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The following are typical main headings:

Introduction, Material and Methods, Results, Discussion and Conclusion

* Introduction: Summarize the rationale for the study with pertinent references. The purpose(s) of the study should be clearly cited.
* Materials and Methods: Identify type of study and describe study subjects and methods used. Provide methods of statistical analysis. Cite references for standard study and statistical methods. Describe new or modified methods. Identify apparatus (with name and address of the manufacturer) used. Generic names of drugs must be given. Manuscripts that describe studies on humans must indicate that the study was approved by institutional Ethical Committee and the subjects gave informed consent. Add statistical analysis.
* Results: Present only important results/ observation in logical sequence in the text, tables or illustrations with relevant statistics
* Discussion: Emphasis new and important results and the conclusion that follows including implications and limitations. Relate observations to other relevant studies.

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1. World Health Organization: WHO recommendations: Obesity: Preventing and Managing the Global Epidemic. Geneva, World Health Org., 2000 (Tech. Rep. Ser. No. 894)

2. Rashid M. Food and Nutrition. In Rashid KM, Rahman M, Haider S eds. Textbook of Community Medicine and Public Health. 4th edn. RHM Publishers 2004; 126-140

3. Sayeed MA, Hussain MZ, Banu A, Rumi MAK, Azad AK. Prevalence of diabetes in a suburban population of Bangladesh Diab Res Clin Prac 1997; 34: 149-155

4. Jarett RJ. Insulin and hypertension (Letter). Lancet 1987; 2: 748- 749

5. Banerji MA, Faridi N, Atulri R, Chiken Rl, Lebovitz HE. Body composition, visceral fat, leptin and insulin resistance in Asian Indian men. J Clin Endocrinol Metab 1999; 84: 137-144 (Abstract)

6. Anwar AKMN & Fariduddin KM, Innovation and re-orientation in medical education and research: Challenges for Ibrahim Medical College. Ibrahim Med. Coll. J. 2007;1(1): 1-2 (Editorial)

Tables

* Each table must be typed on a separate sheet and double spaced. The table number should be Arabic, followed by a brief informative title. Place explanatory matter in footnotes. For footnotes use symbol in this sequence: *, **, etc.

Figures

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Editorial

Diabetes mellitus is associated with chronic complications due to long-term exposure to hyperglycemia. These complications significantly impact the life of subjects with the disease, resulting in decreased quality of life and increased morbidity and mortality. The significant burden of diabetes-related complications entails preventing or delaying its appearance as a primary goal of diabetes management. This issue includes two articles addressing the management of diabetes mellitus and related complications.

One article by Murad et al. proved the blood glucose lowering effect of aqueous extract of Mangifera indica Linn leaves in alloxan-induced diabetic rats. In another paper, Chowdhury et al. evaluated the composition of root canal microflora in diabetic and nondiabetic patients suffering from periapical pathology. The authors concluded that diabetes mellitus can lead to increased strains of bacteria, Epstein-Barr virus and Candida albicans in the root canals of patients suffering from periapical pathosis.

The rest of this issue's articles aim to address contemporary dental and general public health-relevant questions. One original article by Kabir et al. contributed to periodontal inflammatory trends during pregnancy. In contrast, another article by Nargis et al. discussed the serum total cholesterol and LDL levels in postmenopausal women. Further, an article by Parvin et al. evaluated the status of tobacco points of sales and advertisement near some selected educational institutes in Dhaka, Bangladesh.

In conclusion, we should acknowledge that we are far from the optimal knowledge of diabetes and diabetes-related complications. Therefore, more research should be dedicated to providing clinicians with new tools to prevent and manage chronic diabetic complications.

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Contents

Anti-diabetic action of aqueous extract of Mangiferaindica Linn (mango) leaves and Glibenclamide on Alloxan induced diabetic rats - a comparative study

T Murad[1], MI Khan[2], O Yasmeen[3], Z Ahmed[4]

Abstract

Purpose: *The present study has been conceived with the aim to compare the effect of aqueous extract of Mangiferaindica Linn (mango) leaves on blood glucose level in alloxan induced diabetic rats with that of Glibenclamide.* ***Methods:*** *The study was comprised of 18 (eighteen) rats, which were divided into 3 (three) groups each containing 6 (six) rats. Groups were labeled as Group - C (Diabetic control group), Group - D (Diabetic rats treated with aqueous extract of Mangiferaindica) and Group - E (Diabetic rats treated with Glibenclamide1.5 mg/kg body weight) respectively. All the rats received injection Alloxan in a dose of 150mg/kg body weight intraperitoneally, for induction of diabetes on day-1. The duration of the study was 26 days.* ***Results:*** *Administration of 400mg/kg body weight aqueous extract of Mangiferaindica Linn (mango) leaves in Group - D (alloxan induced diabetic rats) produce a significant change in blood glucose level as compared to diabetic control (group C). Administration of 1.5 mg/kg body weight of Glibenclamide in group - E (alloxan induced diabetic rats) also produced reduction in blood glucose level as compared to diabetic control group (group C).* ***Conclusion:*** *Aqueous extract of Mangiferaindica Linn leaves has blood glucose lowering effect in alloxan induced diabetic rats.*

Key words: *Diabetes Mellitus, Mangiferaindica Linn (mango), Glibenclamide* **(J Cont Dent Sci 2020;8(1): 1-8)**

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Introduction:

Diabetes mellitus has increased in alarming way that has created a serious threat to public health globally. It is a major health problem not only in urban but also in the rural areas of Bangladesh, though it is significantly higher in the urban areas compared with the rural areas. Many diabetic people are unaware of their disease in rural areas and do not register themselves in the diabetic clinics or hospitals. Besides many people in rural areas still do not have adequate and appropriate diabetic health care facilities. In our country, a vast majority of the population cannot afford the treatment of diabetes[1, 2].

Majority of diabetes is divided into two broad etiopathogenic categories. In the first category, Type-1 (Insulin Dependent Diabetes Mellitus, IDDM) occurs due to absolute deficiency of insulin. In the other, much more prevalent category, Type-2 (Non-Insulin Dependent Diabetes Mellitus, NIDDM) occurs mostly due to combination of insulin resistance and an inadequate compensatory insulin secretary response. Patients suffering from Type-1 are therefore totally dependent on exogenous source of insulin while patient suffering from Type-2 diabetes can be treated with dietary changes, exercise and medication. About 90% - 95% of all diabetes patients of Bangladesh belong to Type 2 diabetes. Type 2 diabetes has been rising alarmingly throughout the world especially in Bangladesh and reaching the epidemic status[3].

Natural product preparations have historically been the major source of pharmaceutical agents. Analysis of FDA new drug approvals from 1981 to 2002 reveals that natural products continued to play a pivotal role during that time, even if the industry had turned to other discovery strategies[4]. Indeed, more than 90% of current therapeutic classes derive from a natural product prototype and interestingly, even today, roughly two thirds to three quarters of the world's population relies upon medicinal plants for its primary pharmaceutical care[5]. Those "medicinal plants" are either preparations of or natural product substances from plants that has potential utility as pharmaceutical agents[6].

A currently favored hypothesis is that the oxidative stress, through a single unifying mechanism of super oxide production, is the common pathogenic factor leading to insulin

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resistance, beta-cell dysfunction, impaired glucose tolerance (IGT) and ultimately to Type 2 diabetes mellitus. Furthermore, there is convincing evidence that the generation of reactive oxygen species (oxidative stress) may play an important role in the etiology of diabetic complications both macrovascular and microvascular associated with Type 2 Diabetes Mellitus. Mangiferaindica Linn (mango) leaves are a rich source of phenolic compounds with strong antioxidant power, particularly mangiferin, a special xanthone commonly called as 'super-antioxidant'. Because of their potent antioxidant capacity, mango leaves extract can be used in pharmaceutical applications as chemo preventive agent of diseases related with oxidative stress; diabetes mellitus[7].

Recently, we reported the anti-diabetic effect of MangiferaindicaLinn (mango) leaves in experimental diabetic rats[8]. With the background information, this study has been designed to evaluate the anti-diabetic effect of MangiferaindicaLinn extract in experimental diabetic rats. Alloxan has been chosen to induce diabetes mellitus in rats. The blood glucose lowering effect of MangiferaindicaLinn extract has been compared with an oral anti-diabetic drug, Glibenclamide.

Materials and Methods

Place, types and duration of the study

This study has been performed in the department of Pharmacology at Dhaka Medical College, Dhaka. It is an experimental study in animals (rats), allocated duration of the study was 1 year.

Instruments and Accessories:

- Conical flask, funnel, filter paper, gloves, sterile gauge
- Electric digital balance-mettler and Toledo, Switzerland
- Glucometer with kit, ryles tube, syringe, sterile blade
- Grinding machine
- Refrigerator
- Rotator vacuum evaporator-buchilador technique AG, Switzerland

Drugs and Reagents:

- Aqueous extract of Mangiferaindica Linn (mango) leaves
- Alloxan
- Glibenclamide

Collection and Authentication of Plant Material:

The fresh leaves of MangiferaindicaLinn (mango) were collected from a local garden at Savar and was authenticated as Mangiferaindica Linn (mango) leaves by Bangladesh National Herbarium, Mirpur, Dhaka. DACB Accession no 39522.

Preparation of Aqueous Extract:

The fresh leaves of MangiferaindicaLinn (mango) were cleaned and shed dried. The shadow air-dried leaf of MangiferaindicaLinn (mango)was grounded into fine powder with auto-mix blender. Then the fine powder was suspended in equal amount of water and stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at room temperature. This dry mass served as aqueous extract of MangiferaindicaLinn (mango). The extract was subsequently concentrated under reduced pressure to get the corresponding residue, evaporated under vacuum evaporator to obtain final deep green semisolid extract. A total of 30 gm extract was found in this way from 1 kg of leaves.

Animals:

The experiment was carried out on a total number of 18 healthy, 10-12 weeks aged, long Evans Norwegian rats weighing between 150 - 180 gm. The animals were housed in animal house in Dhaka Medical College in clean cages and maintained under standard condition (12 hours Light / 12 hours dark cycle, at 250C and 30-35% humidity) and fed with standard pellet diet and water ad libitum. The rats were purchased from Bangladesh Centre for Scientific and Industrial Research Lab (BCSIR).

Collection of Blood:

Blood was collected from each of the animal following aseptic method. Tail vein was cut at the tip with a sharp sterile blade under local anesthesia.

Determining the effect of aqueous extract of *Mangiferaindica Linn* leaves (AEMIL) and Glibenclamide on blood glucose level in diabetic rats

The experiment was comprised of 18 rats, which were divided into 3 (three) groups, each group containing 6 (six) rats. Groups were labeled as Group - C (Diabetic control), Group - D (Co-treated with Alloxan and Mangiferaindica Linn leaves extract) and Group - E (Received standard drug Glibenclamide). All the rats were fasted over night before collection of blood. The hypoglycemic effect was compared to a standard oral hypoglycemic drug Glibenclamide in diabetic rat. A drop of blood was collected from each of the animals, in the un-anaesthetized state, by cutting the tail at the tip by 0.1 cm with a sharp sterile blade for estimation of fasting blood glucose level. All the rats were fasted over night before collection of blood.

Group - C was given alloxan 150mg/kg body weight intraperitoneally for induction of diabetes on day 1. Before giving Alloxan their fasting blood glucose level was estimated. After injection Alloxan the rats were given standard rat diet and their fasting blood glucose level was estimated again on day 4 and day 26 of the experiment.

Group - D was given Alloxan 150mg/kg body weight intraperitoneally for induction of diabetes on day 1. Before giving Alloxan their fasting blood glucose level was estimated. Three days after Alloxan injection fasting blood glucose was estimated on day 4 of the experiment. Then the rats received aqueous extract of Mangiferaindica Linn leaves 400mg/kg body weight per day orally along with standard rat food for 21 days. Fasting blood glucose level was estimated on day 26 of the experiment.

Group - E was given Alloxan 150mg/kg body weight intraperitoneally for induction of diabetes on day 1. Before giving Alloxan their fasting blood glucose level was estimated. Three days after Alloxan injection fasting blood glucose was estimated on day 4 of the experiment. Then Glibenclamide 1.5 mg/kg body weight per day was given orally along with standard rat diet for 21 days. Fasting blood glucose level was estimated on day 26 of the experiment.

