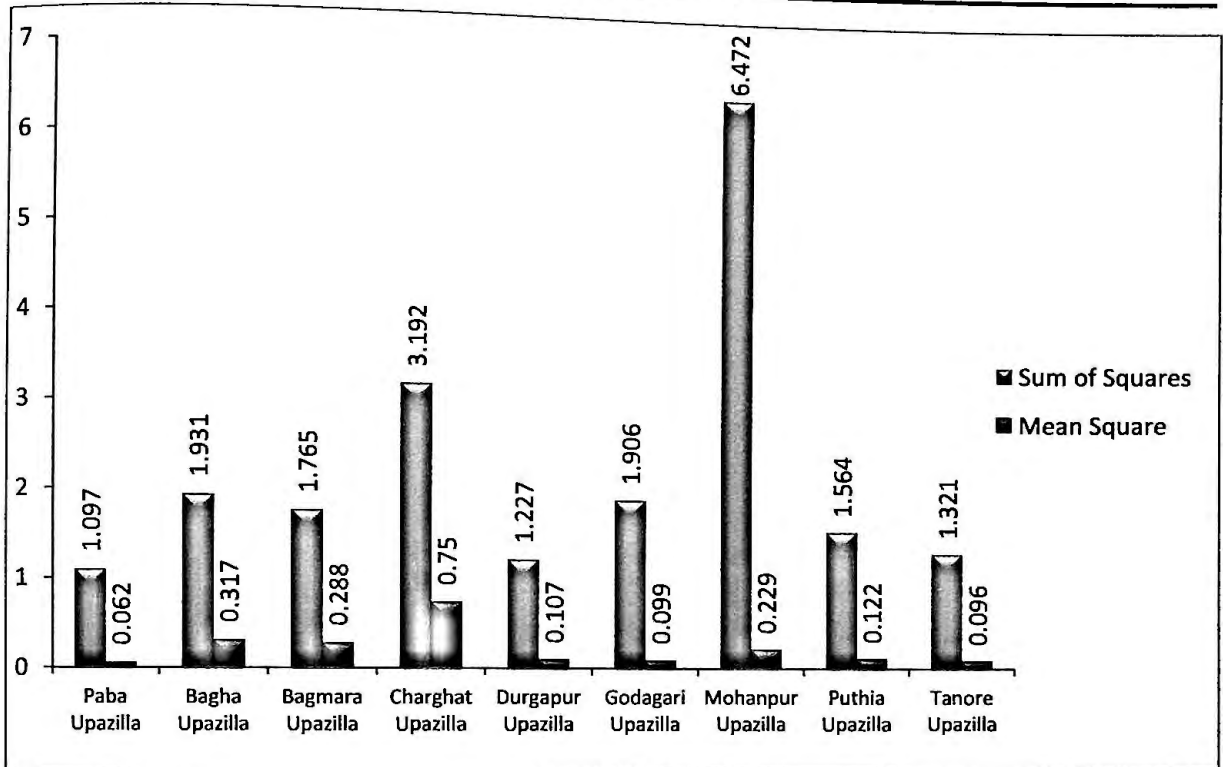
**Figure 4.16:** Fluoride content in drinking water collected from patient of Tanore Upazilla.

Table 16 & Figure 4.16 illustrates among the 60 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 0.231 (Between groups) and 1.090 (within groups). Mean Square 0.077 (Between groups) and 0.019 (within groups). F value 3.955 and signification 0.013 which is significant.



**Figure 4.17:** Maximum and minimum level of fluoride in different Upazila in Rajshahi region.

Fluoride Concentration in water samples collected from different Upazilla ranges from 0.1231 to 2.3254 ppm. Note that Mohanpur Upazilla contains the highest amount of fluoride and Godagari Upazilla contains the lowest amount of fluoride.



**Figure 4.18:** Sum of square and Mean Square of fluoride in different Upazila in Rajshahi region.

The Figure express that the range between sum of squares and mean squares is highest in Mohanpur Upazilla.

## 4.2 Calcium results

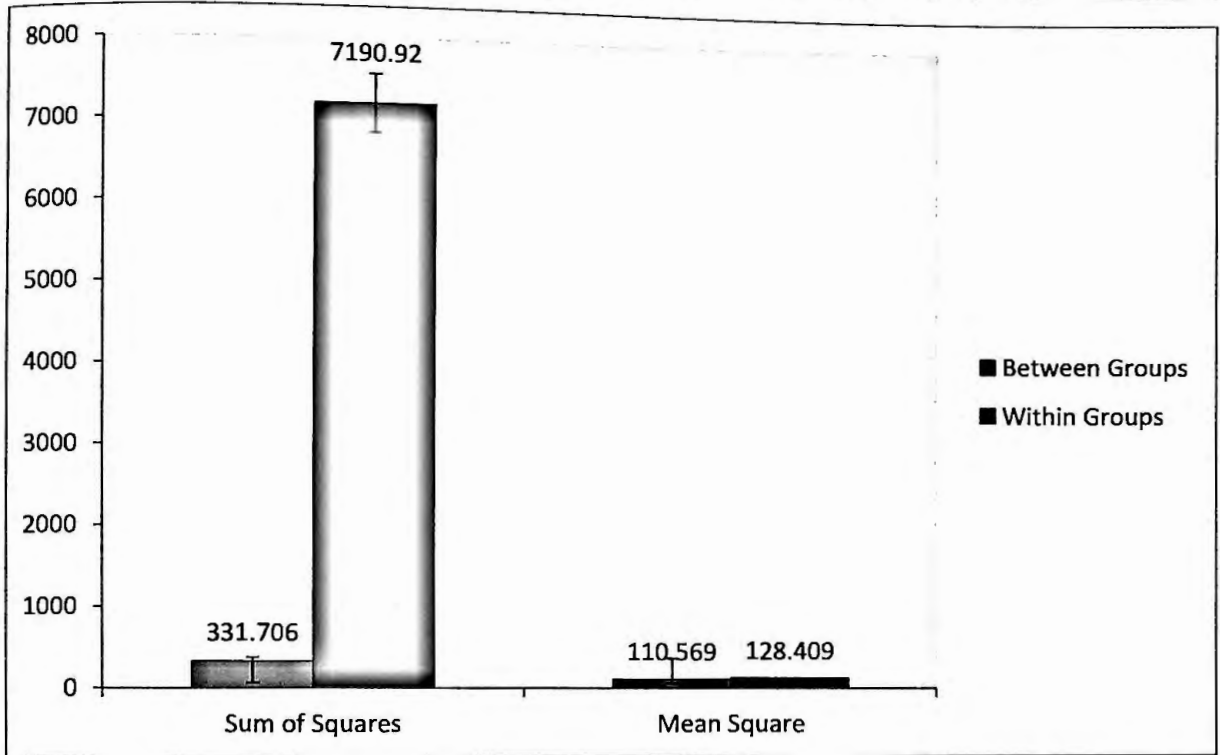
Table 17: Calcium content in drinking water collected from patient of Paba Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	16	16.0247	10.84256	2.71064	10.2471	21.8023	2.31	29.75
B	15	14.6527	9.33329	2.40985	9.4841	19.8213	2.18	31.29
C	15	14.8527	9.62522	2.48522	9.5224	20.1830	2.18	31.29
D	15	10.8397	7.68009	1.98299	6.5866	15.0928	2.22	24.57
Total	61	14.1241	9.43179	1.20762	11.7085	16.5397	2.18	31.29

A=3-20, B=21-40, C= 41-60, D = Above 60

## ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	231.761	3	77.254	.862	.466
Within Groups	5105.763	57	89.575		
Total	5337.525	60			



**Figure 4.19:** Calcium content in drinking water collected from patient of Paba Upazilla.

Table 17 & Figure 4.19 illustrates among the 61 respondents, age were divided in groups 16 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares was 231.761 (Between groups) and 5105.763 (within groups). Mean Square 77.254 (Between groups) and 89.575 (within groups). F value was 0.862 and level of signification 0.466 which is non-significant.

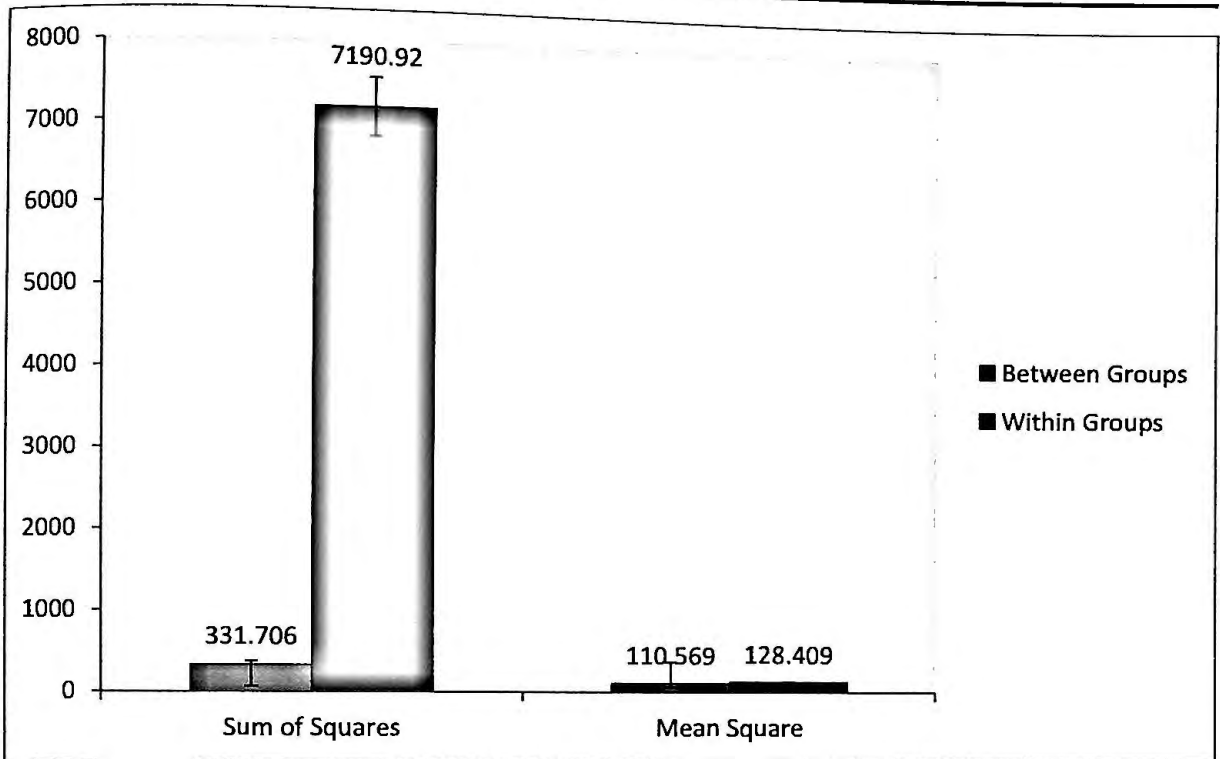
**Table 18:** Calcium content in drinking water collected from patient of Bagha Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	19.3765	13.45700	3.47458	11.9243	26.8287	2.91	39.36
B	15	19.0144	12.60492	3.25458	12.0340	25.9947	2.01	32.59
C	13	19.7003	8.38257	2.32491	14.6348	24.7658	2.39	31.93
D	15	18.4156	10.03856	2.59194	12.8565	23.9748	2.79	31.94
Total	58	19.1069	11.10290	1.45788	16.1876	22.0263	2.01	39.36

