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Informations about Prime Medical College, Rangpur

Prime Medical College is one of the best and largest private medical college in Bangladesh. It was established in 2008. The ideas of establishing this Medical College is to provide standard Medical Education and Health Services to the people at an affordable cost.

The objectives of the institute are :

- To promote and provide education in Medical Science and to Provide training in different discipline of medicine recognized by the postgraduate institutes and universities.
- To conduct research work on the diseases prevalent in the country.
- To conduct research on medical education with the aim of uplifting the quality and standard of medical education in the country.
- To produce and provide skilled manpower in the medical, nursing and paramedical fields.
- To provide quality medical care and heath services to the people at reasonable cost.

The first and foremost objective of establishment of this medical college is to offer MBBS degree under Rajshahi University of Bangladesh and to provide good quality medical graduates, who can fulfill the need of health care prevailing in the country.

Editorial

Obesity in children.

Absar N

Obesity is an well acknowledged nutritional problem in children and adolescent in western world. In America approximately 37-42% of the children and adolescents are overweight to obese. Overweight and obesity is not yet a great health problem in our children and adolescent but it is gradually emerging as a potential threat to the child health. It is a general observation that obesity in children is also on the rise in our country. We can't give any data about this observation as we don't have any national survey on obesity prevalence in our country.

Obesity in children is an outcome of a complex interplay of many factors; genetic, environmental factor, metabolic imbalance, life style and eating habits are among the most important determinants of obesity. It is suggested that 90% of the obesity are idiopathic and 10% are associated with hormonal or genetic cause.

There is no universally approved definition of obesity. However the terms overweight, obese and morbidly obese are used to identify different grades of upward trends of increased weight in children.

These terms are expressed mathematically as follows.

- Overweight: Weight exceeds 20% of expected for height.
- Obese: Weight exceeds 50% of expected for height.
- Morbidly obese: Weight exceeds 80-100% of expected for height.

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BMI (Body mass index),has not been consistently used or validated in children younger than 2 year. However national health and nutrition examination survey (NHANES) of US generated data on BMI in children and percentiles were extra polated from that data. Consensus committee recommended that if BMI exceeds 95th percentiles on curve (1966-1970 NHANES) or exceeds 30kg/m^2 at any age can be marked as obese [1,2]. Obesity is significantly associated with low cardio respiratory fitness in children [3].

Energy imbalance is an important cause of obesity in children. Excess fat accumulates when total energy intake exceeds total energy expenditure. A sedentary life style can cause energy imbalances. Excessive television watching, keeping engaged in video games, excessive computer use and insufficient physical activity is frequently associated with childhood obesity. In infant feeding practice if protein to energy ratio is altered e.g if feedings are supplemented with more carbohydrate and fat, protein remaining same obesity may ensue as a result. Increased incidence of obesity has been observed at 3 years age who were weaned at 4 month with solid feeds [4].

There are some genetic syndromes and hormonal disorders which can cause obesity in children. The genetic syndromes are : Prader-willi syndrome, Pseudo Hypoparathyroidism, Laurence-Moon-Biedl syndrome, Down syndrome, Turner syndrome etc. Besides few hormonal disorders are; Growth hormone deficiency, Growth hormone resistance, Hypothyroidism, Leptin deficiency or resistance to leptin action, Polycystic ovary syndrome, Cushing syndrome etc.

Obesity is an outcome of imbalance between energy intake and energy expenditure.

Dysfunction in the gut-brain-hypothalamic axis has been implicated with obesity. This pathway is again regulated by ghrelin/leptin hormone inter play. In familial obesity leptin deficiency has been reported and replacement therapy caused dramatic weight loss response.

However genetic and hormonal disorder alone are not responsible for obesity. Environmental factors also contribute to develop obesity. The family pattern of food intake, exercise, leisure activity including watching TV are important contributors for development of childhood obesity.

Obesity is associated with increased incidence of reactive airway, poor exercise tolerance and obesity hypoventilation syndrome and eventual right heart failure. Obese children are at increased risk of type-2 diabetes, hypertension and dyslipidemia [5,6].

Disorders like accelerated bone maturation, ovarian hyperandrogenism, gynaecomastia, cholecystitis, pancreatitis and renal disease often emarge as acute complication of obesity. Sleep apnoea and sleep disordered breathing are common in children with obesity.

Among many complications followings are note worthy:

Valgus deformity with slipped capital femoral epiphysises, elevated transaminase cholelithiasis and cholecystitis, depression, eating disorder lower self esteem, gout, colorectal carcinoma etc.

Obesity management has three areas of concern, these are: control of weight gain, reduction in body mass index(BMI) and prevention of long term complication of obesity in childhood.