Briefly the design of the experiment is given on figure 1 below

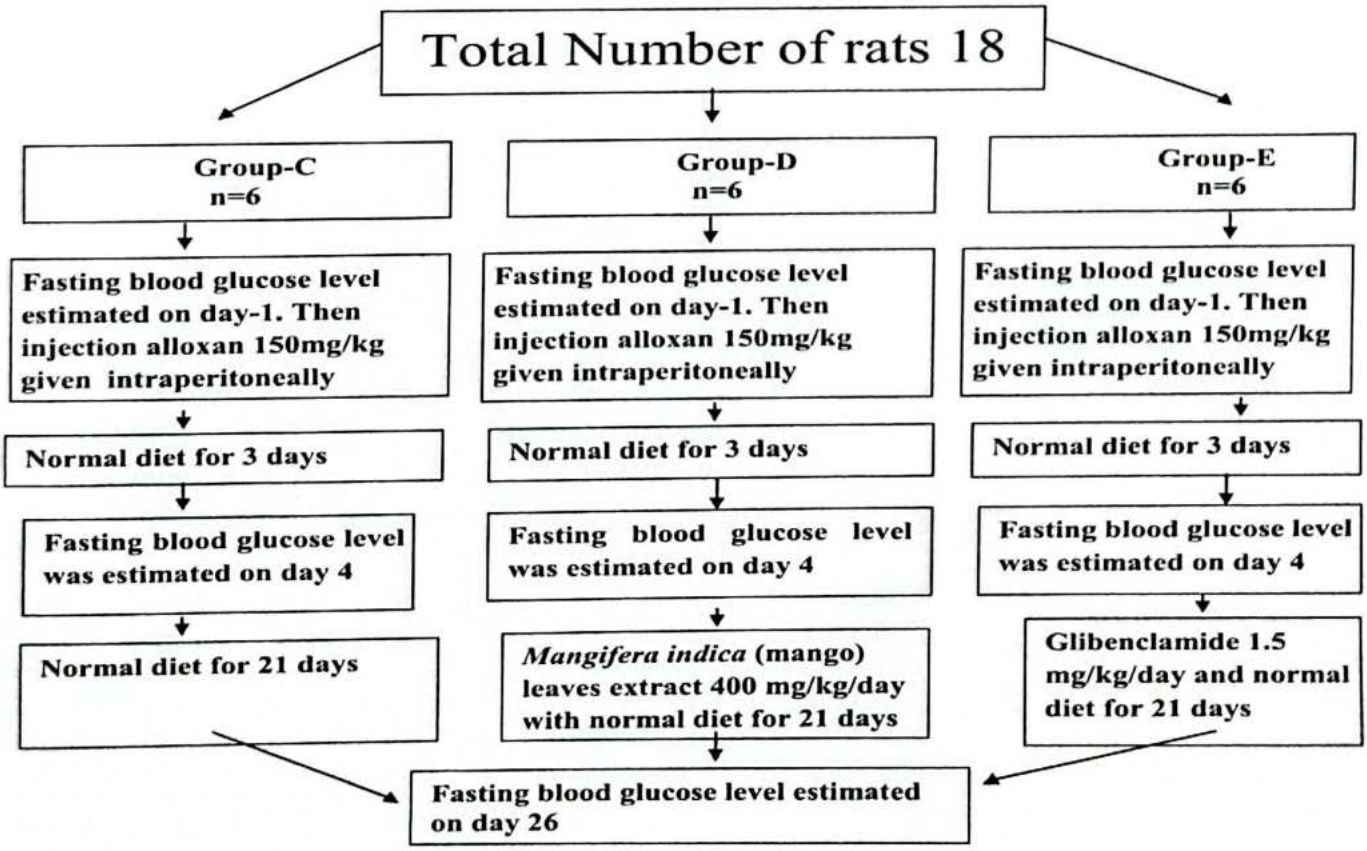

Figure - 1: Design of the Experiment

Biochemical Analysis

Blood glucose estimation was done by electronic glucometer.

Data collection and statistical analysis

Collected data were tabulated and statistical analysis was done by appropriate significant test. Unpaired Student's t test was used to compare the results between individual groups of experiment. The results were presented in tables and by bar diagrams. Each bar diagram represents the (Mean ± SD) of specific groups of rats. Results were considered to be significant when p values were less than 0.05 ($p<0.05$).

Results

Effect of Alloxan on blood glucose level of Group - C, Group - D and Group - E rats on day 4:

In Group - C, the blood glucose levels (mean ± SD) were 5.58 ± 0.31 and 15.58 ± 3.37 on day 1 and day 4 respectively. In Group - D, the blood glucose levels (mean ± SD) were 5.66 ± 0.30 and 15.53 ± 3.30 on day 1 and day 4 respectively and in Group - E, the blood glucose levels (mean ± SD) were 5.70 ± 0.33 and 15.43 ± 3.32 on day 1 and day 4 respectively.

The results are shown in table - 1 and in figure - 2.

Table - 1. Showing the effect of Alloxan on blood glucose level of group-C, Group-D and Group-E rats on day 1 and on day 4:

Group	FBG (mmol/L) on day 1 (Mean ± SD)	FBG (mmol/L) on day 4 (Mean ± SD)
C (n=6)	5.58 ± 0.31	15.58 ± 3.37
D (n=6)	5.66 ± 0.30	15.53 ± 3.30 ^{ns}
E (n=6)	5.70 ± 0.33	15.43 ± 3.32 ^{ns}

Comparison of fasting blood glucose (FBG) level on day 4 with control (Group - C) was done by unpaired student's 't'- test.

ns = not significant

Group - C, Group - D and Group - E were given Alloxan 150 mg/kg body weight single i.p. injection on day-1.

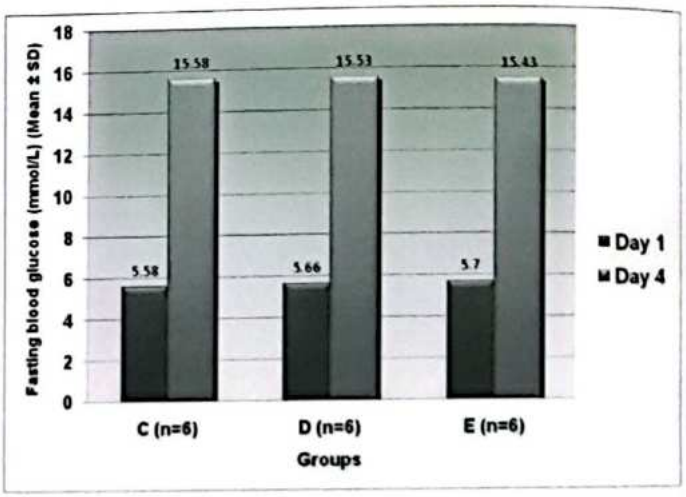

Figure - 2: Bar diagram showing fasting blood glucose level (mmol/L) (Mean ± SD) in Group - C, Group - D and Group - E rats on day 1 and 4.

The vertical bar in the diagram indicates standard deviation.

Effect of aqueous extract of *Mangiferaindica Linn* leaves (AEMIL) and Glibenclamide on blood glucose level in diabetic rats.

In Group - C, the blood glucose levels (mean ± SD) were 5.58 ± 0.31 and 15.692 ± 2.88 on day-1 and day-26 respectively. In group - D, the blood glucose levels (mean ± SD) were 5.66 ± 0.30 and 8.00 ± 1.61 on day-1 and day-26 respectively. In group - E, the blood glucose levels (mean ± SD) were 5.70 ± 0.33 and 7.28 ± 0.59 on day-1 and day-26 respectively.

The results are shown in table - 2 and in figure - 3.

Group	FBG (mmol/L) On day 1 (Mean ± SD)	FBG (mmol/L) On day 26 (Mean ± SD)
C (n=6)	5.58 ± 0.31	15.69 ± 2.88
D (n=6)	5.66 ± 0.30	8.00 ± 1.61***
E (n=6)	5.70 ± 0.33	7.28 ± 0.59***

Table - 2. Showing the Effect of aqueous extract of Mangiferaindica Linn leaves (AEMIL) and Glibenclamide on blood glucose level in diabetic rats

Comparison of fasting blood glucose (FBG) level on day 26 with control (group - C) was done by unpaired student's 't' - test.

***=significant at p<0.001

Group - C - Standard rat diet and water were given.

Group - D - Aqueous extract of Mangiferaindica leaves at a dose of 400 mg/kg b.w./d, standard rat diet and water were given.

Group - E - Glibenclamide at 1.5 mg/kg/day, standard rat diet and water were given.

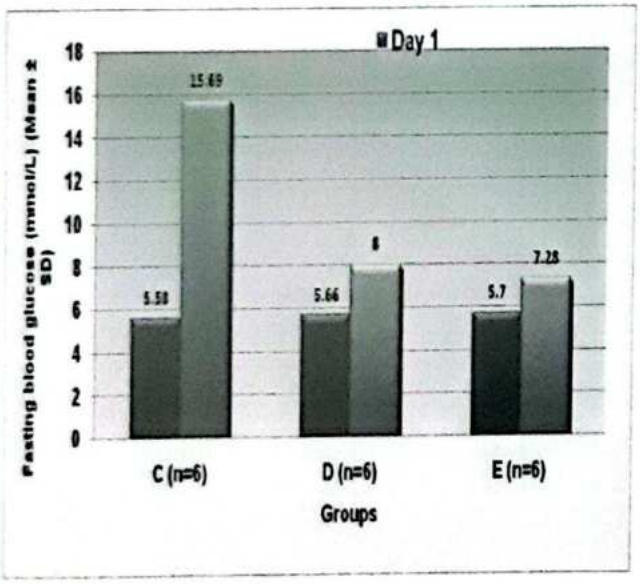

Figure - 3: Bar diagram showing fasting blood glucose level (mmol/L) (Mean ± SD) in group - C, group - D and group -E rats on day 1 and day 26.

The vertical bar in the diagram indicates standard deviation.

Statistical Analysis:

Unpaired student's t test was done between group - C and group - D and between group - C and group - E on day 4; results shows p value>0.05 which was not statistically significant.

Unpaired student's t test was done between group - C and group - D and between group - C and group - E on day 26; results shows p value <0.001 which was statistically significant.

Discussion

This study was carried out to evaluate the effect of aqueous extract of Mangiferaindica Linn (mango) leaves on blood glucose level in experimentally induced diabetic rats. The effect of aqueous extract of Mangiferaindica Linn (mango) leaves on blood glucose level in non-diabetic rats was also evaluated. The glucose lowering effect of Mangiferaindica was compared with a standard oral antidiabetic drug, Glibenclamide. The aqueous extract of Mangiferaindica Linn (mango) leaves was given for 21 days in non-diabetic rats and in alloxan induced diabetic rats.

In the present study diabetes was induced by alloxan. The dose and route of administration of alloxan monohydrate was selected from Etuk et al[9]. According to this study blood glucose level was measured 72 hours after single intra-peritoneal injection of alloxan monohydrate and there was significant rise of blood glucose level in animals. Similar observations were reported by a number of workers.

Ranjith et al[10], investigated the protective and curative effects of Cocos nucifera inflorescence on alloxan-induced pancreatic cytotoxicity in rats. Biochemical parameters such serum glucose, hepatic glycogen, and enzymes involving carbohydrate metabolism (hexokinase, phosphoglucomutase, pyruvate kinase, glucose-6-phosphatase, fructose 1, 6-diphosphatase, glucose-6 phosphate dehydrogenase, and glycogen phosphorylase) were assayed along with pancreatic histopathology.