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.964	3	4.321	.033	.992
Within Groups	7013.673	54	129.883		
Total	7026.637	57			



**Figure 4.20:** Calcium content in drinking water collected from patient of Bagha Upazilla.

Table 18 & Figure 4.20 illustrates among the 58 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 13 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 12.964 (Between groups) and 7013.673 (within groups). Mean Square 4.321 (Between groups) and 129.883 (within groups). F value 0.992 which is non-significant.

**Table 19:** Calcium content in drinking water collected from patient of Bagmara Upazilla.

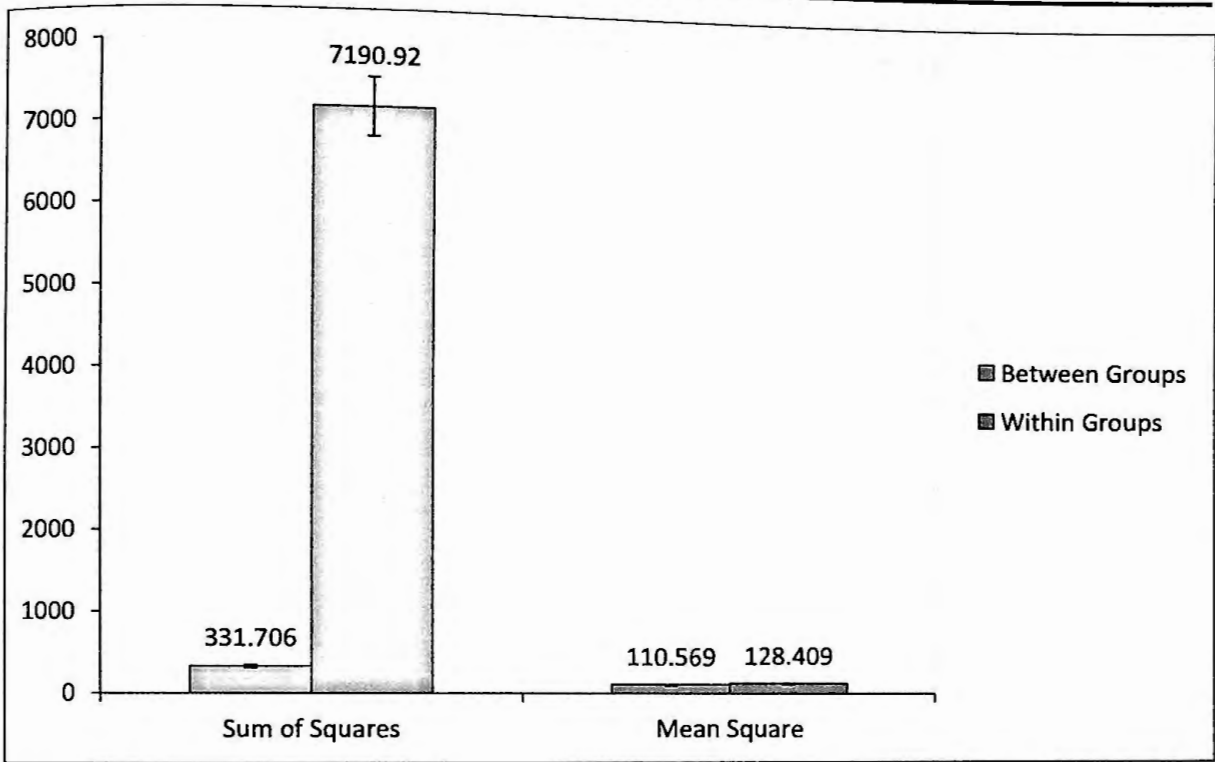
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	13	14.0074	9.15325	2.53866	8.4762	19.5387	2.14	31.41
B	15	21.3142	12.99366	3.35495	14.1185	28.5098	2.35	38.42
C	15	12.4274	7.38782	1.90753	8.3361	16.5186	2.79	24.99
D	15	29.8528	16.91934	4.36856	20.4832	39.2224	2.55	56.61
Total	58	19.5864	13.85391	1.81911	15.9437	23.2291	2.14	56.61

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2799.160	3	933.053	6.189	.001
Within Groups	8140.893	54	150.757		
Total	10940.053	57			





**Figure 4.21:** Calcium content in drinking water collected from patient of Bagmara Upazilla.

Table 19 & Figure 4.21 illustrates among the 58 respondents, age were divided in groups 13 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 2799.160 (Between groups) and 8140.893 (within groups). Mean Square 933.053 (Between groups) and 150.757 (within groups). F value is 6.189 and significant 0.001 which is very highly significant.

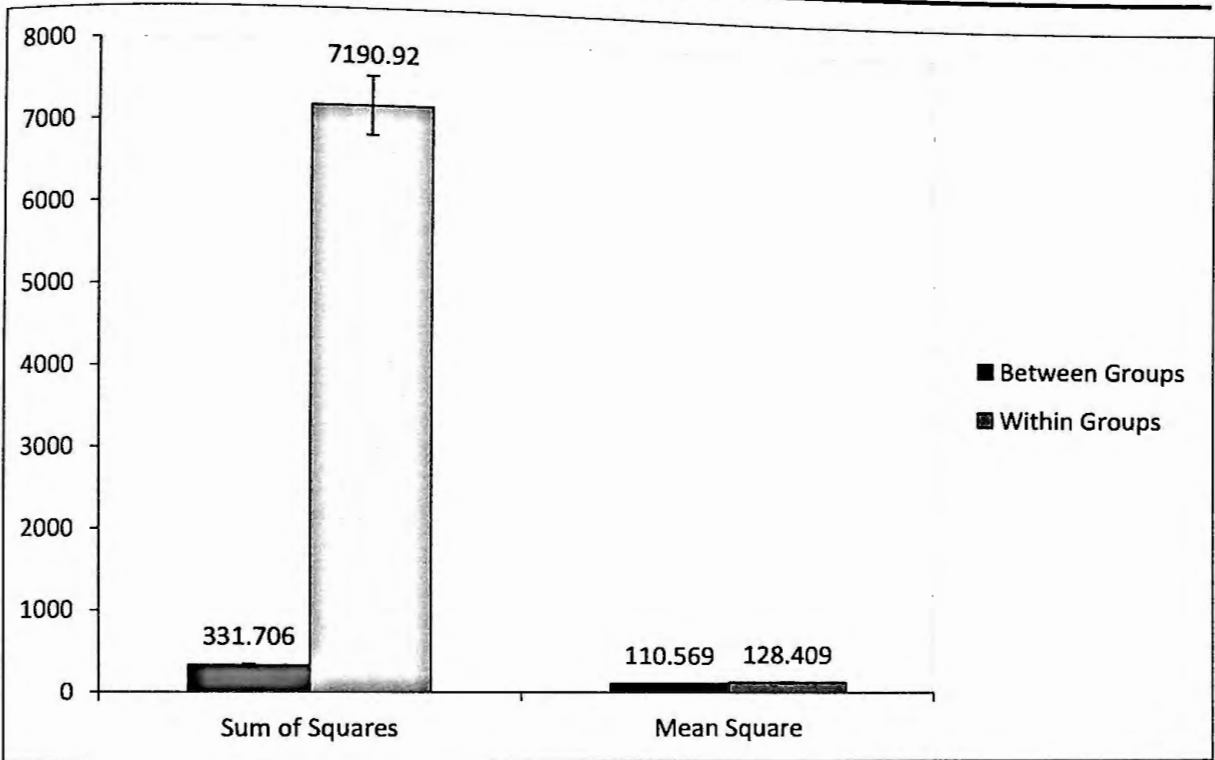
**Table 20:** Calcium content in drinking water collected from patient of Charghat Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	13.5854	6.24009	1.61118	10.1297	17.0410	2.62	22.13
B	15	12.3266	7.69904	1.98788	8.0631	16.5902	2.13	27.94
C	15	16.0942	8.53362	2.20337	11.3685	20.8200	2.56	27.94
D	13	13.2892	8.38009	2.32422	8.2252	18.3532	2.72	24.57
Total	58	13.8423	7.66939	1.00704	11.8257	15.8589	2.13	27.94

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115.493	3	38.498	.642	.591
Within Groups	3237.223	54	59.949		
Total	3352.717	57			



**Figure 4.22:** Calcium content in drinking water collected from patient of Charghat Upazilla.

Table 20 & Figure 4.22 illustrates among the 58 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 13 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 115.493 (Between groups) and 3237.223 (within groups). Mean Square 38.498 (Between groups) and 59.949 (within groups). df 3 (Between groups) and S4 (within groups)- F value 0.642 and significant 0.591 which is non-significant.