Obesity management emphasises on long term dietary management and exercise. To achieve a successful weight management family support is essential. Dramatic weight loss is never encouraged. Gradual reduction in body fat and BMI and maintenance of weight loss is prudent in the management of obesity.

A team work is helpful. Psychiatric assistance for eating disorder, a counselor's involvement, exercise physiologist and nutritionist's help may enhance success in effective obesity management .

Life style modification to increase exercise time and reduce sedentary habit in association with dietary restrictions provides long term weight control in children and adolescences.

To reduce long term complications of obesity in adult is difficult once it has already developed. Preventing the development of obesity in childhood [3] is more effective in reducing long term complications[7,8,9,10,11,12].

Regular follow up is indicated to sustain the obesity control.

Monitoring helps

- Reinforcement of nutritional goals and exercise objective.
- Identification of social and emotional bar riers to therapy.
- Family support and counseiling.
- Assessment of growth.
- Identification and management of obesity related acute and chronic complication.

Medication are not routinely recommended for pediatric obesity.

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Original article

Effects of single dose preoperative intravenous dexamethasone for postoperative analgesia in tonsillectomy patients.

Mohiuddin M[1], Maruf AA[2], Nazrina S[3], Islam Md. R[4]

ABSTRACT

***BACKGROUND**: Dexamethasone is frequently administered in the perioperative period as adjuvant for management of postoperative pain. **OBJECTIVE:** In this study the effects of single dose preoperative intravenous dexamethasone as adjuvant for postoperative pain relief in patients undergoing tonsillectomy was observed. **METHODS**: For this purpose sixty patients of both sexes, aged between 18-50 years, American Society of Anaesthesiologists (ASA category) grade I and II underwent tonsillectomy, were randomly allocated into two groups. Group A (n = 30) patients recieved single dose of dexamethasone 0.15 mg/kg body weight before induction of anaesthesia and Group B (n = 30) received no dexamethasone. Postoperative pain scores were assessed at 2, 6 and 12 hours in both groups using visual analogue scale (VAS). **RESULTS**: There was no significant ($p>0.05$) difference in pain scores after 2 hours postoperatively. Pain scores of Group A at 6 and 12 hours postoperatively were found to be significantly ($p<0.05$) low than Group B. **CONCLUSION**: Single dose preoperative intravenous injection of dexamethasone in tonsillectomy patients reduces postoperative pain in tonsillectomy patient.*

KEY WORDS: Tonsillectomy, Dexamethasone, Postoperative analgesia.

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INTRODUCTION

Tonsillectomy is the most commonly performed otolaryngological procedure. Most of the patients presenting for tonsillectomy are children and young adults[1-4].The postoperative period is usually characterized by throat pain, otalgia, temporary voice changes, poor oral intake and haemorrhage [5]. Post tonsillectomy pain is probably the result of muscle spasm caused by inflammation and irritation of the pharyngeal musculature[6]. For managing postoperative pain after tonsillectomy, different multimodal approaches are available like perenteral opioids, non steroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, nerve blocks and steroids (systemic and topical)[7,8]. Multimodal analgesic approaches have been used as an important strategy to mitigate postoperative pain relief[9]. The effectiveness of adjunct agents; including ketamine [10], gabapentine [11], paracetamol and NSAIDs[12] have been examined in systemic reviews that demonstrate their benefits in reducing postoperative pain with opioid consumption. Steroids may also be used for postoperative pain management after different

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types of surgery including tonsillectomy[8]. Dexamethasone is a corticosteroid commonly used perioperatively to reduce postoperative nausea and vomiting[13] and may have a beneficial role in postoperative analgesia[14].

The aim of present study was to assess postoperative analgesic benefit in tonsillectomy patients who has been administered single dose preoperative intravenous dexamethasone.