Tyagi et al[11], studied the antidiabetic Effect of Anacyclus pyrethrum DC in alloxan Induced diabetic rats. The rats were injected intraperitonially with alloxan monohydrate dissolved in sterile normal saline at a dose of 120 mg/kg b.wt. There was significant rise of blood glucose level. Thus the findings of this study are in well agreement with the findings of other researchers. The dose of Mangiferaindica leaves (400mg/kg of body weight), used in this study was selected in keeping conformity with the dose used in research work by Luca and Mohammed 2012[12]. There was no significant change ($p>0.05$) in the mean value of blood glucose level of non-diabetic rats treated with aqueous extract of Mangiferaindica leaves as compared with normal control. It may be concluded that Mangiferaindica leaves has no effect on blood glucose level of non-diabetic rats. Decreased mean value of blood glucose level was observed in the experimental diabetic group when treated with aqueous extract of Mangiferaindica leaves at a dose of 400 mg/kg of body weight and glibenclamide at a dose of 1.5 mg/kg/day and these changes were significant ($p<0.001$). Therefore, the findings of the study are in well agreement with the findings of the other research works that are sited below.

Sharma et al[13], had shown that 50 % ethanolic extract of the leaves of Mangiferaindicaproduced a significant hypoglycemic effect at a dose of 250 mg/kg, both in normal and streptozotocin-induced diabetic animals. The stimulation of β-cells to release insulin was thought to be part of the mechanism of action.

Dineshkumar et al[14], studied the antidiabetic and hypolipidemic potentials of mangiferin, a major constituent of Mangiferaindica leaves, on streptozotocin induced type1 and type 2 diabetic model rats. Results showed mangiferin possesses a significant antidiabetic as well as hypolipidemic effect.

Morsi et al[15], investigated the effect of aqueous extract of mangiferaindica leaves on blood glucose, total cholesterol (LDL, VLDL, HDL), triglycerides, urea, uric acid, creatinine levels and also did phytochemical screening ofMangiferaindica. Treating diabetic rats with 30, 50 and 70 mg aqueous extract caused a significant improvement in these biochemical measures and best results were achieved by using 70 mg of leaves extract. It was also found that the extract is rich in antioxidants like total phenols and total flavonoides which is responsible for its antidiabetic effect.

Rawi et al[16], tested the hypoglycemic effect of aqueous extracts of leaves of Mangiferaindica and Psidiumguavaja either used individually or in combination on stz induced diabetic rats. The result showed prolonged treatment of the diabetic rats with each of the tested extracts as well as with glibenclamide for three weeks had more beneficial effect on OGTT. Fasting glucose was lower than that of the first and second weeks treated groups. At the end of the experiment the decrease in blood glucose concentration was statistically highly significant.

Luka CD et al[12], studied the antidiabetic property of aqueous extract of Mangiferaindicaleaf on normal and alloxan-induce diabetic rats. The aqueous extract ofMangiferaindica leaf was administered orally at a dose of 400mg/kg of body weight to both normal and alloxan induced diabetic rats. Twenty-four (24) rats were divided into four groups of 6 rats in each group of which two groups were made diabetic and the other two groups were normal. One of the diabetic groups was treated with the extract and the second served as diabetic control. The alloxen was given through intraperitoneal route at a concentration of 150 mg/kg of body weight. The administration of the extract lasted for 21 days. Effect of the extract on glucose, cholesterol, triglycerides, high density lipoprotein and protein concentration were analyzed. The toxic effect of the extract was determined using biochemical enzyme markers. The phytochemical screening of the aqueous extract and ethanol extract showed the presence of flavonoids, tannins, cardiac glycosides, resins, sterols, balsam and saponins. Treatment of the diabetic rats with aqueous extract showed significant ($p<0.05$) reduction in the level of glucose, cholesterol, triglyceride and enzyme

activities. Therefore, Mangiferaindica aqueous extract showed that it possesses hypoglycemic and hypolipidemic properties and showed no toxic effect on the liver at the concentration applied and may be used for the management of diabetes mellitus.

The exact mechanism of aqueous extract of Mangiferaindica leaves in reducing blood glucose level is not well understood. Numbers of phytochemical screening was done to identify the phytochemicals present in different parts of Mangiferaindica. The aqueous extract of leaves of Mangiferaindica showed the presence of flavonoids, tannins, saponins, cardiac glycosides, resins, sterols and balsam. The hypoglycemic effects produced by leaves extract may be due to the presence of these bioactive constituents; flavonoids, tannins, alkaloids, steroids and trepenoids[12]. Various parts of Mangiferaindica viz. leaves, fruits, stem bark, heartwoods and roots have been reported to yield mangiferin - a polyphenolic antioxidant and major bio-active constituent. Mangiferin has been reported in various parts of M. indica: leaves[17], fruits[18], stem bark[19, 20], heartwood[21] and roots[22]. Antidiabetic activity of M.indica leaves may be due to this mangiferin. The antidiabetic activity of mangiferin probably due to some extrapancreatic actions[23]. Aderibigbe et al[24] and Amrita et al[25] demonstrated that Mangiferaindica water extract may interfere with intestinal absorption of glucose in the gut by various mechanisms when given with a simultaneous glucose load in diabetic rats. Murugandan et al[26] suggested that both pancreatic and extra pancreatic mechanism might be involved in its antidiabetic effect. The extra pancreatic mechanism could be - i) stimulation of peripheral glucose utilization ii) an enhancement of glycolytic and glycogenic processes Saxena et al[27] and/or iii) a glycemic reduction through inhibition of glucose intake. Basha et al[28] suggested that extract may possess insulin like effects on peripheral tissues by either promoting glucose uptake or metabolism, by inhibiting hepatic gluconeogenesis Ali et al[9]or absorption of glucose into the muscles and adipose tissues Kamanyi et al[30], by the stimulation of a regeneration process and revitalization of the remaining beta cells Shanmugasundaram et al[31].

In this study, it was observed that aqueous extract of Mangiferaindica leaves has blood glucose lowering effect in alloxan induced diabetic rats but not in normal rats. The result suggested that the aqueous extract of Mangiferaindica leaves may be used as blood glucose lowering agent in the treatment of diabetes mellitus. However, to validate this claim and to evaluate the mechanism of action, more studies would be necessary, such as measurement of plasma insulin level, lipid hydroperoxidation level, haemoglobinA1C, lever glycogen level and free radical in the tissues after treatment with aqueous extract of Mangiferaindica leaves. Despite all this limitations, the interpretation of the results obtained in this study which was made carefully and cautiously are satisfactory and encouraging.

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Does diabetes mellitus alter the composition of root canal microflora in patients with periapical pathology?

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Abstract

Background and purpose: *Diabetes Mellitus impairs innate and acquired immunity. Moreover, compromised peripheral circulation of a diabetic patient favours microbial colonisation by further reducing host defence. Accordingly, root canal microflora should vary among diabetic and non-diabetic patients. Therefore, this study aimed to evaluate the composition of root canal microflora in diabetic and non-diabetic patients suffering from periapical pathology.* ***Methods:*** *Of thirty-one samples, sixteen were obtained from diabetic patients (case) and fifteen from non-diabetic patients (control). 1.5 ml Eppendorf tube, Anaerogen 2.5 litre Gas Kit (Oxoid Ltd. UK) and Anaerobic Gas Jar were used to transport collected samples to the laboratory. Blood agar and MacConkey agar media were employed for aerobic cultures of bacteria. Blood agar and Chocolate agar media containing Vitamin K were employed for anaerobic cultures. Sabouraud agar medium was used for the isolation of Candida albicans. Virus detection was done with RealLine CMV and EBV Quantitative (Str-Format), CE-IVD (BIORON-GERMANY) and RealLine DNA Extraction Kit.* ***Results:*** *Bacteria were isolated from all diabetic and non-diabetic patients. The number of bacterial strains isolated from diabetic patients was higher than non-diabetic patients. However, Epstein-Barr virus and Candida albicans were only isolated from 4 diabetic patients.* ***Conclusion:*** *Diabetes mellitus can lead to increased strains of bacteria and the presence of Epstein-Barr virus and Candida albicans. In the root canal systems of patients suffering from periapical pathology.*

Key Words: *Root canal microflora, Periapical pathology, Diabetes Mellitus, Immunosuppression, Host defence*

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Introduction

The steps in developing an endodontic infection include microbial invasion, multiplication and pathogenic activity. Once microorganisms have invaded and multiplied, the pathogenic process is related to the microorganisms' number, virulence, and the host's resistance. The virulence of the microorganisms may be affected not only by their total numbers but also by their synergistic relationships.[1] This hypothesis is supported by the fact that the obligate anaerobe ***Prevotella*** has never been recovered as the sole isolate in the endodontic environment.[2] Since periapical pathosis is caused by the oral microflora in a compromised host with a weakened defence mechanism, their composition should alter with increased opportunist organisms. In support of this, Haapsalo suggested that ***Bacteroides buccae*** may have a specific role in developing an acute opportunistic infection in periodontitis.[3]

Once the dental pulp becomes necrotic, the root canal system becomes a privileged sanctuary for bacteria (mainly) and other microorganisms, bacterial by-products, and degradation products of both the microorganisms and the pulpal tissue.[4-6] Though the microflora found in a root canal is usually mixed and becomes predominantly anaerobic over time, it may also vary according to the routes it reaches the site. Thus, among bacteria, ***Streptococcus, Actinomyces, Lactobacillus, Propionibacterium, Eubacterium, Veillonella*** and ***Peptostreptococcus*** are commonly found in the pulp beneath deep dentinal lesions;[7]

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gram-negative bacteria predominate in traumatically devitalised but non-exposed teeth.[8] Bacteria from the sulcus and periodontal pocket can also be the source of root canal infection.[9] To support this hypothesis, Spirochetes are enumerated to differentiate an abscess of periodontal origin from that of endodontic origin.[10]

Although fungi are members of oral microbiota, particularly Candida species, they have been only occasionally found in primary root canal infections,[11-14] even though a recent molecular study has reported the occurrence of Candida albicans in 21% of the samples from primary root canal infections.

Because viruses require viable host cells to infect and use the cell machinery to replicate the viral genome, they cannot survive in a root canal containing necrotic pulp tissue. Accordingly, viruses in the root canal have been reported only for non-inflamed vital pulps of patients infected with the human immunodeficiency virus (HIV).[15] Conversely, herpes viruses have been detected in apical periodontitis lesions, where living cells are abundant. Of the eight human herpes viruses currently identified, the Human Cytomegalovirus (HCMV) and the Epstein-Barr virus (EBV) have been linked to the pathogenesis of diverse forms of periodontal diseases.[16,20]

Diabetes Mellitus (DM) is a complex metabolic disorder characterised by abnormalities in carbohydrate, fat and protein metabolism resulting either from a deficiency of insulin (Type 1) or from target tissue resistance to its cellular metabolic effects (Type 2). Hyperglycemia is the most clinically crucial metabolic aberration in DM and the basis for its diagnosis. Chronic hyperglycemia in uncontrolled diabetic patients is associated with ophthalmic, renal, cardiovascular, cerebrovascular and peripheral neurological complications.[21] It has also been well established that hyposalivation, gingivitis, periodontitis and periodontal bone loss are associated with DM, especially when poorly controlled.[22,23]

Infectious diseases are a common and severe complication of DM and hyperglycemia. The increase of infections in patients with DM depends on an immunosuppressive condition brought about by impaired innate and acquired immunity. For instance, functions of neutrophils such as phagocytosis, chemotaxis and cytokine production are decreased in mice suffering from DM and hyperglycemia.[24] DM also affects fibroblastic proliferation and is known to be associated with anaerobic infection.[25] Moreover, compromised peripheral circulation of a diabetic patient favours microbial colonisation by further reducing host defence. Therefore, in an immunocompromised diabetic patient suffering from apical periodontitis, the composition of root canal microflora should vary from a non-diabetic patient suffering from a similar lesion. In such polymicrobial conditions, routine culture and sensitivity tests could devise a combined antimicrobial therapy to address the microbial burden of the infected root canals, especially in patients with risk factors such as uncontrolled DM.

Therefore, this study aimed to evaluate the composition of root canal microflora in diabetic and non-diabetic patients suffering from periapical periodontitis so that the successful elimination of endodontic infection and selection and administration of empiric antibiotics will be more straightforward. Furthermore, the use of antiviral and antifungal agents may become rational. Therefore, the research hypothesis tested was- in patients suffering from periapical pathosis, impaired immunity due to diabetes mellitus may alter the composition of root canal microflora.

Materials and methods

This study was conducted following ethical approval and clearance under the Ethical Review Committee of Dhaka Dental College & Hospital (DDCH) and Bangladesh Institute of Research

and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM).