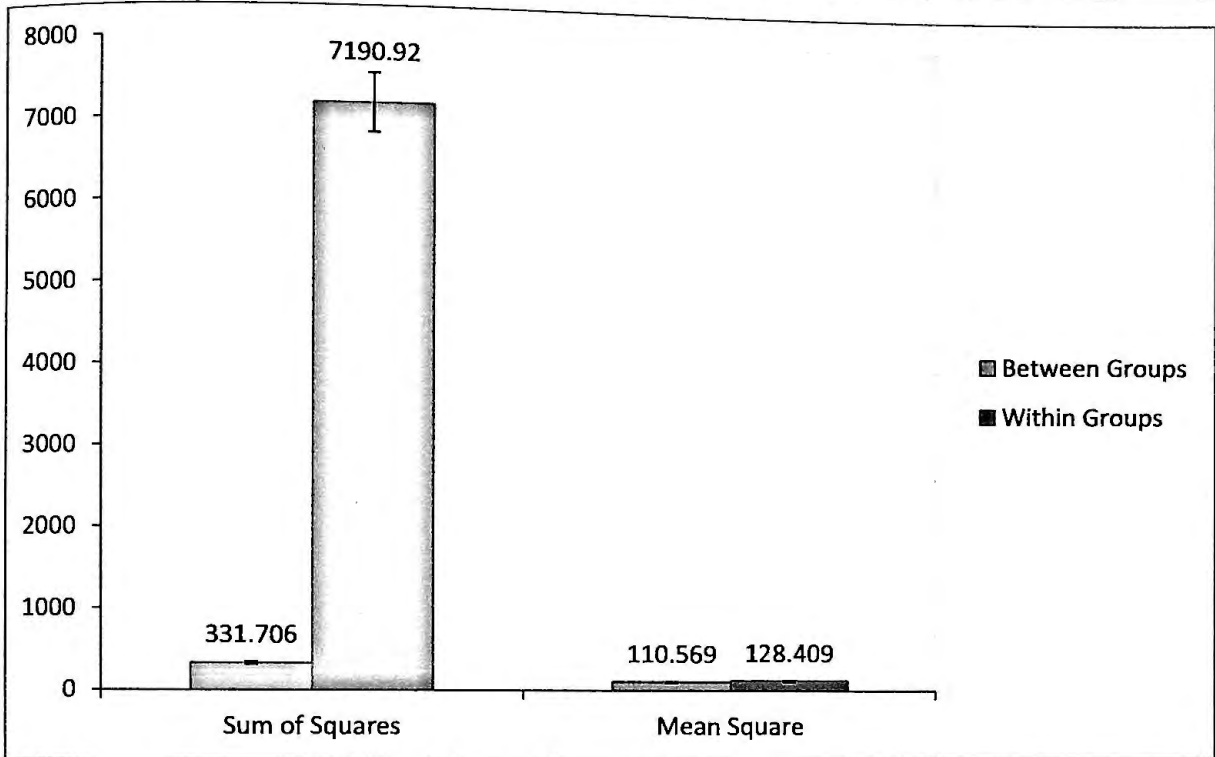
**Table 21:** Calcium content in drinking water collected from patient of Durgapur Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	19.6894	10.59976	2.73685	13.8194	25.5593	2.10	32.4
B	15	20.4962	11.82465	3.05311	13.9479	27.0445	2.61	34.1
C	17	15.4064	9.62516	2.33444	10.4576	20.3552	4.82	33.2
D	16	12.9031	8.51897	2.12974	8.3637	17.4426	2.26	27.5
Total	63	17.0023	10.39924	1.31018	14.3832	19.6213	2.10	34.1

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig-
Between Groups	603.568	3	201.189	1.945	.132
Within Groups	6101.369	59	103.413		
Total	6704.937	62			



**Figure 4.23:** Calcium content in drinking water collected from patient of Durgapur Upazilla.

Table 21 & Figure 4.23 illustrates among the 63 respondents, age were divided in groups 15 respondents were in A groups (3-20), 15 respondents were in B groups (21-40), 17 respondents were in C groups (41-60), 16 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 603.568 (Between groups) and 6101.369 (within groups). Mean Square 201.189 (Between groups) and 103.413 (within groups). F value was 1.945 and significant was 0.132 which is non-significant.

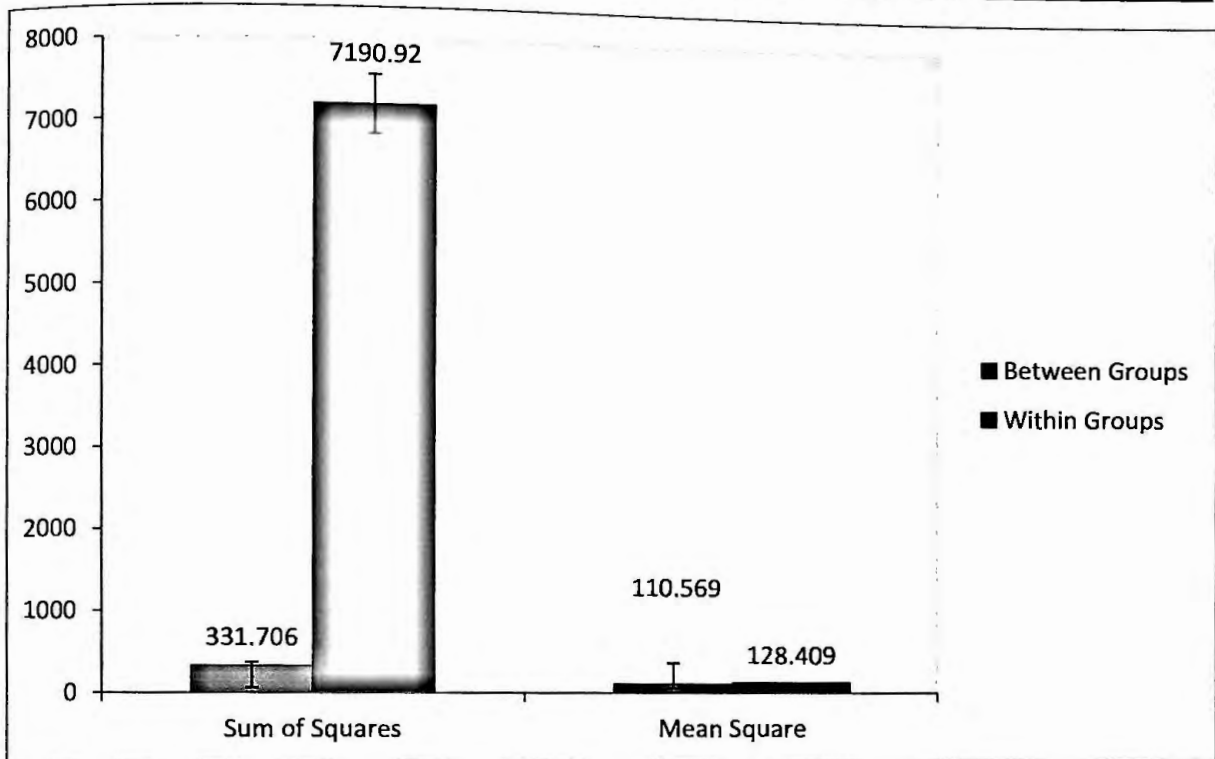
**Table 22:** Calcium content in drinking water collected from patient of Godagari Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	19.8579	10.01010	2.58460	14.3145	25.4013	2.53	33.93
B	14	17.7326	12.98716	3.47096	10.2341	25.2312	2.50	34.19
C	17	14.9470	9.57575	2.32246	10.0236	19.8704	3.85	29.94
D	17	28.1126	18.15633	4.40356	18.7775	37.4477	2.46	51.66
Total	63	20.2879	13.88722	1.74963	16.7905	23.7854	2.46	51.66

**A=3-20, B=21-40, C= 41-60, D = Above 60**

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1619.954	3	539.985	3.082	.034
Within Groups	10337.047	59	175.204		
Total	11957.002	62			



**Figure 4.24:** Calcium content in drinking water collected from patient of Godagari Upazilla.

Table 22 & Figure 4.24 illustrates among the 63 respondents, age were divided in groups 15 respondents were in A groups (3-20), 14 respondents were in B groups (21-40), 17 respondents were in C groups (41-60), 17 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 1619.954 (Between groups) and 10337.047 (within groups). Mean Square 539.985 (Between groups) and 175.204 (within groups). F value is 3.082 and significant 0.034 which is significant.