MATERIALS and METHODS

This prospective comperative study was conducted in Prime Medical College Hospital, Rangpur, in one calender year from July 2012 to June 2013. For this total sixty patients (30 patients in each group) of both sexes were selected randomly for elective tonsillectomy, age ranged were from 18 to 50 years, physical status were I and II according to American Society of Anaesthesiologists (ASA category). Patients with psychiatric illness, having history of peptic ulcer disease, hypertension, diabetes, tuberculosis, osteoporosis and heart diseases were excluded from the study. The study was conducted after approval from the ethical review committee of PMC. Pre-anaesthetic check up was done 24 hours prior to surgery. Then the procedure was explained to the patient and written consent was taken from each patient. During the preoperative interview, patients were instructed how to assess postoperative pain by using the Visual Analogue Scale (VAS) 0-10, 0=no pain, 10=the worst imaginable pain. All eligible patients were randomized in to two groups. Patients in group A (n=30) were administered intravenous dexamethasone 0.15 mg/kg body weight before the induction of anaesthesia. Group B patients (n=30) were not administered injection dexamethasone in addition to other drugs. Operation was done under general anaesthesia with controlled ventilation. All patients received oral diazepam (5 mg) at night before surgery. Again, Pethidine 1 mg/kg body weight was given intravenously before induction of general anesthesia. Induction was done with thiopentone 5 mg/kg body weight intravenously. After intubation with vecuronium 0.1 mg/kg body weight, anesthesia was maintained with 70% nitrous oxide in oxygen, halothane 0.5-1% and muscle relaxation was maintained with incremental doses of vecuronium. Patient's heart rate, blood pressure, respiratory rate, and SpO_2 were monitored and recorded in every 5 minutes interval. After completion of operation the patients were extubated after reversal of muscle relaxant with neostigmine and atropine and then admitted to the postoperative ward. Postoperatively, the pain was assessed in both the groups at 2, 6 and 12 hours using Visual Analogue Scale (VAS). During this period of 12 hours if the patient required additional analgesia, it was provided in the form of intravenous ketorolac 0.5 mg/kg body weight, with a maximum dose of 30 mg. All statistical analysis was carried out by using SPSS (Statistical Package for Social Sciences) version 17.0 for windows. All results are expressed as mean ± standard deviation (SD) or in frequencies as applicable. Results are considered statistically significant if $p<0.05$.

RESULTS

Patient's demographics were similar and fairly comparable in both groups and differences were statistically not significant ($p>0.05$) (Table I).

Duration of surgical procedure and duration of anesthetic procedure were similar in both groups and the differences were statistically not significant ($p>0.05$) (Table II).

Table I: Distribution of the subjects according to Demographic data.

Characteristics	Group A (n=30)	Group B (n=30)	P Value	Results
Age (years)	22.71±4.13	23.09±4.03	0.097	NS (student 't' test, unpaired)
Body weight (Kg)	50.42±5.21	51.17±5.13	0.281	NS(student 't' test, unpaired)
Sex				
Male	16 (53.33%)	17 (56.67%)	0.789	NS(chi square test)
Female	14 (46.67%)	13 (43.33%)	0.768	NS(chi square test)
ASA physical status				
I	27 (90%)	26 (86.67%)	0.812	NS(chi square test)
II	3 (10%)	4 (13.33%)	0.776	NS(chi square test)

Values are expressed in Mean ± SD and Percentage NS= Not significant; n= number of subjects

Table II: Distribution of the subjects according to perioperative data

Variables	Group A (n=30)	Group B (n=30)	Value
Duration of surgery (minutes)	37.91±7.32	38.17±7.12	0.816^{NS}
Duration of anaesthesia (minutes)	48.63±8.13	47.76±8.67	0.821^{NS}

Values are expressed in Mean ± SD, n= number of subjects Statistical analysis was done by unpaired students 't' test, NS= Not significant; n= number of subjects

Table III: Distribution of the subjects according to Mean pain score (VAS) after surgery.

Measurement time	Group A (n=30)	Group B (n=30)	P Value
After 2 hour	2.59±1.7	2.86±1.6	0.098^{NS}
After 6 hour	3.18±0.77	4.01±0.89	0.035^{*}
After 12 hour	2.31±1.1	3.5±0.91	0.029^{*}

Values are expressed in Mean ± SD,n= number of subjects Statistical analysis was done by unpaired students 't' test, NS= Not significant,*= $p<0.05$

In all cases operating conditions were pronounced satisfactory by the concerned surgeon. The pain intensity was measured by visual analogue scale (VAS) in both groups in postoperative ward at 2, 6, and 12 hours. The mean VAS was less in group A than group B at 2, 6, and 12 hours postoperatively. The difference of mean values between the two groups at 2 hours postoperatively was statistically not significant ($p>0.05$). However, the pain scores were found to be significantly ($p<0.05$) lower at 6 and 12 hours postoperatively in the group A than group B (Table III).

DISCUSSION

There is a long period of practice of using glucocorticoids to reduce inflammation and postoperative pain in many surgical procedures [15-18]. Corticosteroids are steroids that bind with high affinity to its receptor in the cytosol of cells. There are multiple sites of action at which glucocorticoid-activated receptors produce anti-inflammatory and immunosuppressive effects [19]. Dexamethasone is a synthetic glucocorticoid with high potency and long duration of action having half life of 2 days, but with no mineralocorticoid activity. In addition to reducing inflammation, dexamethasone can also reduce post operative nausea and vomiting

(PONV). Again, Prostaglandins are one of the main inducers of inflammation after tissue injury, and one mechanism by which glucocorticoids reduce prostaglandin synthesis is by inhibiting the expression of cyclooxygenase-2 without affecting cyclooxygenase-120. Studies used dexamethasone for postoperative pain relief had observed mostly positive results, especially with surgical procedures involving a large amount of tissue trauma, such as orthopedic and neurological surgery [18]. Dexamethasone when added to solutions for intravenous regional nerve blocks has been demonstrated to reduce pain and in addition to acetaminophen supplementation it can reduce pain for the first 24 hours after surgery[21].