Thirty-one teeth from thirty-one diabetic and non-diabetic adult patients suffering from periapical pathosis and visiting the Conservative Dentistry & Endodontics Department of DDCH for endodontic treatment between April 2013 and September 2013 were included in the study. Of them, sixteen samples were taken from the root canals of sixteen individual teeth of sixteen diabetic patients and were included in the case group. In addition, another fifteen samples taken from the root canals of fifteen individual teeth of fifteen non-diabetic patients were included in the control group. First, patients' information about the diseased condition was recorded using appropriate questionnaires, a careful history, clinical examination and investigation. Then, after ensuring informed consent and proper aseptic techniques, the sample collection procedures were carried out.

The teeth were cleaned and isolated from the oral cavity with cotton rolls. The access cavities were prepared with sterile burs without water spray. Disposable insulin syringes were introduced to collect pus and exudations from the root canals in appropriate situations. When pus or exudation was absent, sterile reamers were inserted into the root canals just short of the apical constriction (if any), followed by sterile paper points to collect the necrotic debris. When introduced, paper points were retained in position for 60 seconds. In cases where a dry canal was identified, another sterile paper point, moistened in sterile normal saline (0.9% NaCl solution), was used to ensure viable sample collection. The sample collections were not preceded by irrigation with any chemically active agent.

Collected samples were transported to BIRDEM for culture by 1.5 ml Eppendorf tubes for aerobic cultures and by 2.5-litre anaerobic gas jars where Anaerogen 2.5-litre Gas Kits achieved anaerobic conditions. Blood agar and MacConkey agar media were employed for aerobic cultures of bacteria. Blood agar and Chocolate agar media containing Vitamin K were employed for anaerobic cultures. Sabouraud agar medium was used for the isolation of Candida albicans. Virus detection was done by specific polymerase chain reactions (PCR) with RealLine CMV and EBV Quantitative (Str-Format), CE-IVD (BIORON-GERMANY) and RealLine DNA Extraction Kit.

After collection, all data were checked and immediately rendered anonymous. Descriptive statistics were used to analyse the data. Means and standard deviations were used to represent the variables' average and typical spread of value.

Results

Of 31 patients, 17 were male, and 14 were female. Sixteen patients had diabetes, and 15 were non-diabetic. Figure 1 shows the percentage distribution of non-diabetic, controlled diabetic and uncontrolled diabetic patients against sex. The percentage of non-diabeties patients was higher in males and uncontrolled diabeties in females.

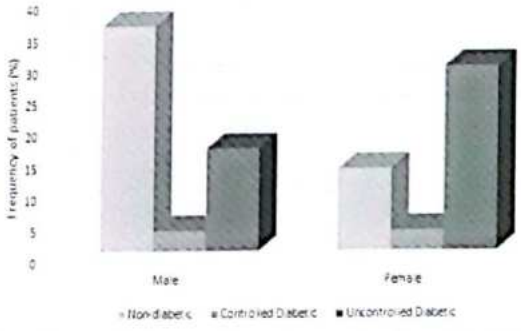

Figure 1: Bar chart showing the percentage distribution of non-diabetic, controlled diabetic and uncontrolled diabetic patients (N = 31) against sex.

Table 1 shows the types of microorganisms isolated from the patients. Bacteria could be isolated from the samples of all the patients regardless of their sex and diabetic/non-diabetic conditions. However, viruses and fungi could only be isolated from 4 diabetic patients.

Table 1: Types of microorganisms isolated from the patients (N = 31).

Microorganisms isolated	From diabetic patients (n = 16)	From non-diabetic patients (n = 15)
Bacteria	16	15
Virus (Epstein-Barr virus)	3	0
Fungus (Candida albicans)	1	0

Table 2 shows that 4 bacterial strains could be isolated from non-diabetic patients. In addition, Streptococcus viridans were found in all the patients.

Table 2: Isolation frequency and percentage of bacteria from non-diabetic patients (n = 15)

Bacterial strains (4)	Isolation frequency	Isolation percentage (%)
Streptococcus viridans	15	100
Coagulase-negative staphylococcus	1	6.7
Staphylococcus aureus	2	13.3
Pseudomonas stutzeri	4	26.7

On the contrary, Table 3 shows that 7 bacterial strains could be isolated from diabetic patients. Again, Streptococcus viridans were found in most of the patients.

Table 3: Isolation frequency and percentage of bacteria from diabetic patients (n = 16)

Bacterial strains (7)	Isolation frequency	Isolation percentage (%)
Streptococcus viridans	13	81.3
Enterobacter	1	6.3
Staphylococcus aureus	5	31.3
Pseudomonas stutzeri	1	6.3
Escherichia coli	1	6.3
Gram-positive cocci	1	6.3
Gram-negative cocci	1	6.3

Discussion

Interestingly, in the present study, among 31 respondents, the number of male patients was higher in the non-diabetic group than in their female counterparts (Figure 1). Moreover, uncontrolled diabetes mellitus was found more in females. Due to social and religious conditions, females mostly remain at home, lacking physical activities. Therefore, they might suffer more from diabetes mellitus.

After the initial phases of microbial invasion and multiplication, the pathogenic process of an endodontic infection is related to the number and virulence of the microorganisms and the host's resistance. Chronic and uncontrolled hyperglycemia in diabetic patients leads to periodontal complications and infections resulting from impaired innate and acquired immunity.[21-23] Moreover, compromised peripheral circulation favours microbial colonisation by further reducing host defence. Therefore, in an immunocompromised diabetic patient suffering from apical periodontitis, the composition of root canal microflora should vary from a non-diabetic patient suffering from a similar lesion. Our results are in agreement with this hypothesis. In the current study, although bacteria could be isolated from all the patients regardless of their diabetic or non-diabetic conditions, the number of isolated bacterial strains was higher in the case of diabetic patients (Table 3) compared to their non-diabetic counterparts (Table 2). These findings also align with a previous report by Sharma et al.[26] Moreover, according to our results, PCR revealed the presence of EBV in three diabetic patients and culture with sabouraud agar media revealed the presence of Candida albicans in one diabetic patient (Table 1). Guggenheimer et al.[27] also reported similar findings.

Therefore, the results of the current study prove our research hypothesis that- in patients suffering from periapical pathosis, impaired immunity due to diabetes mellitus may alter the composition of root canal microflora.

Previous reports also suggested that fungi (mainly Candida albicans)[11-14] and viruses (commonly EBV and HCMV)[16-20] can be isolated from infected root canals of immunocompromised patients.

While Candida albicans, if retained, can lead to post-endodontic complications, EBV and HCMV impair local host defence, possibly leading to bacterial overgrowth. This assumption has been substantiated by the increased number of bacterial strains found in the case of diabetic patients in the present study. Perhaps a more significant fact is that EBV and HCMV have also been implicated with cellular dysplasia, leading to nasopharyngeal carcinoma. Therefore, administering antibiotics alone may be insufficient for successful outcomes in such conditions; a combined therapy to eradicate bacteria, viruses, and fungi is paramount to achieving long-term success. Failure to administer suitable antifungal and antiviral drugs may lead to treatment failure, tooth extraction, morbidity and even mortality, particularly in immunocompromised individuals. Therefore, our traditional belief of prescribing only antibiotics in these situations must be substituted with a combined antimicrobial approach incorporating antivirals, antifungals and antibiotics based on culture and sensitivity tests.

In the present study, although anaerobic conditions were ensured by gas packs and gas jars while transporting the collected samples, it is almost impossible to maintain anaerobic conditions during sample collection from root canals. This might have contributed to the failure of isolation of some obligatory anaerobic bacterial strains. Moreover, identifying bacteria employing polymerase chain reaction (PCR)-based methods is more sensitive than traditional culture-based methods. Therefore, PCR-based methods could have isolated bacterial strains more efficiently if employed in the present study. Future studies should evaluate the association of incidence of nasopharyngeal carcinoma in patients suffering from periapical pathosis due to root canals infected with EBV and HCMV.

Conclusion

In this study, data was presented to show that the composition of root canal microflora can alter with a patient's systemic conditions. For example, immunosuppressed conditions like diabetes mellitus can lead to increased strains of bacteria and the presence of EBV and Candida albicans in the root canal systems of patients suffering from periapical pathosis.

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Assessment of Periodontal inflammatory trends during pregnancy in a selective hospital of Dhaka City; A call for quality of antenatal care while promising evidences are emerging

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Abstract

Introduction: *Gum bleeding and periodontal inflammatory trends are predominant during pregnancy. Has almost similar clinical feature but pregnancy gingivitis is diagnosed by symptom based criteria. This is the major public health issue to meet the challenges for maternal and child health. There is ongoing debate about the importance of radiological feature, microscopically cellular changes, presence of calculus, pocket, etc. Aim of this study was to assess the prevalence of gum bleeding feature during pregnancy and associated risk factors among women, describe the features or situation of gum bleeding during pregnancy among women and to find out the risk factors of gum bleeding during pregnancy in a selective hospital of Dhaka City.* ***Methods:*** *This observational study was conducted in the department of Dental Public Health, City Dental College and City General Hospital, Dhaka, Bangladesh, having ethical approval through institutional ethical review board of City Dental College. Study was performed during the period of one year (January 2019 to December 2019) and a total of 84 patients (mean age 21.07 years had all the pregnant women with history of gum bleeding) were studied to determine the Periodontal inflammatory trends during pregnancy.* ***Results:*** *This study showed that periodontal inflammation and pregnancy was significantly (P 0.001) associated. Inflammatory gingival changes occurs after pregnancy were present in 74 out of 84 (88.09%) among them severe changes occurs in 24 (28.57%) cases (P-0.03). Other significant findings were gum bleeding, xerostomia, candidiasis, burning mouth and abnormal taste. In this subjects (after 1 months of delivery), the symptoms was lower in subjects with lesions compared with those without oral lesions (no inflammatory changes in 55 patients (65.47%) p <0.001.The study finally may contribute to provide essential dental health service to the pregnant women for prevent & controlling of their dental health problem.Periodontal intervention results in a significantly decreased incidence for preterm delivery. Pregnancy without periodontal treatment is associated with significant increases in probing depths, plaque scores.*

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Introduction

Infections may play a significant role in the induction of births and prematurity. Periodontal disease could be a risk factor for pregnancy outcomes such as preterm birth, and low birth weight[1]. The placenta is a very good line of defense to protect a human fetus from the elements. But it is known for some time that it isn't an impenetrable barrier. Tobacco and alcohol, for example, can travel through the mother's system and into the baby's system causing illnesses and birth defects in many cases[2]. Smoking, alcohol use and drug use may contribute to produce an alteration, disruption or teratogenic consequence. New research suggests a new risk factor – periodontal disease. Systemic inflammation and its chemical mediators play a major role in the pathogenesis of preterm delivery, including pre-eclampsia[3], intrauterine growth restriction,[4] and preterm delivery[5]. Chronic infections like intrauterine infection and chorioamnionitis are linked to both preterm birth [6]

and elevated CRP levels[7]. Furthermore, periodontal disease has been associated with increased risk of preterm low birth weight [8], low birth weight [9], and preterm birth.[10] In Bangladesh district level healthcare have no adequate support for dental treatment by government hospital. Mother and child welfare center also sister organization of Bangladesh government under the Ministry of Health & Family Welfare. Managerial structure and health facilities under Directorate General of Family Planning (DGFP) operates 97 MCWCs (maternal and child welfare centers: 24 in union level, 12 in upazila level and 61 in district level), where patient came for treatment, who have no ability to go a private hospital or clinic. Especially the antenatal women who came for regular cheek up. They have dental problems but unfortunately in mother and child welfare center, there are no support for dental treatment. For this reason, data needs to be collected and analyzed so that programs are set up to effectively to reach all segments of the population (Mahfuz at all. 2012). Maintaining good oral health may have a positive effect on cardiovascular disease[11], diabetes[12], and other disorders. Physiologic changes during pregnancy may result in noticeable changes in the oral cavity[13]. Oral changes due to the complex physiologic alterations occurring in pregnancy are believed to be related to fluctuations in levels of estrogen and progesterone[14], leading to an increase in oral vasculature permeability and a decrease in host immune competence, thereby increasing susceptibility to oral infections[15]. Gum bleeding affects 36-100% of pregnant women[16]. Any adverse infectious or inflammatory systemic challenges to the mother or fetus[2, 17].