**Table 23:** Calcium content in drinking water collected from patient of Mohanpur Upazilla.

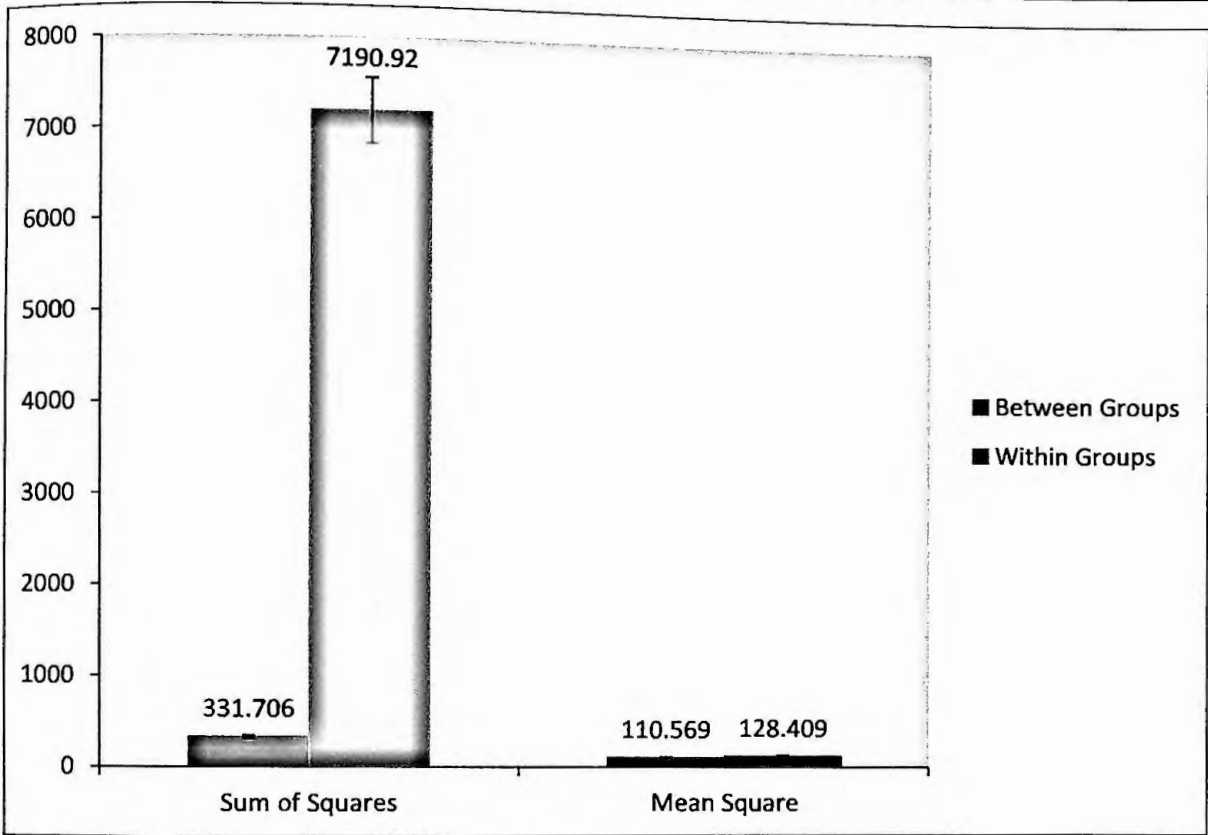
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	13	12.4611	6.95254	1.92829	8.2597	16.6625	3.63	23.73
B	13	13.3152	9.66360	2.68020	7.4756	19.1549	2.10	33.26
C	14	20.2379	10.85188	2.90029	13.9722	26.5036	2.31	33.96
D	14	15.9835	9.61866	2.57069	10.4299	21.5372	2.68	29.02
Total	54	15.5962	9.64804	1.31293	12.9628	18.2296	2.10	33.96

A=3-20, B=21-40, C= 41-60, D = Above 60

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	499.146	3	166.382	1.876	.146
Within Groups	4434.342	50	88.687		
Total	4933.488	53			





**Figure 4.25:** Calcium content in drinking water collected from patient of Mohanpur Upazilla.

Table 23 & Figure 4.25 illustrates among the 54 respondents, age were divided in groups 13 respondents were in A groups (3-20), 13 respondents were in B groups (21-40), 14 respondents were in C groups (41-60), 14 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 499.146 (Between groups) and 4434.342 (within groups). Mean Square 166.382 (Between groups) and 88.687 (within groups). F value was 1.876 and significant 0.146 which is non-significant.

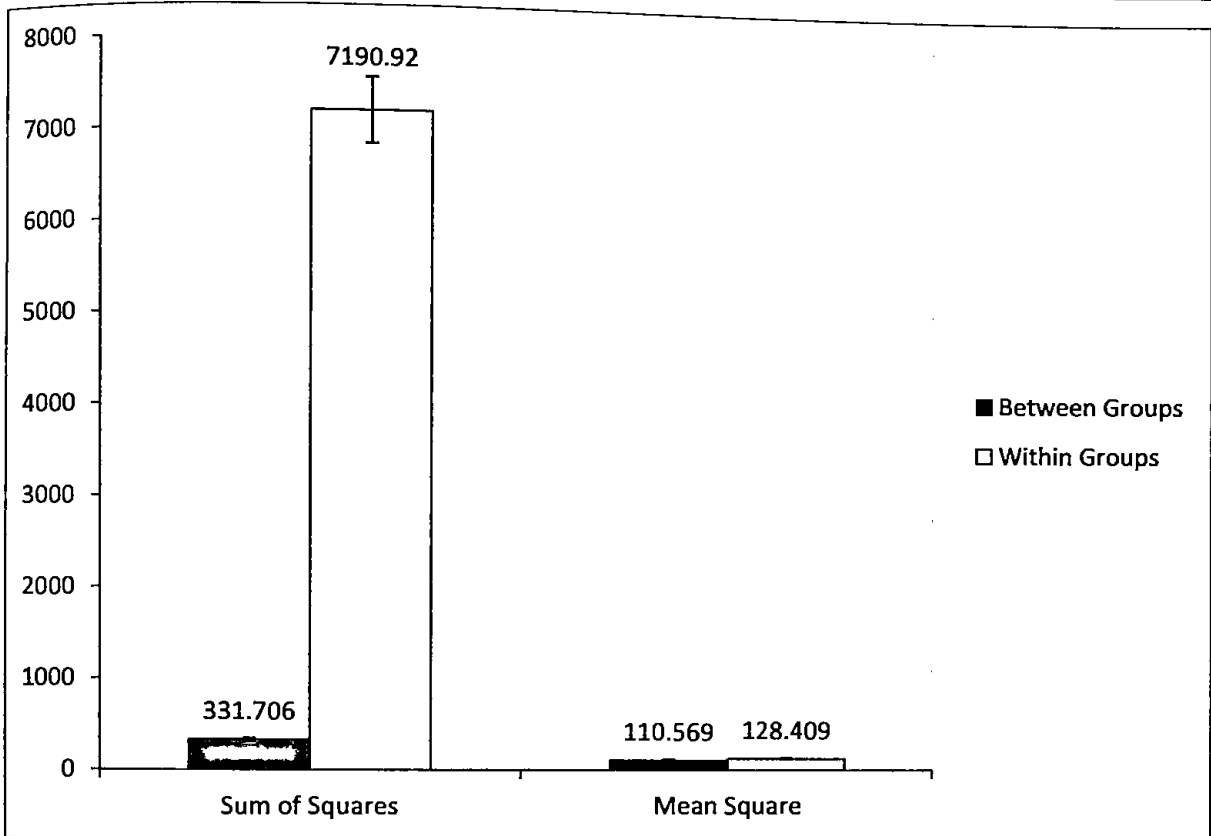
Table 24: Calcium content in drinking water collected from patient of Puthia Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence		Minimum	Maximum
					Lower Bound	Upper Bound		
A	13	15.7269	6.53039	1.81120	11.7806	19.6731	5.92	24.93
B	15	30.0226	13.91121	3.59186	22.3188	37.7263	8.35	52.17
C	15	20.5253	7.67548	1.98180	16.2748	24.7759	8.86	33.60
D	15	16.0218	7.03610	1.81671	12.1253	19.9182	4.48	26.30
Total	58	20.7413	10.83589	1.42282	17.8921	23.5904	4.48	52.17

A=3-20, B=21-40, C= 41-60, D = Above 60

## ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1953.815	3	651.272	7.421	.000
Within Groups	4738.931	54	87.758		
Total	6692.746	57			



**Figure 4.26:** Calcium content in drinking water collected from patient of Puthia Upazilla.

Table 24 & Figure 4.26 illustrates among the 58 respondents, age were divided in groups 13 respondents were in A groups (3-20), 13 respondents were in B groups (21-40), 15 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). According to ANOVA analysis Sum of Squares 1953.815 (Between groups) and 4738.931 (within groups). Mean Square 651.272 (Between groups) and 87.758 (within groups). F value 7.421 and level of significance .000 which is highly significant.

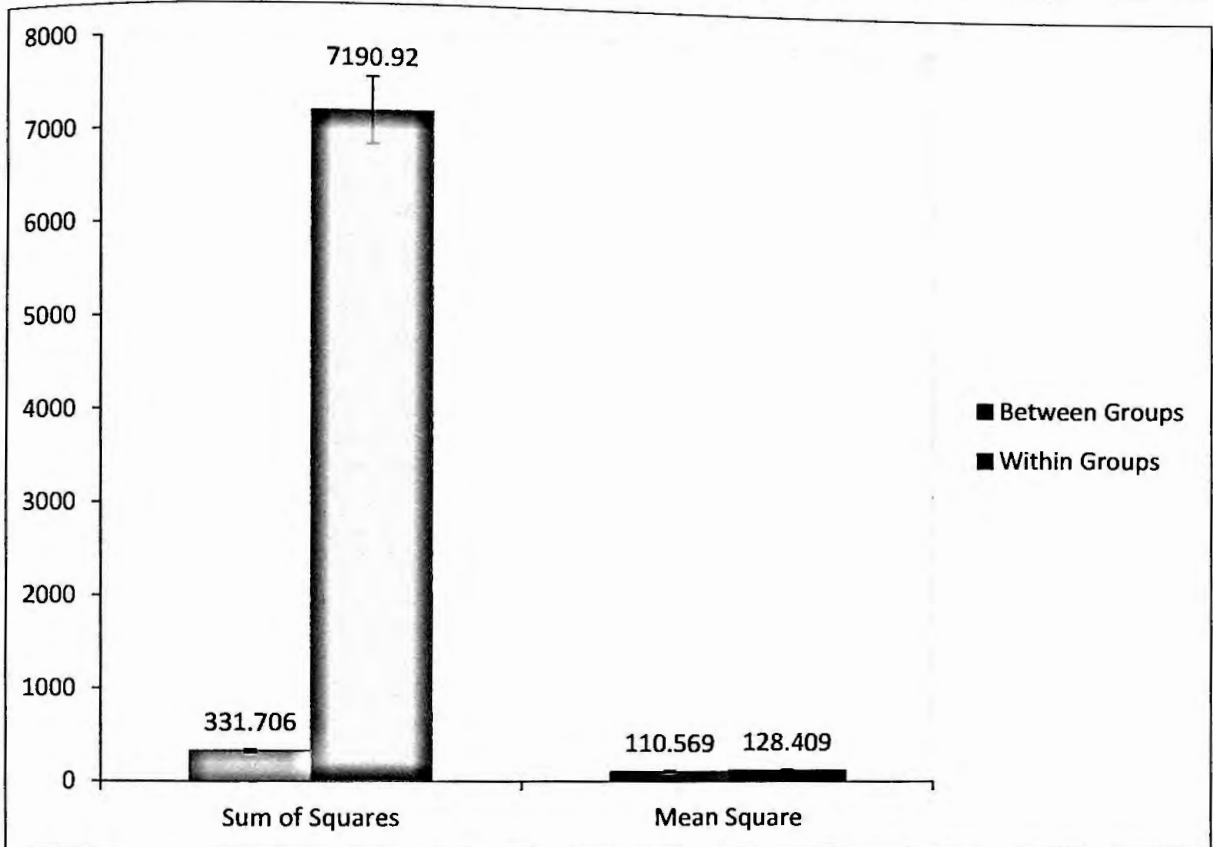
**Table 25:** Calcium content in drinking water collected from patient of Tanore Upazilla.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	15	16.8702	12.64442	3.26477	9.8680	23.8725	2.61	35.96
B	16	21.5628	16.28819	4.07205	12.8834	30.2421	3.85	71.27
C	14	18.1169	5.80400	1.55118	14.7657	21.4680	2.50	24.92
D	15	15.3006	6.18225	1.59625	11.8770	18.7242	4.28	27.20
Total	60	18.0201	11.29168	1.45775	15.1031	20.9370	2.50	71.27