Materials and Methods

A cross-sectional descriptive type of study was designed and done in the Department of Dental Public Health, City Dental College, Dhaka, from January 2019 to December 2019, based on gum bleeding predominant pregnant woman attended in the department of gynecology, City Dental College and City General Hospital. A total of 84 consecutive patients were included in the study (Purposive sampling technique was followed). A partially structured questionnaire which was duly pre-tested was used to collect data from the respondents and their (pregnant women) gum bleeding and periodontal condition were examined as per standard WHO guideline, dental calculus, periodontal condition scoring was recorded in the concerned portion of the questionnaire.Patients were evaluated thoroughly and who meet the condition (developed periodontal inflammatory trends during pregnancy) was included in the study and those who are unwilling to participate in the study i.e.; did not give consent to participate in an interview are excluded.Informed consent was taken from each patient. Patient`s name and particulars were recorded in the case record file. Initial evaluation of the patients by history and clinical examination (as per Standard WHO guideline) was performed and recorded in the performed data collection sheet. Demographic profile, vital sign, oral health status was noted. Prior to commencement of this study the respective authority approved the research protocol. All the patients included in this study were informed about the nature, risk and benefit of the study. Proper permission was taken from the department and institution concerned for the study.

The data were screened and checked for any missing value and discrepancy. Then data were analyzed by computer aided statistical software SPSS version 21 and level of significance was set at 0.05. Both qualitative and quantitative tests were performed. For comparison between groups, chi-square test was performed for qualitative variables and student-*t* test was performed for quantitative variables. All the information collected for the study was utilized only for

the purpose of research and was not disclosed to anyone outside the research team. Verbal consent was taken from all participating patients .The participation was completely voluntary. Their right to refuse to participant in the study (If they wished so) was respected.

Results

Table - 1: Demographic Data (n=84)

Total no of patients	84
Age (years) means - SD	21.07-4.7
Symptom duration (months)	4 (15)
Monthly income (Average taka)	15000

The table 1 describe the demographic data of 84 patients, where age (years) means - SD was 21.07-4.7and their monthly average income was tk 15000.

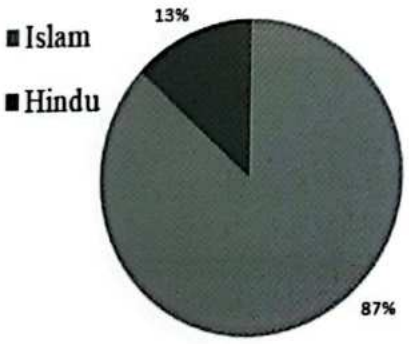

Figure-I: Distribution of religion of pregnant women (n=84)

Table-2: Educational Qualification of the Pregnant Women (n=84)

Education	Frequency	Percent
Primary	35	41.70
Secondary	23	27.36
Higher secondary	19	22.61
Graduate	04	04.76
Masters	02	02.38
Illiterate	01	01.19

According to table 2 majority (41.7%) of the participants completed their primary education, where 4.76% were graduate and 1.19% were illiterate.

Table-3: Occupation of the Pregnant Women(n=84)

Occupation	Frequency	Percent
Service	11	13.10
Housewife	69	82.14
Day Labor	01	1.19
Student	03	3.57
Farmer	00	00.00
Total	84	100.00

In the occupation category, 82.14% participants were serving as housewife, 13.1% occupied with service, 1.19% were day laborer and 3.57% were students.

Table-4: Pattern of inflammatory gingival changes (n=84)

Stage	No of patients
Mild	22 (26.19 %)
Moderate	38 (45.24 %)
Severe	24 (28.57 %)

Table-5: Distribution of the Grade of Gingivitis (N=84)

Grade	Frequency	Percent
Grade-0	06	7.14
Grade-1	26	30.95
Grade-2	50	59.52
Grade-3	02	2.39
Total	84	100.0

According to gingivitis grading, 59.52% were suffering with Grade-2 gingivitis, 30.95% with Grade-1, 2.39% with Grade-3 and 7.14% with Grade-0.

Table-6: Pattern of develop symptoms after pregnancy (n=84)

Age group	-20 years	21-30 years	-30 years	
Increased	22	28	24	P= .003
Not increased	00	06	04	

Table-7: Relation with gum bleeding after delivery after 30 days (n=84)

Condition of gum bleeding after delivery	Respondents		
	Frequency	Percentage	
Yes	29	34.53	P= .001
No	55	65.47	
Total	84	100.0	

Among the participants, 65.47% were responds with no gum bleeding after delivery where 34.53% had the complaints of gum bleeding after the delivery.

Table-8: Segmental distribution of the systemic diseases and relationship with gum bleeding (n=84)

Systemic diseases	Respondents		
	Frequency	Percentage	
DM	1	1.19	
Others (HTN)	5	5.95	P=0.019
None	78	92.86	
Total	84	100.00	

The table 8 reveals that, majority (92.86%) of the participants with gum bleeding had no systemic diseases where 1.19% suffering with DM and 5.95% suffering with Others (HTN) systemic diseases.

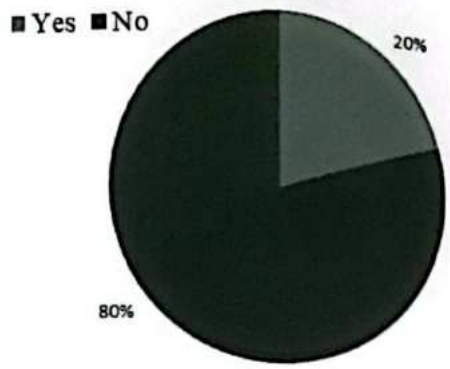

Figure-2: Visit of the Pregnant Women to Dentist (n=84)

Table -9: Reasons of visit of the Pregnant Women to Dentist (n=84)

Reason	Frequency	Percent
Pain	09	10.71
Sensitivity	03	3.57
Gum Bleeding	23	27.37
Bad odor	11	13.07
Not Applicable	38	45.28
Total	84	100.0

Among the participants, 27.37% were visited dentist for gum bleeding, 13.07% for bad odor, 10.71% for tooth ache.

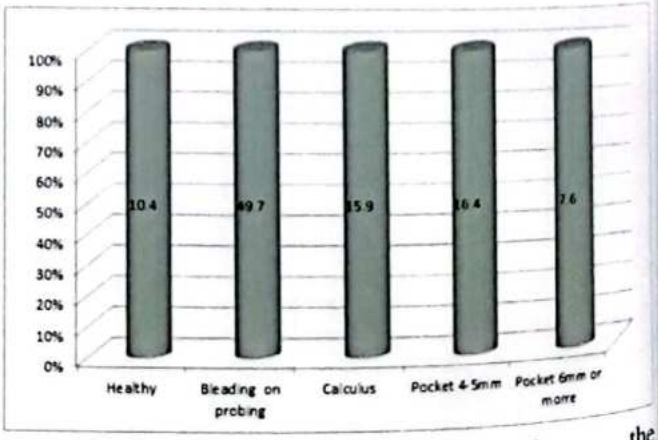

Figure-3: Percentage of the respondentsperiodontal trends according to the periodontal status (measured by community Periodontal Index) (n=84)

Table-10: Distribution of the Tools used for Brushing (n=84)

Tools	Frequency	Percent
Brush	56	66.66
Finger	23	27.39
Meswak	05	5.95
Total	84	100.0

According to table 10, 66.66% of participants use toothbrush for brushing where, 27.39% use finger.

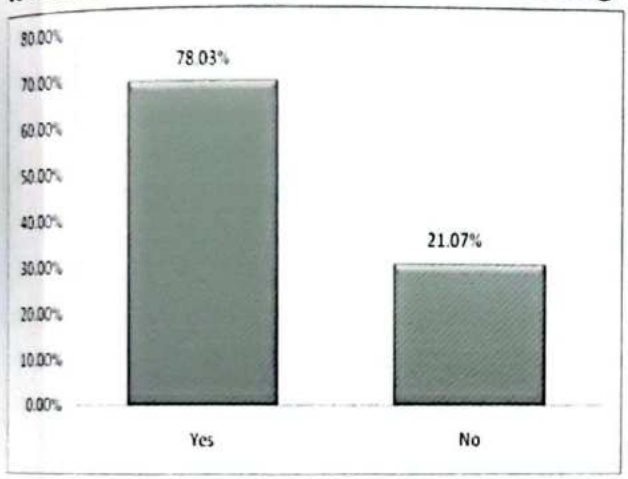

Figure-4: Distribution of oral tobacco use per day

Table-11: Other significant findings were gum bleeding, xerostomia, candidiasis, burning mouth and abnormal taste others .

Reason	Frequency	Percent
Vomiting / Gastro-oesophagal reflex	42	50
Xerostomia	9	10.7
Candidiasis	8	9.5
Burning Mouth	12	14.3
Abnormal Taste	13	15.5
Total	84	100.0

Among the participants, 50% were suffering with Vomiting / Gastro-oesophagal reflex, 15.5% with Abnormal Taste, 14.3% with burning mouth and 10.7% with Xerostemia.

Discussion

The compartmentalization involved in viewing the mouth separately from the rest of the body must cease because oral health affects general health by causing considerable pain and suffering and by changing what people eat, their speech and their quality of life and well-being. Oral health also has an effect on other chronic diseases[18] (Petersen PE. 2003). Oral health is an important part of good overall health, which is especially important during pregnancy. Pregnancy is particularly important to access oral health care because the consequences of poor oral health can have an impact not only on the woman but also on the developing fetus (American Academy of Periodontology. 2010). During pregnancy, lots of physiological changes occur in body as a result of increased production of estrogen and progesterone that may influence the oral health status. Hormonal changes during pregnancy have been suggested to predispose women to oral diseases[19] (Acharya S et al. 2013). From the periodontal perspective, the effects of hormonal levels on the gingival status of pregnant women may be accompanied by increased levels of Bacteroides, Prevotella and Porphyromonas (Durlacheret al. 2006). Gum disease during pregnancy is linked to diseases such as: low weight at birth, giving birth too early in the pregnancy (Saini R et al. 2010).

A total of 84 purposively selected subjects were studied with inflammatory periodontal changes on the basis of their symptoms suggestive gum bleeding according to CDC infection control guideline criteria[18] .Among them mean age were 21.07 years and all subjects were female pregnant having gum bleeding. The education level of respondents, almost two-third 35 (41.70%) of them finished primary education and only 01 (1.19%) of respondents have no formal education (Shown in table-2).

Majority of the respondents 69 (82.14%) were housewife (table-3). Majority of the respondents had moderate inflammatory gingival changes 38 (45.24 %) after pregnancy (table-4. Pregnancy

gingivitis (bleeding gums) is extremely common. Studies report that 30-100% of all pregnant women have bleeding gums (Eleni Markou, et. al 2009).

This study was carried out to assess the Periodontal inflammatory trends during pregnancy in a Akhter Jahan Mirza General Hospital, City Dental College, Dhaka. As this hospital is a hospital for a specific geographical location, specific population, specific mode of payment so the study could not represent the wide range of Bangladeshi population.

Among them most of the respondents 50(59.52%) had grade-2 gingivitis (table-5). The pattern of developed gum bleeding after pregnancy were increased in 21 to 30 years of age mostly 28 (33.34%) and symptoms developed were statistically very much significant (P=0.003) to pregnancy (table-6). Gradually the symptoms of inflammatory gingival changes became improved after 30 days of pregnancy, most of the respondents 55 (65.47%) had no gum bleeding and the relation with gum bleeding after 30 days of delivery is very much proportionate, p value is 0.001, which is statistically significant (table-6). Among all the respondents most of the respondents 78 (92.86) had no systemic diseases, few are had hypertension, Polycystic ovary etc 5 (5.95%) , Diabetics 1 (1.19%), and systemic diseases are not always statistically significant to gum bleeding, P value is 0.019 (Table-7). In Bangladesh most of the patients visit the Dental office only when they have pain or an emergency dental problem (Ahmend S, 1997). In this study most of the pregnant women (80%) visit to their Dentist (figure-2, and the reason had mostly 38 (45.28%) gum bleeding, others were pain 09(10.71%), Sensitivity 03 (3.57%), bad breath 11 (13.07%). In this current study, the periodontal status was generally poor; bleeding on probing and periodontal pockets was recorded in 49.7% and 15.9% of the subjects. (Figure-3),The study showed that out of all pregnant women majority (66.66%) of them used brush for cleaning teeth, (27.39%) used fingure for cleaning teeth, and rest (5.95%) used meswak for cleaning teeth, which depicted in the table-10. Poor oral health behavior and tobacco consumption were identified as associated risk factors for periodontitis in the current study, Out of all pregnant women, majority (78.3%) did not take oral tobacco, and rest of them (21.7%) took oral tobacco (Figure-4). In this current study (Table-11) the others pregnancy related significant oral findings were most of all 42 (50%) Vomiting / Gastro-oesophagal reflex, 9 (10.7%), xerostomia, 8 (9.5%) candidacies, 12 (14.3%) burning mouth and 13 (15.5%) had abnormal taste. Barriers to access to maternal and oral health care facilities are distance to the primary health care facility, proper knowledge, health education counseling, treatment burden, lack of intersectoral coordination as a result of inadequate impact of the oral health care work force and facilities and also no programme to take care of the oral health care improvement needs of the pregnant mother.

Conflict of interest statement

All authors stated explicitly that there are no conflicts of interests in connection with this article.

Conclusion

A consistent link between periodontal inflammation and pregnancy complications are observed, not explained by common risk factors or hormonal factors. Inter-professional coordination between maternal healthcare, gynecologists and dental health care professionals is required to reduce the risk factors. So this study might open a new era in ameliorating the suffering of the people and will provide for the most clinically-effective approach to manage periodontal inflammation during pregnancy and associated risk factors in Bangladesh and preterm birth. This study can also be used as a base line study for further investigation. Prospective studies with large sample size are needed to validate these findings and to give treatment and follow up.

Limitation of this study

Sample size was small. Radiological (OPG/CT/CBCT/MRI) and others serological, Microbiological evaluation was need in every follow-up, which could demonstrate different frequency or values.A shortcoming of our study was the inability to blind the patient for uses of oral drugs or agents. And the lack of OPG/ CBCT/ CT and/or MRI examination is a limitation of our study since we can assume that a certain proportion of the patients we included might have been classified as having actual periodontal changes.

Recommendation

Studies with larger sample size and longer period including radiological, Microbiological and serological investigation with longer follow-up time could be a good predictor forfind the appropriate value. The mechanism of action and mode of action behind this phenomenon requires further evaluation.

Acknowledgement

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Original Article

Study on Status of Tobacco Point of Sales and Advertisement near Some Selected Educational Institutes in Dhaka City, Bangladesh

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Abstract

Introduction: *In Bangladesh, 18th and 4th highest tobacco producer in the world and South East Asia. Exposure to POS tobacco promotions is associated with adolescent smoking initiation, impulse cigarette purchases, and relapse among those trying to quit. Furthermore youths exposed to POS promotions exhibit higher levels of brand recognition and positive imagery of tobacco products.* ***Methods:*** *This work was a descriptive type of cross-sectional study. The aim of the study was to determine tobacco point of sale (POS) and advertisement prevalence within 100 yards of some selected schools of Dhaka, Bangladesh This study was carried out from February to November in 2014. A total of 97 schools were selected following the systematic sampling method from four ddifferent areas of Dhaka city. Data were collected from May to June 2014 with pretested structured questionnaire and interview checklist. All points of sale within a 100-yard from main entrance of schools were observed for type of product sold and advertisement with checklist.* ***Results:*** *Result showed presence of tobacco point of sale within 100 yards of school was near about 99 % within the study area. General stores selling tobacco products were the common type of point of sale at more than half of the observation spots. In about two-third spots there was POS tobacco advertising. Packet arranged within glass box was the most common form in more than half spots. Almost all spots sold cigarettes and more than half spots sold Zarda. Presence of bidi/cigarette bud was significantly associated ($p=0.000$) with administration of school. Number of POS near that school were weakly correlated with Number of students of a school [$r(95) = 0.168$, $p> 0.05$], Number of teachers of a school [$r(95) = 0.156$, $p> 0.05$] and Number of stuffs of a school [$r(95) = 0.122$, $p> 0.05$].* ***Conclusion:*** *Incorporation of ban on tobacco point of sale near/ within 100 yards of educational institute are needed to reduce the prevalence of tobacco use through preventing smoking uptake during adolescence*

Key Words: *Smoking, Tobacco Point of Sales, Tobacco Advertisement, Prevalence* ***(J Cont Dent Sci 2020;8(1):22-30)***

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Introduction

In Bangladesh, 18th and 4th highest tobacco producer in the world and South East Asia respectively, 43.3 percent people (15 years and above) use tobacco in either smoking or other forms (GTSS, 2009). WHO study (2004) suggests that tobacco use is liable for 57,000 deaths and 382,000 disabilities in Bangladesh. To reduce the continuously growing concern on tobacco use and health disaster, Government of Bangladesh (GoB) imposed ban on public smoking, cigarette advertising, and public display of tobacco products. But the market responds in the opposite way; cigarette sales increase along with the rate of smoking over time. Since the last couple of years tobacco industry has been facing lots of obstacles in doing business around the globe including Bangladesh. But the players in the industry did not sit idle, rather they have modified the situation in their favor and some companies set their selling target highest ever in Bangladesh.[1]

Tobacco Point of sale (POS) is broadly defined as a place in the wholesale or retail environment where tobacco products are sold. Point of Sale (POS) promotion is a variety of activities in the retail environment to increase sales of tobacco products. This could include price discounts, branded product giveaways, tobacco advertising

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signs, branded functional items and the display of the tobacco products themselves. Targeted marketing (i.e., promoting different products in different socioeconomic areas) increases the power of POS promotion by segmenting customers into groups and tailoring advertising to appeal to them. This market segmentation is based on characteristics such as consumer or potential consumer demographics, product use, location or lifestyle.[2]

Exposure to POS tobacco promotions is associated with adolescent smoking initiation, impulse cigarette purchases, and relapse among those trying to quit. Furthermore youths exposed to POS promotions exhibit higher levels of brand recognition and positive imagery of tobacco products. POS promotions are more prominent in low-income neighborhoods, stores near schools, areas near schools with high smoking prevalence, and shopping areas frequented more often by adolescents.[3] POS promotion may decrease a smoker s chances of cessation or encourage former smokers to return to tobacco use by acting as a cue to smoke. One-third of smokers who had recently quit reported being tempted to smoke by POS promotions.[2]

POS advertising weakens the effectiveness of tobacco control laws, and exposes the population to tobacco advertising, promotion, and sponsorship-Research studies shows that the youth are particularly vulnerable to POS advertising and promotion. Display at point-of-sale is aimed at keeping cigarette visible to the public and normalizing the product .[4] The tobacco industry denies that their marketing is targeted at young nonsmokers, but it seems more probable that tobacco advertising and promotion influences the attitudes of nonsmoking adolescents, and makes them more likely to try smoking.[5]

Materials & Method

Objectives

The study was conducted to determine tobacco point of sale (POS) and advertisement prevalence within 100 yards of some selected schools of Dhaka, Bangladesh.

Study design

This work was a descriptive type of cross sectional study.

Study sites

The study was conducted within the 100 yards of the main entrance of the ninety seven selected educational institutions in Dhaka city were observed for type of product sold and advertisement. Three sites (in front, right side, left side) for each school were considered as a spot for observation. For this study Dhaka city was divided into four areas according to the socio-economic condition. From each area eight primary schools, eight secondary schools and 8 higher secondary schools were selected. Equal numbers of government and non-government schools were selected purposively. When government school was unavailable, those were replaced by non-government schools.

Study period

The study was conducted from February to November 2014.

Selection criteria

Inclusion criteria

(a) Sales point must be a vendor of tobacco product (either smoked and/or both), either as shop or street hawkers.

(b) Representative of school authority, irrespective of gender.

Exclusion criteria

a) Involuntary participation

Sample size

This type of study has not been done in our country before. But almost similar study was done in neighboring country in India. The prevalence of POS

within 100 yards of educational institutes [out of 30 schools] was 87 percent [n=26, 95 percent CI] in the study, was done in Ahmedabad city of India. Using this prevalence the sample size was calculated 174 (through valid standard formula 95 percent CI and 5 percent standard error). But this was not achievable within given study period. So sample size was purposively calculated. According to 2011 census of India and Bangladesh population of Ahmedabad city was 55,70,585 and area is 464 km2 which is respectively about half of Dhaka population and 1/3 of Dhaka city area. So for this study calculated sample size was 3 fold of that of Ahmedabad city study. Therefore purposively selected sample size was at least 90. As the sample needed to collect from four areas in 3 categories, 8 schools were selected from each category, therefore 96 schools were needed. So, for this study proposed sample size was 96.

In this study sample size was purposively selected as 97.

Sampling Technique

Dhaka was divided into four (4) areas. From each area 8 primary schools, 8 secondary schools and 8 higher secondary schools was selected. Equal number of government and non-government schools was purposively selected. Where government school is unavailable, those were replaced by non-government schools

Data Collection procedures

The study mainly focused on observational information and an interviewer administered questionnaire. Observations like presence of POS, types of tobacco sold, presence of direct or indirect advertisement, and use of any promotion and also any activities seen against tobacco control was noted by checklist.

Statistical analysis

Data were analyzed using appropriate software following a planned analysis strategy. The plan had largely followed the objectives of the research. Comparative analysis had been done primarily with descriptive statistics. Cross-tabulation and chi-square test had been performed as a part of data analysis.

Ethical Issues

Ethical permission for carrying out the study was taken from local ethical committee of Bangladesh Medical Research Council in due time. Institutional permission was taken from the concerned authority prior to conduct the study. Written informed consent was taken from positive respondents only with maintaining their full autonomy. Privacy as well as confidentiality of each participant was maintained strictly.

Results

Results of the study are presented in sections as prevalence of tobacco POS, types of POS, advertisements at POS etc. in this study data were analyzed by comparing with different categories mentioned as:

Between four different areas: Comparison of several findings between four different areas about existence and type of POS. The simple total tabulation result of several items was compared dividing the school into four areas.

Between Govt. and Non-govt. school: Comparison of several findings between govt. and non-govt. schools. The simple total tabulation result of several items was compared dividing the school into govt. and non-govt. school.

Between primary, secondary, higher secondary school: Comparison of several findings among primary, secondary, higher secondary schools. The simple total tabulation result of several items was compared dividing the schools into primary, secondary, higher secondary school.

1. Presence of Tobacco point of sale (POS)

Table 1: Presence of Tobacco point of sale (POS) / Tobacco point of sale prevalence (POS)

Number of tobacco point of sale	Frequency	Percent
0	1	1.0
1	3	3.1
2	5	5.2
3	5	5.2
4	14	14.4
5	5	5.2
6	5	5.2
7	9	9.3
8	2	2.1
9	5	5.2
10	3	3.1
11	7	7.2
12	3	3.1
13	4	4.1
14	4	4.1
15	5	5.2
16	4	4.1
18	3	3.1
19	1	1.0
21	1	1.0
23	1	1.0
24	1	1.0
25	1	1.0
26	1	1.0
27	1	1.0
31	2	2.1
35	1	1.0
Total	97	100.0

Table 1 shows the density of tobacco point of sale near schools. There were four POSs each near 14.4 percent schools, seven POSs each near 9.3 percent schools. At about 30.9 percent schools there were more than 12 POSs and At 9.3 percent schools there were more than 20 POSs. Only one (1) percent school had no POS around it. That means tobacco POS were present within 100 yards of 99.0 percent schools.