**A=3-20, B=21-40, C= 41-60, D = Above 60**

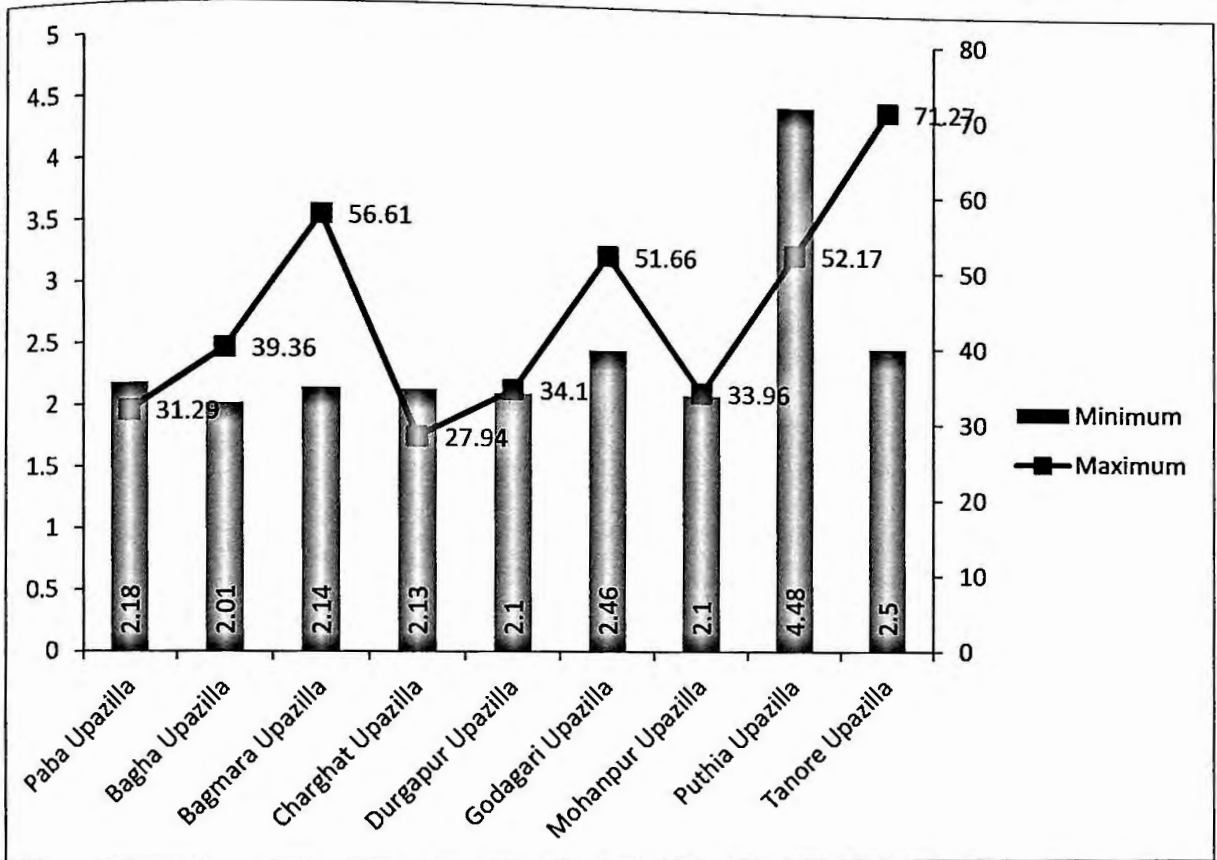
### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	331.706	3	110.569	.861	.467
Within Groups	7190.920	56	128.409		
Total	7522.626	59			



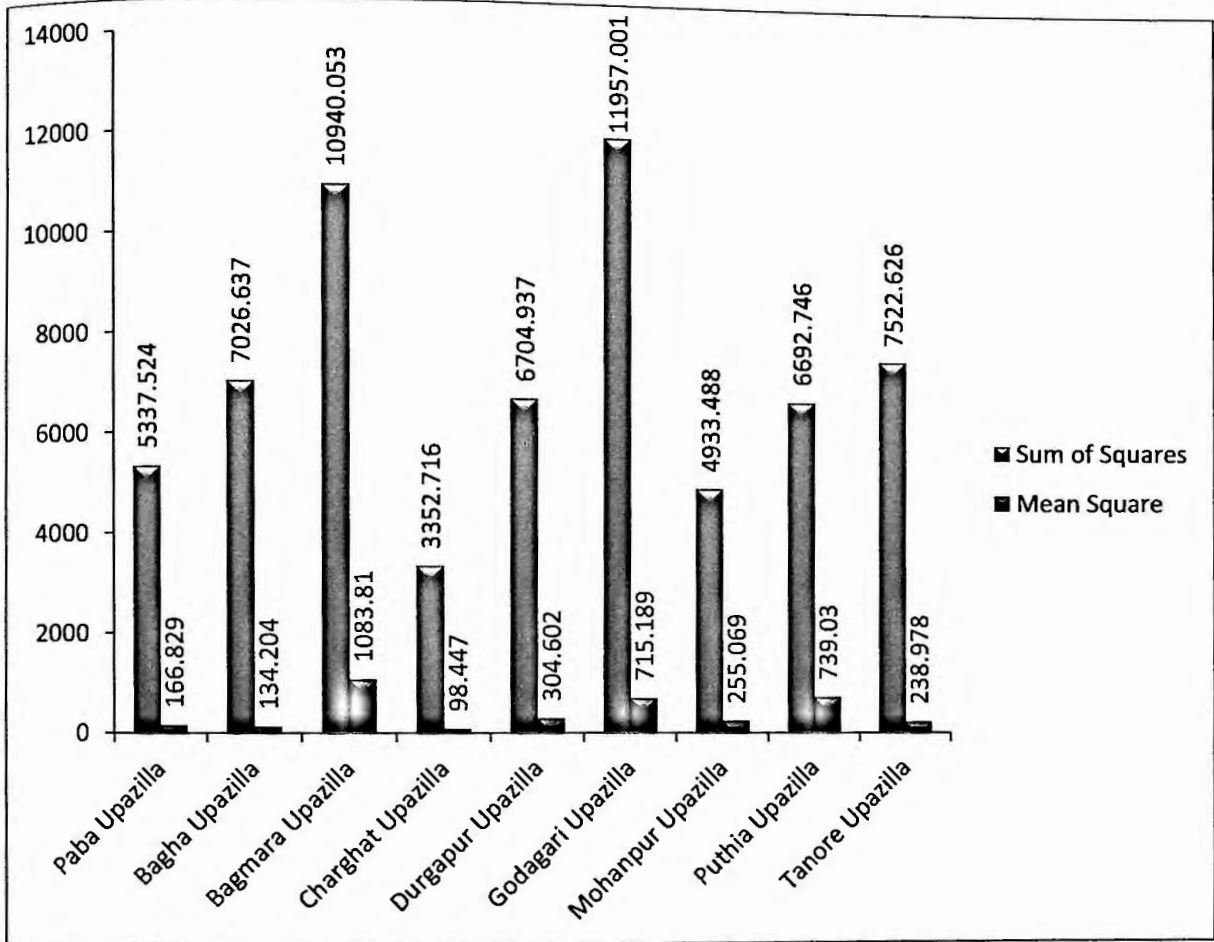
**Figure 4.27:** Calcium content in drinking water collected from patient of Tanore Upazilla.

Table 25 & Figure 4.27 illustrates among the 60 respondents, age were divided in groups 15 respondents were in A groups (3-20), 16 respondents were in B groups (21-40), 14 respondents were in C groups (41-60), 15 respondents were in D groups (Above 60). On ANOVA analysis Sum of Squares 331.706 (Between groups) and 7190.920 (within groups). Mean Square 110.569 (Between groups) and 128.409 (within groups). F value was 0.861 and significance 0.467 which is non-significant.



**Figure 4.28:** Maximum and minimum level of Calcium in different Upazilla in Rajshahi region.

Calcium Concentration in water samples collected from different Upazilla ranges from 27.94 to 71  $\text{gml}^{-1}$ . Note that Tanore Upazilla contains the highest amount of fluoride and Charghat Upazilla contains the lowest amount of fluoride.



**Figure 4.29:** Sum of square and Mean Square of Calcium in different Upazila in Rajshahi region.

The figure Express that the range between sum of squares and mean squares is highest in Godagari Upazilla.

### 4.3 Microbiological analysis

**Table 26:** Microbiological analysis of drinking water collected from patient of different Upazilla.

Name of the upazillas	N	Mean	Std. deviation	Std. Error	95% Confidence Interval for mean	
					Lower Bound	Upper Bound
Paba	20	33.75	9.453	2.114	33.642	33.855
Mohanpur	20	40	21.052	4.709	39.906	40.094
Bagha	20	39.37	19.74	4.416	39.15	39.59
Bagmara	20	41.67	24.013	5.372	41.384	41.938
Durgapur	20	44.36	31.232	6.987	43.966	44.709
Puthia	20	37.5	16.118	3.605	37.3198	37.6802
Charghat	20	36	13.473	3.014	35.849	36.1507
Tanor	20	31.5	6.96	7.046	31.423	31.577
Godagari	20	39.2	19.40	4.340	38.983	40.11

### ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4384.09	8	3555.65	66.37	.000
Within Groups	287216.91	531	53.569		
Total	291601	539			

Table 19 express that the microbiological analysis which was done by membrane filtration method. In this study degree of freedom are 8 and 539. The table of F distribution at 5% level of significance is 66.37.



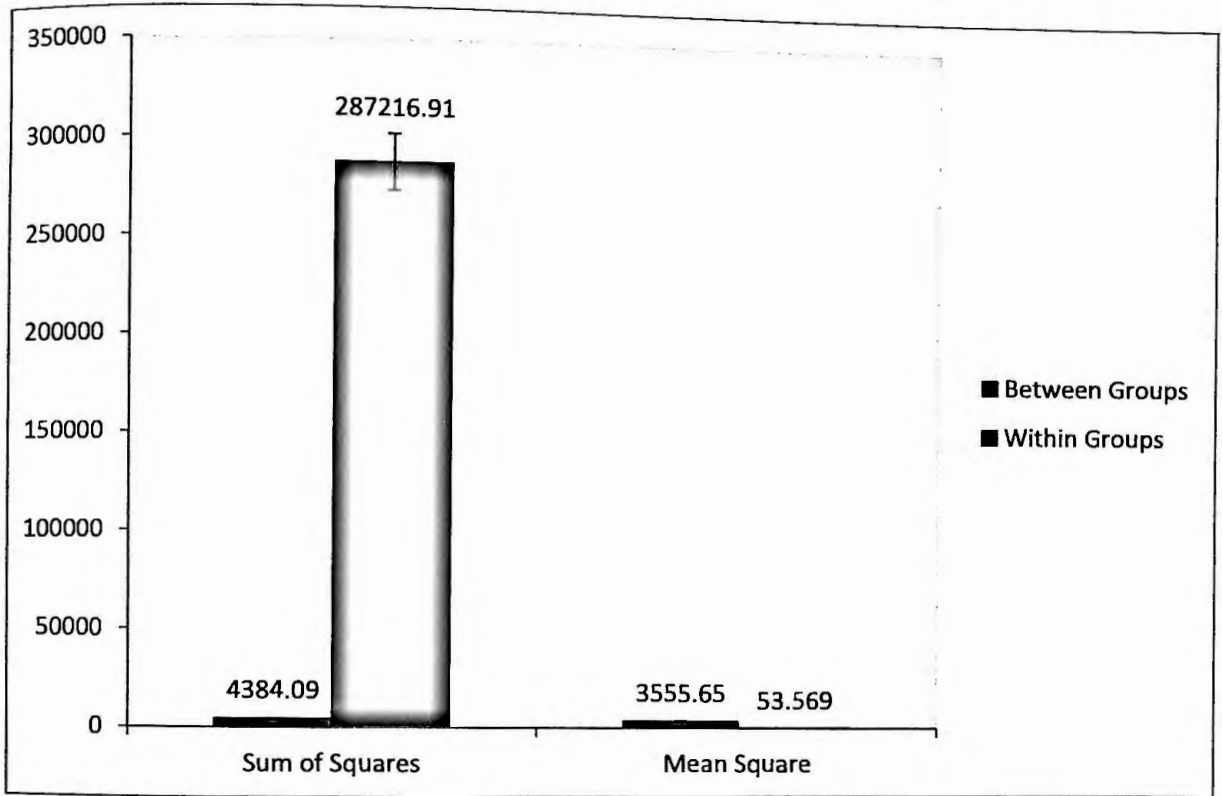


Figure 4.30: Microbiological analysis of drinking water collected from patient of different Upazilla.

**CHAPTER V**  
**DISCUSSION, CONCLUSION AND**  
**RECOMMENDATION**

# DISCUSSION, CONCLUSION AND RECOMMENDATION

## 5.1 Discussion

Despite exceptional reduction within the prevalence of caries in developed countries, caries continues to be an extremely prevailing illness among kids and adult in developing countries. According to the National Institute of Dental and craniofacial analysis, regarding 92% of adult (ages 20-64) have caries teeth in rural areas. Preventing at caries is difficult because the incidence of the illness is extremely high generally population in upazilla and have it happens in economically derived people that cannot give the commercially on the market oral hygiene product. Though current interest is specializing in the potential preventive role of certain nutrients.

Dental caries may be a multi-factorial malady influenced by several factors together with age, sex, diet, microorganisms, trace parts, saliva, genetic predisposition and tooth morphology. In the gift study, we discover slightly high variety of tooth decay cases among females ( $P=0.510$ ) as compared to males ( $P=0.490$ ) suggesting that females square measure additional at risk of tooth decay than males (Table 1). This will be attributed to variety of facts, together with early teeth eruption in ladies as compared to boys, variations in dental attending because of lack of monetary independence on the a part of females and worry of medical man among male and feminine and additionally to distinction in dietary pattern between housewives and dealing man.

Among the type of most frequent caries in the patients, we found the occlusal pits and fissure caries ( $P=0.808$ ) as highly prevalent (Tables 2 and 3). The smooth surface caries ( $P=0.125$ ) ranked second followed by root caries ( $P=0.058$ ) and recurrent caries ( $P=0.010$ ) at third and fourth position respectively. Highest prevalence of occlusal pits and fissure caries can be correlated with the architecture of these sites, which is more retentive to carry food substances and is not fully exposed to flushing action of saliva. The biofilm tends to form and mature in these locations on the tooth including approximal surface cervical to the contact point, and along the gingival margin especially during eruption. These areas are relatively protected from mechanical wear by tongue, cheeks, abrasive food, and tooth brushing. Thus, these are the sites where caries lesions may become visible. Similar observations have been obtained by many scientists indicating that, there is a relative lack of proper preventive procedures for such type of caries. The smooth surface caries is generally correlated with the long term poor oral hygiene and long term accumulation of plaque which is a very common practice among the rural area due to lack of awareness regarding the dental hygiene.

About 85.57% people in rural region used shallow well for the drinking water. Only 6.73% used well water and 7.69% used surface water for drinking purpose (Table 4). It has been verified that dental caries is a process due to formation of acid by fermentation of sugar through acidogenic bacteria that lead to decalcification of dental enamel. But this acid is usually neutralized with the buffering action of saliva and the dental caries is prevented. When fermentable carbohydrate was not added to the saliva, putrefaction replaced fermentation, alkalinity replaced acidity, and no decalcification is usually observed. Fluoridated water may reduce the amount of acid in their mouth and relatively be protected from dental caries. But the content of fluoride is variable in the rural areas of Bangladesh.

Community water fluoridation is a public health tool that benefits the entire community by providing affordable access to prevent tooth decay. It has been proven safe and effective for more than 70 years and was listed as one of 10 great public health achievements of the 20<sup>th</sup> century by the Centers for Disease Control and Prevention.

While easy access to community fluoridation has proven benefits, there are many reasons why communities may not choose to fluoridate their water, including misinformation and myths about current programs, and challenges' ensuring their water is optimally fluoridated once a program is established.

Action for Dental Health advocates for fluoridation based on evidence of efficacy and cost effectiveness to bring the population receiving optimally fluoridated water to 80% by 2020.

More than 70 years of research has systematically shown that an optimum level of Fluoride in community water is safe and effective in preventing caries by a minimum of 25% in each youngsters and adults. Just by drink, Americans will like fluoride's cavity protection whether or not they area unit reception, work or faculty. The Centers for malady management and bar named community water addition one in all 10 great public health achievements of the 20<sup>th</sup> century.

In the gift study we have a tendency to found that in some geographic area Fluoride content in drink but traditional level (<.7ppm).

About 60 respondents in each upazilla of in Rajshahi region were selected and collected sample are investigated. The information regarding dental caries patients were taken in a data sheet. Investigation was done in two categories. One was chemical analysis and other was microbiological analysis of the drinking water of

the respondents Fluoride content in drinking water was collected from the patient the F value was found variable which was up to 16.126. The significant result was found in Tanore, Puthia, Upazilla. Highly significant was observed in Bagha, Bagmara, Charghat, Durgapur Upazilla. Whereas Others non-significant result was found in Paba, Godagari, Mohonpur Upazilla. In case of calcium, F value was analyzed as variable which was recorded up to 41.151. The level of significant was different in each upazilla. In Godagari Upazilla is significant. In Paba, Bagha, Charghat, Durgapur, Mohonpur and Tanore the result were extremely important on the microbiological analysis that represent the *Escherichia coli* and coliforms contamination accounted for twenty 29.80% and 88.2% severally.

Among the different age group studied we found highest number of cases in between 21 to 30 year peoples (Table 5). It might be suggested that, the development of dental caries is a long term process, and the habit of sugar consumption is relatively high among the teenagers (In the form of chocolates and other sticky sugar rich food), but the peoples usually don't attend the hospitals, until and unless they feel unbearable pain in the mouth. So we have found most number of dental caries patients among this group. But the number of cases continually increased up to the age of 21-30 ( $P=0.269$ ) and later on these cases got declined. But a more detailed and long term study is required to analyze the long term effect of age on the development of dental caries among the population. However caries is traditionally reported as DMFS (Decayed, missing filled surface)/ DMFT (Decayed, missing filled teeth) index, but this index neither a ratio nor a percentage and does not reflect the part of population having caries. Beside it this index does not work with the study of type of caries and many other issues, so we did not analyzed this index during our study. These results on prevalence of dental caries can be correlated with other findings.

The inverse relationship between Fluoride in water and cavity is well established. with none dietary modification topical Fluoride in either dentifrice, mouth rinses dental caries by between 20 and 40% however doesn't eliminate cavity. The metallic element concentration of plaque powerfully influence the balance between Diamond State and remineralisation of enamel Rugg Gum *et al.*, found Associate in Nursing inverse relationship between the start of metallic element and dental caries entombment.