2. Existence of tobacco point of sale

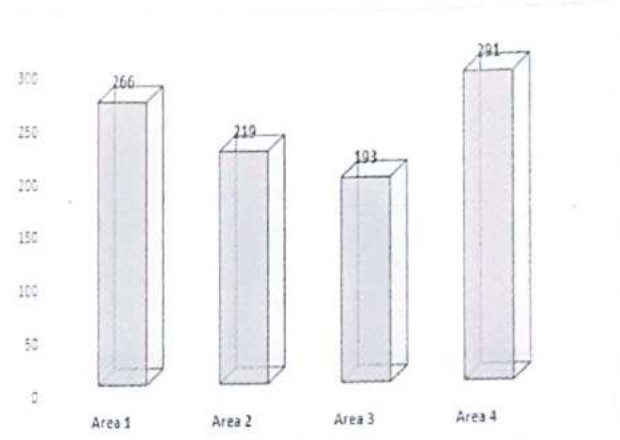

Figure 1: Existence of tobacco point of sale

Figure 1 shows existence of point of sale (POS) in different areas. Out of 969 POS, 291 were present in area four (Airport to Uttara). In area one (Gulshan to Banani), two (Mirpur, Muhammadpur, Dhanmondi) and three (Old Dhaka), there were 266, 219 and 193 POS respectively. Prevalence of tobacco point of sale within 100 yards of school was 99 percent.

3. Distribution of tobacco point of sale

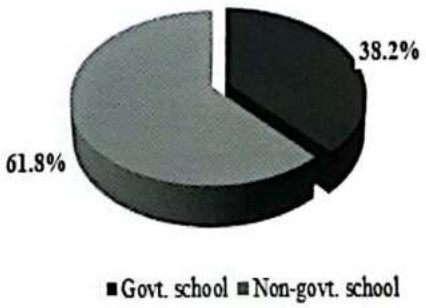

Figure 2: Tobacco Point of Sale by Government and Non-government school

Figure 2 shows distribution of point of sale of tobacco products within the study area according to administration of school. Among the schools point of sale was 61.8 percent near non-government schools and 38.2 percent near government schools. But the ratio of schools was almost equal.

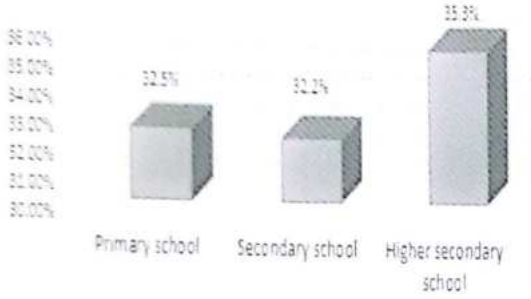

Figure 3: Tobacco Point of Sale by type of school

Figure 3 shows distribution of point of sale of tobacco products within study area according to type of school (primary, secondary, higher secondary). Point of sale was more common near higher secondary schools i.e. 35.3 percent, than primary and secondary schools.

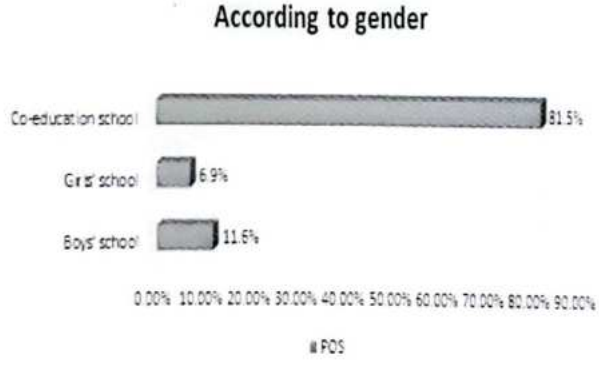

Figure 4: Tobacco Point of Sale by gender of the students of school

Figure 4 shows distribution of tobacco point of sale (POS) within study area according to gender of students. POS was more common near co-education schools i.e. 81.5 percent than boys' only and girls' only schools.

Figure 5: Location of tobacco point of sale around schools

Location of tobacco point of sale is showed in figure 5. At about 78.1 percent schools tobacco POS were situated within the lane i.e. beside the boundary and 21.9 percent in front of main entrance.

4. Tobacco point of sale advertisement (Presence of tobacco advertisement in point of sale)

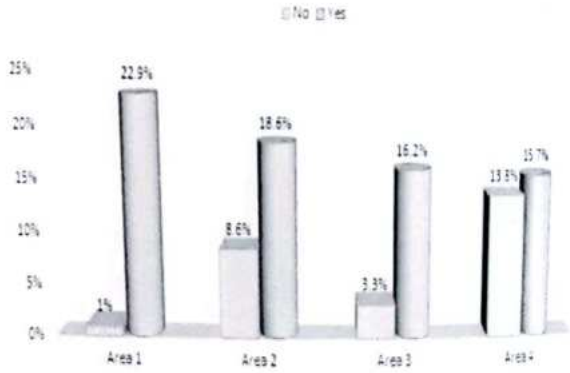

Figure 6: Existence of tobacco advertisement in Point of Sale

Figure 6 shows presence of tobacco advertisement in point of sale (POS) within study area. Among the 218 spots tobacco POS was present at 210 spots. Among them in 70.6 percent spots there was POS tobacco advertising. POS advertising was highest at area one (Gulshan, Banani) and gradually decreased to area two (Mirpur, Muhammadpur, Dhanmondi), three (Old Dhaka) and four (Airport to Uttara).

5. Evidence of smoking within 100yards of school

Table 2: Presence of bidi/cigarette bud within 100 yards of school

Number of buds	Number of bidi/cigarette bud											
	0-25		26-50		51-75		76-100		>100		Total	
	n	%	n	%	N	%	n	%	n	%	n	%
Over all	115	52.8	55	25.2	18	8.3	10	4.6	20	9.2	218	100
Administration												
Government	35	41.2	18	21.2	7	8.2	9	10.6	16	18.8	85	100
Non-government	80	60.2	37	27.8	11	8.3	1	0.8	4	3.0	133	100
Quality												
Primary	35	46.7	27	36	3	4	3	4	7	9.3	75	100
Secondary	38	56.7	11	16.4	9	13.4	3	4.5	6	9	67	100
Higher secondary	42	55.3	17	22.4	6	7.9	4	5.3	7	9.2	76	100
Gender												
Boys	9	45	5	25	2	10	2	10	2	10	20	100
Girls	19	76	3	12	1	4	1	4	1	4	25	100
Co-education	87	50.3	47	27.2	15	8.7	7	4	17	9.8	173	100

Table 2 shows presence of bidi/cigarette bud nears schools. Among 218 spots in 52.8 percent spots there was collection of 0-25 bidi/cigarette bud.

6. Association between Presence & number of POS, type of tobacco product available in POS location of shop, number of bidi/ cigarette bud , presence of tobacco advertisement with administration of school , level and type of school

Table 3: Probability value by chi-square test

	Presence of POS	Number of POS	Location of shop	No. of bidi/ cigarette bud	Type of tobacco available in POS	Presenc e of advertisement
Administration of school	0.165	0.498	0.612	0.000	0.113	0.430
Level of school (primary/ secondary/ higher secondary)	0.654	0.360	0.359	0.229	0.130	0.610
Type of school (Boys'/Girls'/Coed)	0.349	0.592	0.057	0.446	0.592	0.471

Table 3 reveals the association between Presence of POS, number of POS, location of shop, number of bidi/ cigarette bud, type of tobacco product available in POS, presence of tobacco advertisement, sale to minor and sale by minor etc with administration of school (govt. and non-govt.), level of school

(primary/ secondary or higher secondary school) and type of school (boys'/ girls' or co-education). Results obtained from chi-square test shows that Presence of bidi/cigarette bud was significantly associated (p=0.000) with administration of school.

Discussion

1. Presence of tobacco point of sale

In term of prevalence, 99 percent schools had tobacco sale points within 100 yards of their main entrance. Among the 218 spots tobacco POSs were present at 210 spots. The result was more alarming than a study of 6 where 87 percent had tobacco sales within 100 yards of their entrance. A Mumbai study observed tobacco vendors and advertisements within 500 meters of schools, and showed that 13 percent vendors and 10 percent advertisements were located within 100 meters.[7]

Existence of point of sale (POS) in different location showed that number of tobacco POS increases as we proceed from older Dhaka to newer Dhaka i. e. proportion was lower at area three (3) (old Dhaka) and gradually increased at area two (2), area one (1) and higher at area four (4). Male sellers were common at about 92.9 percent spots and female sellers were only about 7.1 percent meaning that female participation in cigarette retailing is still insignificant. According to Islam (2012) around 95 percent retailers have been found male. But these female sellers may not prohibit their children from smoking while usually mothers do. From observation at 17.0 percent sites seller was found minor. But according to Islam (2012) around 7.56% retailers were minor.[8]

General stores selling tobacco was almost equally common in area 2 (Mirpur, Muhammadpur, Dhanmondi), 3 (Old Dhaka) and 4 (Airport to Uttara) but was less common in area 1 (Gulshan, Banani). Hand carried or shoulder carried box were less visible in area 1, 2 and 3. This finding was competent with the finding of a study at Naogaon where 76 percent shops sold tobacco products with other products and the rest one-fourth shops found to be dedicated tobacco shops.[9] In a study from Lebanon it is found that 90 percent were small convenience stores or small grocery.[10]

The ratio of POS to govt. and non-govt. school was almost same. The ratio of POS to higher secondary schools, primary schools and secondary schools was also almost equal. But POS were more common near co-education schools (81.5percent) than boys only and girls only schools. Number of POS near that school were weakly correlated with Number of students of a school [$r(95) = 0.168$, $p > 0.05$], Number of teachers of a school [$r(95) = 0.156$, $p > 0.05$] and Number of stuffs of a school [$r(95) = 0.122$, $p > 0.05$].

2. Presence of tobacco advertisement

One year following the ban on tobacco advertisement at POS, 70.6 percent spots in a large were non-compliant with the new law. It was found that tobacco advertisements were prevalent predominantly inside stores and to a smaller extent visible from outside. This was no surprise, given the low level of compliance with the advertising ban. It is assumed that retailers may not be familiar with all provisions in the law. It was also noticed that tobacco products were placed (plate 1, 4, 8) along with candy, grocery items in many of stores (not measured). Placement of tobacco near daily commodity is suggested to encourage adolescents to see tobacco as benign and commonplace. In Guatemala, 60 percent of stores had cigarette advertisements. High and middle socioeconomic status neighborhood stores had more indoor cigarette advertisements. And this is consistent with this study too In Argentina, 80percent of stores had cigarette advertisements and few differences were observed by neighborhood socioeconomic status. 12 In Lebanon 62 percent of tobacco retail outlets had cigarette advertising; exterior advertising was found in 14 percent of stores, Interior advertising was observed in 60 percent of stores. Tobacco products were placed within 1 meter of candy in 81 percent of stores and within 1 meter of the cash register in 98 percent of stores. 10 In India, tobacco advertising in violation of the law was found around 57 percent of schools, product displays around 83 percent of schools and single sales around 70 percent of schools.[6]

The commonest violation of point of sale advertisements was the Packet arranged within glass box (more than half) and point of sale (POS) followed by branded showcase supplied from vendor (nearly one-third) . Arranging packet wall is an indirect form of advertising. At Naogaon, before intervention tobacco pack wall was found at 35 percent shops but after intervention it reduced to 8 percent.[9] Implementing POS tobacco display bans does result in lower exposure to tobacco marketing and less frequent impulse purchasing of cigarettes.[11] In a study packet wall had been observed in almost every area and of different size and types. Posters had also been observed in different size, types and colors in almost every area. Big size packet and fire box had been observed in both urban and rural areas but not so frequent. Symbol had been observed in urban areas and not so frequent.[11] Other form of advertisement included sticker, flyer, leaflet etc. In the tobacco control law of 2005, there was a provision for the retailers to distribute leaflet (size not exceeding 5.5 x 8.5 inches and color limited to black & white) to the customers describing their tobacco products. Misinterpreting this clause companies are distributing colored flyer and also displaying them at POS. compared to Naogaon study with 1 percent flyer, this study found 7.5 percent flyer in Dhaka city. No billboard, signboard, wall writings or banner was found in the study area and this is analogous to the study at Naogaon.[9] According to Global Adult Tobacco Survey Bangladesh 2009 38 percent of adults noticed cigarette advertisements and 14 percent of adults noticed smokeless tobacco advertisement at points of sale. It was noted that advertisement of tobacco products through leaflets, posters and flyers at points of sale are common.