## 5.2 Conclusion

The fluoridation of drinking water often has both support and opposition unit within different communities. This is controversial due to the fact that fluoride and calcium have been found to have beneficial effect at optimal level. This is intentionally added to many public water supplies at higher concentration which cause toxic effect. The environmental protection agency regulates the quantity of Fluoride that will be gift publicly water to shield against Fluoride cyanogenetic result. Beverage could also be a contributor of atomic number 20 in diet and will be necessary for those that square measure marginal for atomic number 20 intake. However in geographic area there's no target that facet. They need Fluoride in water from natural supply. That is kind of optimum so, there cavity prevalence depends not solely Fluoride and atomic number 20 content in their beverage however conjointly others factors. The count of passageway coliform was satisfactory. The conclusion of the study is that wherever there's smart exposure to Fluoride and atomic number 20, sugar consumption could be a moderate risk issue for cavity in the general public in geographic area. Sugar consumption is probably going to be an additional powerful indicator for risk of cavity in persons UN agency don't have regular exposure to Fluoride and atomic number 20. The unclean coliforms don't seem to be to blame for cavity as a result of they're not cariogenic microorganism.

### **5.3 Recommendation**

The perception of the patient of dental caries about their drinking water and caries prevention is important in rural area of Rajshahi region in order to develop effective measures. Patient's health behaviors, habits and practices usually have a direct influence on their oral health.

Following recommendation to improve the food and drinking habit about oral health among the people in the upazilla level-

- People within the upazilla level should receive applicable preventent steering and conjointly have to be compelled to appreciate the advantages of Fluoride and atomic number 20 to the oral health and well-being of them.
- Need to have access to instructional activities in oral health so as to place into observe data the data they need and conjointly acquire new knowledge necessary to avoid dental issues.



# REFERENCES

## REFERENCES

- Abok Elisha; Michael wardayi okoth, cantherine Nkirote kunyanga and Bernard ochicng Aliwa; Microbiological Quality and contamination level of water sours in Isiolo county country in Kenya" (2018) Article ID 2139867.
- Acharya Turkesar, "Membrane filtration in water treatment removal of micsopollutionts", Chemistry of Advanced Environmental Purification Processes of Water (2012). pp. 199-248.
- Akhter P, Baloch N, Mohammad D, Orfi SD and Ahman N (2004). Assessment of strontium and calcium levels in Pakistani dict. J. Environ. Rabioact.73:247-256.
- Almedon AM, Blumein TU and Manderson L (2009). Hygiene evaluation procedures; Approaches and methods for assessing water and Sanitation-related hygiene practices, Practical Action Publishing, 22(9): 8-7.
- Ando M, Tadano M, Asanuma S, Tamura K, Matsushima S, Watanabe T, Kondo T, Sakurai S, Ji R, Liang C and Cao S (1998). Health effects of indoor fluoride pollution from coal burning. Environmental Health Perspectives, 106(5): 239-244.
- Anony M (1990). Drinking Water After of China, Beijing, China Cartographic Publishing House, pp, 91-92.
- Antia FE (1962). The dental Caries in experience of school going children in the city of Bombay. J Indian Dent Assoc. 39: 325.
- APHA (1992).Standard methods for the examination of water and waste water 18thed. American public health Association Washington DC.

- Bagramian RA, Garecia-Godoy F and Volpe AR (2009).The global increase in dental caries. A pending public health crisis, *American Journal of Dentistry*, 22(1): 3-8.
- Bali RK, Mathur VB, Talwar PP and Channa HB (2004).National Oral health Survey, Srilanka 2002-03, Ministry of health and Nutrition, pp. 1-176.
- Balogun SA, Akingbade AO, Oyekunle MA and Okerentugba PO (2014). Physiochemical and microbiological profile of drinking water sold in Abeokuta, Ogun State. *Nigeria Nature and Science*, 12:103-105.
- Barrard WR, Wordstrom DK (1982). Fluoride in precipitation-I.Methodology with fluoride-selective electrode. *Atmospheric Environment*, 16: 99-103.
- Bell RA, Quandt, SA, Spangler JG and Case D, (2002). Dietary calcium intake and supplement use among older African American, White and native American women in a rural southeastern community. *J. Am Diet. Assoc.* 102(6): 844-847.
- Beltran-Aguilar ED; Barker LK, Carto MT, Dye BA and Goocla BF (2005). Griffin Social Surveillance for dental caries, dental sealants, tooth retention. Edentalism and enamel fluorosis- United states, 1999-2002 *MMWR surreally Summ*, 54(3): 1-43.
- Bhaskar DJ, Sardana V, Aswini YB and Aruna DS (2011). Prevalence of dental caries and treatment needs among 12 year old School going children in rural Bagalkote, Karnatake, *J. Indian Dent Assoc.* 5(1): 85-86.
- Boubetra A, Nestour FL, Allaert C and Feinberg M (2011).Validation of alternative methods for the analysis of drinking water and their applications to *Escherichia coli*. *Applied and Environmental Microbiology*, 77(10): 3360-3367.

- Bourgeois D, Nihtila A and Mersel A (1998). Prevalence of caries and edentulousness among 65-74-year-olds in Europe/D. Bourgeois, A. Nihtila, and A. Mersel. *Bulletin of the World Health Organization*, 76(4): 413-417.
- CaO SR and Li YF (1992). The evaluation of indoor air quality in areas of endemic Fluorosis caused by coal combustion. In *Proceedings of the XIX conference of the International Society for Fluoride Research*, Kyoto, Japan, Department of Hygiene and Public Health, Osaka Medical College, p.38.
- Cao SR *et al.*, (1992). Study on preventive and control measure on coal-combustion type endemic fluorosis in the Three Gorges area in China. *Proceeding of the Fourth. National Academic conference on Endemic Fluorosis*. *Chinese Journal of Endemic Disease*, 11: 6-21.
- Charlton KE, Steyn K, Levitt NS, Zulu JV, Jonathan D, Veldman FJ and Nel JH (2005) Diet and Blood pressure in south Africa. Instance of foods containing sodium potassium, calcium and magnesium in three ethnic groups *Nutrition* 21(1): 39-50.
- Chen EJ *et al.*, (1988). A nation wide survey on drinking water quality and water borne diseases in China. *Beijing, Monitoring, Chinese Academy of Preventive Medicine*, pp. 95-99.
- Choubisa SL *et al.*, (1997). Fluorosis in some tribal villages of Dungapur district of Rajasthan, India. *Fluoride*, 30: 223-228.
- Cowson RA and Odell EW (2002). "Dental Caries:" *Oral Pathology and Oral medicine*, 7(3): 36-52.
- Daida Y, Novotny R, Grove, JS, Acharya S and Vogt TM (2006). Ethnicity and nutrition of adolescent girls in Hawaii. *J. Am. Diet Assoc.* 106: 221-226.

- Damle SE and Patel AR (1994). Caries prevalence and treatment need amongst children of haravi, Bombay, India. *Community Dent Oral Epidemiol*, 22(1): 62-63.
- David R ferguson; Dr. A Shuttle worth, Dr. D.K whittler, "Fluoride and dental caries": *Oral bioscience*( 2012,) 1 (10) 195-208.
- De Oliveira KMH, Memezio MA, Romualdo PC, de Silva RAB, de Paula E, Silva FWG and Kuchler EC (2017). Dental Flossing and Proximal Caries in the Primary dentition: A systemic Review. *Oral Health and preventive dentistry*. 15(5): 427-434.
- Dean HT (1942).Epidemiological Studies in United Stater. In: Moulton FR, ed. Fluoride and dental health. Washington, DC, American Association for the Advancement of Science (AAAS Publication No.19).
- Desh JK, Sahoo PK, Bhuyan SK and Saboo SK (2002). Prevalence of dental caries and treatment needs among children of Cuttak (Orissa). *J. Indian Soe Pedod Prev Dent*, 20(4): 139-43.
- Dhara V, Join A, Dyke VTE and Kohli A (2007). Prevalence of Dental Caries and Treatment Needs in the School Going Children of Rural Areas in Udaipur district. *J. Indian, Soe Pedod Preve Dent*, 25(3):119-21.
- Dr. Shabeel PN (2009). Classification of dental caries, <http://www.apexiondental.com>.
- Dr. Tirthankar Debnath (2002). Prevention of dental diseases; public health and preventive dentistry, 2: 114-127.
- FAO/WHO (2001). Human vitamin and mineral Requirement. Report of a Joint FAO/WHO Expert consultation. Food and Agriculture Organization of the united Nations, Rome, and world Health organization Geneva, p. 1-303.

- Featherstone JDB (2004). The continuum of dental caries-evidence for a dynamic disease Process. **83**:39-42.
- Fejerskov O, Nyvad B and Kidd EA (2008). Pathology of dental caries." in fejerskov O, Kidd EAM (eds) Dental caries: The disease and its chemical management Oxford. Blackwell manksgaurds, 1.2: pp. 20-48.
- Fitzgerald J *et al.*, (2000).Groundwater quality environmental health implications, Anargu Pitjantjatjara. Lands, South Australia, Canberra, Burue of Rural Sciences, **6**: 89.
- Fuhong R and Shuqin J (1988). Distribution and formation of high- fluoride groundwater in china Environmental Geology and water Science, **12**(1): 3-10.
- Galan P, Arnaud MJ, Czernichow S, Dlabroise AM, Preziose P, Bertraise S, Franchisseur C, Maurel M, Favier A and Hecbeg S (2002). Contribution of Mineral Waters to Dictary . Calcium and Magnesium in A French Adult Polulation J. Am Dict. Assoc. **102**: 1658-1662.
- Gangil R, Tripathi R, Patyal A, Dutta P and Mathiur KN (2013). Bacteriological Evaluation of Packaged, Bottled water, Sold significance, Veterinary world, **6**(1): 27-30.
- Goel R, Vedi A, Veersha KL, Sogi GM and Gumbhir RS (2015). Oral hyגיע Practices and dental caries prevalence among 12 and 15 years school children in Ambala; Haryana- A cross-sectional Study, J Elin Exp Dert. **7**(3): 374-79.
- Hardie JM (1982). The microbiology of dental caries "Dental update. **9**(4): 199-200, 204-4, 206-4.

- Hiremath A, Murugaboopathy V, Ankola AV, Hebbal M, Mohandoss S and Pastay P (2016). Prevalence of Dental Caries Among Primary School Children of India, A Cross-Sectional Study, 10(10): 47-50.
- Holloway PJ: Moore, W.J (September, 1983) "The role of sugar in the etiology of dental caries" Journal of Dentistry 11(3): 18.
- Homa Amini, Hammersmith, Mark D Sicgal, Paul S, Casamassimo (2019). Ohio Dentists Awareness and Incorporation of the Dental home Concept, 41(1): 20-24
- Ingle NA, Dubey HV, Kaur N and Gupta R (2014). Prevalence of dental caries among School children of Bharatpur city, India, Journal of International Society of Preventive and community dentistry, 4(1): 52-55.
- IPCS (2002). Fluorides Geveva, World Health organization, International Programme on Chemical Safety, (Environmental Health criteria 227), pp. 25-31.
- IPES (1984). Fluoride and fluorides Geveva, World Health organization, International Programme on chemical safety, Environmental Health criteria, pp. 1-99.
- Irene W and Kimaru, "Using Classical EDTA titration to measure calcium and Magnesium in intravenous fluid Bajs" (2018). Journal of chemical education. 95(12).
- Janssen PJCM; Janus JA, Knaap AGAC (1988). Integrated Criteria document Fluoride Effects. Beethoven, National Institute of Public Health and Environmental Protection.(Appendix to Reports No. 75847005).
- Jolly SS *et al.*, (1968). Epidemiological, Clinical and biochemical study of endemic dental and skeletal fluorosis in Punjab, British medical Journal, 4: 427-429.

- Joshi N, Sujan S, Joshi K, Parekh H and Vave B (2013). Prevalence, Severity and related factors of dental caries in School going children of vadodara city- An epidemiological Study. *J Int. Oral Health*, 5(4): 35-39.
- Karunakaran R, Somasundaram S, Gawthaman M, Vinodh S, Manikandan S and Gokulnathan S (2014). Prevalence of dental caries among School going children in Namkkal district: A cross sectional study. *J. Pharm Bioallicd Seci.* 6(1): 160-61.
- Kassebaum NJ, Smith AGC, Bernabé E, Fleming T D, Reynolds AE, Vos T, Murray CJL and Marcenes W (2017). Global, Regional, and National Prevalence, Incidence and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors, GBD 2015 Oral Health Collaborators, 69(4): 380-387.
- Kidd EAM (2004). The Scientific basis for caries control and practical detail for delivery of caries control to the individual home been given, 38(3): 305-13.
- Laudenbach JM and Simon Z (2014). Common Dental and Periodontal Diseases: Evaluation and Management. *The medical clinics of North America* 98(6): 1239-1260.
- Li Y *et al.*, (2001). Effect of long term exposure to fluoride in drinking water on risks of bone fractures *Journal of Bone and Mineral Research*, 16(5): 932-939.
- Liang C, Ji R and Cao S (1997). Epidemiological analysis of endemic fluorosis in china. *Environmental carcinogenesis and eco to axiology review*, 15(2): 123-138.



- Liu JW *et al.*, (1987) Measurement of low level fluoride in water and water based products using a fluoride electrode and an ion analyzer with automatic calibration program. *Journal of Micronutrient Analysis*, **3**:295-305.
- Lukaes JR and Langaespada LL (2006). Explaining Sex difference in dental caries prevalence: Saliva, normores and "Life history" *Etiologias Am J ham Biol*, **18**: 450-55.
- Madigan MT and Martinko JM (2006). *Brock-Biology of Micro-organism 11th Ed*, Pearson, USA. pp. 705.
- Mahesh KP, Joseph T, Verma RB and Jayanthi M (2005). Oral health status of 5 years and 12 years school going children in chennai city an epidemiological study. *J Indian Soc Pedod Prev Dent*, **23**:17-22.
- Margherita Fontana (2018). The tooth decay process: How to reverse it and avoid a cavity": National institute of dental and craniofacial research.
- Marsh PD, Head DA, Devine, DA (2015). Dental Plaque as a biofilm and a microbial community- Implications for treatment. *Journal of Oral Biosciences*. **51(5)**: 185-191.
- Masironi R, Koirtiyohann SR, Ricrec JO and Sehamsehula RG (1976). Calcium content in river water, trace element concentrations in toenails and blood pressure in village populations in New Guinea. *Sci Total Environ*. **6**: 41-53.
- Maures JK *et al.*, (1990). Two year carcinogenicity study of sodium fluoride in rats. *Journal of the National Cancer Institute*, **82**: 1110-1126.
- Monica cheesbrough (2012). Water related diseases and testing of water supplies, **2**:148-151.

- Morr S, Cuartas EMD, Alwattar BMD, Joseph M and Lane, MD (2006). How Much Calcium Is in Your Drinking Water? A Survey of Calcium Concentrations in Bottled and Tap Water and Their Significance for Medical Treatment and Drug Administration, *HSS, J.*, 2(2): 130–135.
- Mr. Brain Oram, “Drinking Water testing Private well Owners and well water testing city drinking water” (2020). Educational guide for private well users and city wear customers.
- Mulu W, Tazebew Demilie T, Yimer M, Meshesha K and Abera B (2014). Dental caries and associated factors among primary school children in Bahir Dar city: A cross-sectional study, *23*:7-949.
- Murray, J. J, World Health Organization, International Dental Federation & W.K. Kellogg Foundation. (1986). Appropriate use of fluorides for human health, World Health Organization. <https://apps.who.int/iris/handle/10665/39103>
- Nisha Rijal, “Water treatment by member filtration technique.” *Environmental Water*, (2019). pp. 135-154.
- NTP (1990). NTP Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies) Research Triangle Park, NC, National Institutes of Health, National Toxicology Program, *393*:1-448.
- Olajire AA and Imeokparia FE (2001). Water quality assessment of Osan River: Studies on inorganic nutrients. *Environ. Morit.* 69(1): 17-28.
- Oludairo O and Aiyedun J (2016). Contamination of commercially packaged sachet water and the public health implications: and Overview," *Bangladesh Journal of veterinary Medicine*, 13(3): 73-81.

- Pip E (2000). Survey of bottled drinking water available in Manitoba, Canada, *Environ. Health perspcet.*108(9): 863–866.
- Radhakrishna M, Hasecna M, Nisha KV and Malaya PS (2003). Bacteriological Study of bottled drinking water marketed in Mangalore. *J. common. Dis*, 35: 123-128.
- Rahman SS, Rasul CH, Kashem MA and Biswas SS (2010). Prevalence of dental caries in the primary dentition among village people, 43:1-2.
- Ralph R, Steinman and Leonora J (1971). Relationship of fluid transport through dentition to the incidence of dental caries" *Journal of Dental Research* 50(6): 36-43.
- Reid IR (2002). Relationship among body moss, its components, and bore, PMID, 11020400.
- Roberts Thomson P, Patterson, K.A. Walker, J.G. (2013). In: *Pathology* 51(6): 669-672.
- Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SK and Kirkham J (2000). The chemistry of enamel caries. *Critical revicnes in oral Biology and medicine*, 1(4): 481-95.
- Saravanan S, Kalyani V, Vijayaroni MP, Jayakodi P, Felix J, Arunmoshi P, Krishnan V and Kumar SP (2008). Caries prevalence and treatment needs of rural school children in Chidambaram Taluk, Tamil Nadu, South India, *Indian J Dent Res*, 19(3): 186-90.
- Sehwendick F, Dörfer CE, Sehlatmans P, Foster Page LF, Thomson WM and Paris S (2015).Socioeconomic Inequality and caries: A systemic Review and meta-Analysis. *Journal of Research*. 94(1): 10-18.

- Shah N, Pandey RM, Duggal R, Mathur VP, Rajan K (2007) Oral Health in India: A report of the multi centric study, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India and World Health Organisation Collaborative Program, <https://www.scienceopen.com/document?vid=c5b5586f-b6c6-453f-bd75-b351e197878d>
- Shouric KL (1941). Dental caries in Indian children Indian, J medical Res **29**: 709-21.
- Silk H (2014). Discusses of the moutn" Primary care: Clinics in office practice **41(1)**: 75-90.
- Silver Stone LM (May 1983) "Demineralization and enamel caries: new concepts" **10 (4)**261-73.
- Sjörs H and Gunnarsson U (2002). Calcium and pH in north and central Swedish mire waters, J. Ecol. **90(4)**: 650-657.
- Slooff W, Eerens HC, Janus JA and Ros JPM (1988) Basis document fluoride Bilthoven, Netherlands, National Institute of Public Health and Environmental Protection (Report No 758474005) pp. 1-191.
- Sukhabogi J, Shekan C, Hamced I, Ramana I and Sandhu G (2014). Oral health statur among 12 and 15 years old children from government and private schools in Hydenabad, Andhra, Pradesh, India, Annals of medical and Health sciences research. **4(3)**: 272-277.
- Tewari A and Chawla HS (2002).Study of Prevalence of dental caries and treatment needs among children of Cuttack (Orissa).J. Indian Sue Pedodprev Dent. **20(4)**: 139-43.

- USEPA (1982); Drinking water criteria document on fluoride Washington, Dc, US Environmental Protection Agency, Office of Drinking water (TR-823-5).
- USEPA (1985); National Primary drinking water regulations; fluoride, final rule and proposed rule. US Environmental protection Agency. Federal Register, 50(220): 47142-47171.
- Wong A Young DA, Emmanouil DE, Wong LM, Waters AR and Booth MT (2013). Raisins and oral health. Journal of food Science. 78(1): 26-29.
- Yang CY (1998). Calcium and magnesium in drinking water and risk of death from cerebrovascular disease stroke 29: 411-414.
- Yewe-Dyer M (1993). The definition of Oral health Br Bent J. 174(7): 224-25.