Almost all spots sold cigarettes and Zarda was sold at more than half spots. Another study showed that in Guatemala and Argentina, all stores sold cigarettes and most had tobacco products in close proximity to confectionery. No sale to minor sign was not reported in this study. In Guatemala fewer stores had 'No smoking' or 'No sales to minors' signs. Compared to Guatemala, 'No sales to minors' signs were more prevalent in Argentina.[12]

Conclusion

One strategy for combating the smoking epidemic is to reduce the prevalence of tobacco use through preventing smoking uptake during adolescence. Even infrequent experimental smoking in youth may increase the risk of smoking in adulthood. Many studies document an association between exposure to tobacco advertisements and smoking initiation. A total ban of tobacco advertising and promotion around the world is a key policy measure of the World Health Organization Framework Convention on Tobacco Control. Bangladesh has implemented a total ban on tobacco advertisement and marketing though it lacks proper implementation and regular monitoring. As a result tobacco industry is being successful with changing social norms related to smoking. For the school going adolescents, atmosphere within and outside school both are influential not to initiate smoking. Persuasion by teachers to discourage smoking/smoking initiation may counter the effect of tobacco point of sale, tobacco advertisement at the vicinity of school to some extent. In this study Prevalence of tobacco point of sale within 100 yards of school was 99 percent. General stores selling tobacco products were the common type of point of sale at more than half of the observation spots. At more than two-third spots there was POS tobacco advertising. Packet arranged within glass box was the most common form in more than half spots. To protect adolescents and school-going children there should be customized strategy. Implementation of promotive program should be targeted to school personnel, community leaders to alleviate the avertable risk factors for youngsters' smoking initiation.

Recommendation

To justify the role as a signatory of FCTC Government of Bangladesh has enacted laws for tobacco control. The GoB has from time to time, taken measures through its functionaries, including legislations, to control tobacco consumption. The actual implementation of these rules has been postponed repeatedly, apparently because of constant tactics by the tobacco industry.

So there is no proper enforcement of these laws. From this study researcher wish to make following recommendations:

1. Incorporation of ban on tobacco point of sale near/ within 100 yards of educational institute.
2. Motivating school heads about their responsibility to control tobacco use.
3. Reinforcement of ban on direct or indirect tobacco advertising, promotion and sponsorship at the point of sale.
4. Incorporating definite guideline to sale tobacco and tobacco display guideline at sale point.
5. Continue tobacco control surveillance and evaluation.

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Original Article

Serum Total Cholesterol and LDL- Cholesterol Levels in Postmenopausal Women

S Nargis[1], HL Roy,[2] R Bhuyan[3], A Sultana [4]

Abstract

Background: *Menopause has an effect on lipid profile which leads to increased risk of development of coronary heart disease, obesity and other risk factors.* ***Aims and Objectives:*** *The aim of this study was to investigate the association of serum total cholesterol & LDL cholesterol in postmenopausal women in Bangladesh.* ***Materials and Methods:*** *A cross-sectional study was carried out in the Department of Biochemistry, Mymensingh Medical College, Mymensingh, from January 2015 to December 2015. This study included 50 postmenopausal women as case compared with 50 apparently healthy premenopausal women as control. Fasting total cholesterol & LDL cholesterol were measured.* ***Results:*** *The mean value of serum total cholesterol and LDL- cholesterol were 231.51-23.8 mg/dl & 169.03-27.33 mg/dl respectively in group B(Case) and176.81-18.36 mg/dl & 105.17-17.52 mg/dl respectively in group A (Control). The levels of serum total cholesterol & LDL- cholesterol were significantly increased in postmenopausal women.* ***Conclusion:*** *The present study may facilitate the clinicians and gynecologists to update their knowledge in regard to lipid profile of postmenopausal women.*

Key Words: *Postmenopausal women, total cholesterol, LDL cholesterol.*

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Introduction

Menopause is defined as the cessation of menstruation for a period of longer than one year. It begins with changes in ovarian function and gradual decrease in the production of estrogen and other hormones.[1] During menopause, women face various physiological, psychological and biochemical changes. The adverse effects of menopause are attributed to a decrease in the estrogen level, which leads to alterations in body mass index, insulin levels and also to increase the risk of hypertension, cardiovascular diseases, osteoporosis, diabetes mellitus, cancer and other degenerative changes in postmenopausal females.[2] Lack of the protection of estrogen has been the major reason. However, several physiological changes which develop during menopause may also influence the risk of cardiovascular disease, such as ageing, increased obesity or android pattern of body fat distribution, decreasing resting metabolic rate and physical activity.[3] The hormonal changes associated with menopause i.e low plasma levels of estrogen and marked increase in luteinizing and follicle stimulating hormone levels exert a significant effect on the metabolism of plasma lipids and lipoproteins.[4] Lipid profile abnormalities in the menopausal women are common health hazard all over the world. There is derangement of lipoproteins profile independent of age.[5] Menopausal women have higher plasma levels of total cholesterol (TC), low density lipoprotein cholesterol (LDLC), when compared with premenopausal women. Hypercholesterolemia is a key factor in the development of atherosclerosis.[6] Ovarian estrogen seems to be inversely related to the development of cardiovascular disease and elevated serum lipid levels.[7] A stronger relationship was found between cardiovascular disease morbidity, mortality and surgical menopausal women.[8] Some researchers reported that the rate of metabolic syndrome (hypertension, hyperglycemia and dyslipidemia) was higher in surgical menopausal women than that of natural menopausal women.[9]

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It has also been suggested that estrogen level drops abruptly in women with bilateral oophorectomy whereas gradually in natural menopausal women.[10] Hysterectomy alone cannot increase risk of cardiovascular disease, but hysterectomy may lead to subsequent ovarian failure.[11] After the age of 50 years, the risk of myocardial infarction increases among the oophorectomized women, compared with women who retain their ovaries.[12] However, after natural menopause, ovaries continue to produce significant number of androgens, which are converted to estrogen peripherally.[13] Some investigators observed that hysterectomy with bilateral oophorectomized women had higher level of TC than those of natural menopausal women.[14] Smaller LDL particles (LDL-III) are considered more atherogenic than larger more species because of their increased susceptibility to oxidation and their increased residence time in plasma.[15,16] After menopause, there is loss of ovarian function. This results in adverse changes in glucose and insulin metabolism, body fat distribution, coagulation, fibrinolysis and vascular endothelial dysfunction.[17] In this study, estimation of serum total cholesterol & LDL cholesterol of postmenopausal women and to compare with the apparently healthy premenopausal women has been made.

Materials and Methods

This cross-sectional study was carried out in the Department of biochemistry, Mymensingh Medical College and the subjects were collected from the outpatient department (OPD), Mymensingh Medical College Hospital, Mymensingh during the period of January 2015 to December 2015 with a view to compare serum total C & LDL- C level between postmenopausal and healthy subjects. In this study 50 cases of postmenopausal and another 50 premenopausal women were selected as control. Informed written consent was taken before taking any interview. Women with any chronic illness, history of hysterectomy & oophorectomy, taking medication that causes amenorrhea (e.g. antidepressant, antipsychotic) were excluded from the study. Fasting total-C & LDL-C were collected for biochemical analysis. Relevant information was recorded in a pre-formed data collection sheet designed for the study. All statistical analysis was done by using Statistical Package for Social Science (SPSS) using version 20. Results were calculated as mean - SD (standard deviation). Statistical significance of difference between two groups were evaluated by using unpaired `t test and 95% confidence limit was taken as level of significance.

Results

A total of 100 subjects were included in the present study. Subjects were classified into group A (Control) and group B (Case). Group B comprising of (n = 50) postmenopausal women aged between 45-55 years served as case. Group A comprising of (n = 50) apparently healthy women aged between 30-40years served as control. In group B (Case, n = 50) the mean value of serum total cholesterol and LDL cholesterol were 231.51-23.88mg/dl and 169.03-27.33mg/dl respectively. In group A (Control, n = 50) the mean - SD of serum total cholesterol, and LDL-cholesterol were 176.81-18.36 mg/dl and 105.17-17.52 mg/dl. There were significant difference (P <0.001) in case of serum total cholesterol, and LDL 'cholesterol between group A and group B.

Table 1: Serum total cholesterol & LDL cholesterol of the subjects.

Biochemical variables	Group A (Control, n = 50) Mean -SD	Group B (Case, n = 50) Mean -SD	P value
Total cholesterol (mg/dl)	176.81-18.36	231.51-23.88	<0.001
LDL cholesterol(mg/dl)	105.17-17.52	169.03-27.33	<0.001

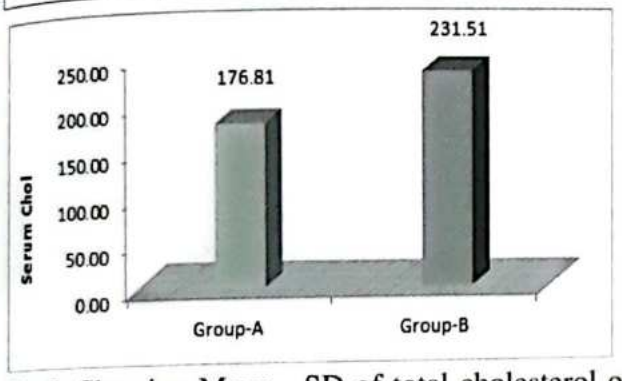

Fig I: Showing Mean - SD of total cholesterol of the subjects

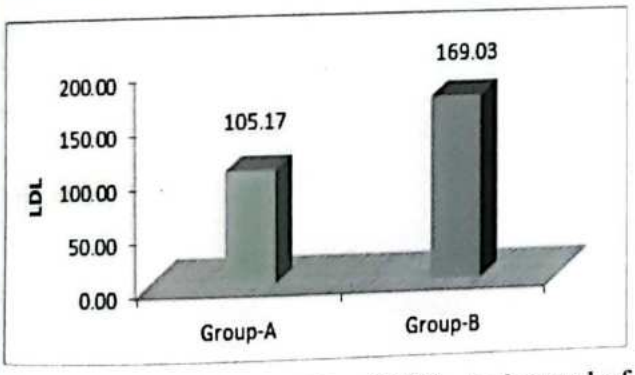

Fig II: Showing Mean - SD of LDL-cholesterol of the subjects.

Discussion

The present study was done to observe the various changes of serum TC & LDL-cholesterol levels in postmenopausal women compared with premenopausal women. Related medical history and clinical information of the subjects were taken by questionnaires from all the individuals included in this study. Fifty apparently healthy premenopausal women aged between 30-40 years were selected as group A (control) and fifty postmenopausal women aged between 45-55 years were selected as group B (case) in this study.

Our finding in case of cholesterol is in agreement with that of Igweh et al, where they found high cholesterol level compare to control.[7] Our findings are also in agreement with findings in the other studies by Igweh et al.[18] A similar observation was also made by Carr et al in postmenopausal women.[5] High level of LDL-cholesterol in postmenopausal women compared to control was observed in our study which is in agreement with Usoro et al.[19] This study was also supported by Igweh et al.[7] A similar observation was also made by Carr et al and Mathews et al in postmenopausal women.[5,9] A major effect of estrogen on lipid metabolism is up to the regulation of LDL receptors, resulting in increased clearance of LDL particles by hepatocytes and reduction in plasma LDL-Cholesterol, stated by Lamarche et al.[20] During menopausal period estrogen level is low; so this action is hampered, resulting in increased LDL- cholesterol level. But the finding of Schnatz et al, in menopause supplementation with calcium/vitamin D increased mean serum 25-OHD3 concentrations 38%, and higher concentrations of the metabolite were associated with lower LDL-cholesterol.[21]

Conclusion

Menopause has an effect on serum total cholesterol and LDL-cholesterol which leads to increased risk of development of coronary heart disease, obesity and other risk factors. The available knowledge of menopause and its effect on human can alleviate the risk factor of coronary heart disease and other cardiovascular alteration. The findings of this study will make it easier for doctors and gynecologists to stay up-to-date with their knowledge of total cholesterol and LDL-cholesterol levels of post-menopausal women.

Limitation of the study

Sample size was small & data was collected from

only one medical college, due to limited time frame. Overall, these limitations suggest that future research should aim to address these limitations and build upon the current findings to further advance the field.

Acknowledgement

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Conflict of Interests

The authors have no conflicts of interest to declare.

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