In this study we have administered intravenous dexamethasone 0.15 mg/kg body weight before induction of anaesthesia. By giving this intermediate dose of dexamethasone (0.11-0.2 mg/kg body weight), beneficial effects on postoperative pain relief, a reduction in opioid consumption as well as decrease in nausea and vomiting can be achieved [22].

In our study we assessed the effects of preoperative single dose dexamethasone (0.15 mg/kg body weight) for treatment of postoperative pain after tonsillectomy, where the pain scores were found to be significantly lower in patients at 6 and 12 hours postoperatively who were given dexamethasone preoperatively as compared to patients who did not receive the dexamethasone. Mathew et. al. [23] studied the effects of single preoperative dose of dexamethasone sodium phosphate in patients undergoing tonsillectomy by hot (electrocautery) and sharp (cold) dissection method and they have found that use of steroid with cold dissection method to be the most effective in reducing postoperative pain. Pain scores were significantly lowered, oral intake was improved in steroid group as compared to placebo group. In another study conducted by Carr et. al. [24] in which they had also observed the effects of single preoperative intravenous dose of dexamethasone on postoperative pain, in patients undergoing tonsillectomy. Pain was assessed by visual analogue scoring system for 10 days. The other group received a placebo. There was no statistically significant difference between the two groups, but the dexamethasone group had a trend to report less pain over first several days. Single dose was not associated with adverse effects, so the risk-benefit ratio may be favourable for this practice. McKean et. al. [25] reported in a study that perioperative dexamethasone administration improved pain scores, reduced analgesic requirements, allowed earlier oral fluid intake and improved postoperative swallowing and quality of oral intake. Stewart et. al.[26] also reported that dexamethasone reduced postoperative pain and analgesic requirements after adult tonsillectomy. These results may be attributed to the anti-inflammatory effect produced by dexamethasone by reducing local oedema and pain. These results are similar to patients receiving steroids for acute pharyngitis, where improvement in symptoms is mainly due to pain relief secondary to anti-inflammatory action of steroids[27-30]. These results were validated in the present study. Though several studies supported the results we obtained from this study but some researchers like Tewary et. al.[31] found that steroids were found to have no appreciable effect on the postoperative pain relief. In our study we have found no adverse events attributable to preoperative use of dexamethasone. No reports in different studies have been found about the complications from the use of a single intravenous dose of corticosteroid during tonsillectomy[23-25].

CONCLUSION

Single dose preoperative intravenous injection of dexamethasone reduced post tonsillectomy pain significantly at 6 and 12 hours after surgery. So, it may be used as suitable adjuvant as multimodal postoperative analgesia in tonsillectomy patients along with routinely prescribed NSAIDs.

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Original article

Estimation of HbA_{1c} by HPLC and TINIA method in patients with hemoglobin E trait.

Sheme ZA[1], Muttalib M.A[2], Choudhury S[3], Hoque S[4].

ABSTRACT

BACKGROUND: *Hemoglobin A_{1c} (HbA_{1c}) is a well-established indicator of glycemia in diabetes mellitus patients. The presence of genetic variants of hemoglobin such as Hb E can profoundly affect the accuracy of HbA_{1c} measurements.* ***OBJECTIVE:*** *The objective of our study is to compare the HbA_{1c} values measured by High Performance Liquid Chromatography (HPLC) and Turbidimetric Inhibition Immunoassay (TINIA) in diabetic patients having hemoglobin variant.* ***METHODS:*** *This study was performed in BIRDEM General Hospital within a period of two months from December 2013 to January 2014. For this purpose 50 diabetic patients were selected. Among the study subjects 27 cases had undetectable HbA_{1c} by HPLC method and they were included in group Ia. 23 cases had below normal HbA_{1c} levels by same method and they were included in group Ib. HbA_{1c} of both group patients were again analyzed by TINIA method. Age matched healthy 20 volunteer without diabetes that served as control, denoted as Group II and they were also analyzed by both HPLC method and TINIA method. Fasting blood sugar (FBS) was measured in group Ia and group Ib and correlation of FBS with HbA_{1c} by two methods were done in both groups. Alkaline electrophoresis was done to confirm hemoglobin variant in both cases and in control group.* ***RESULTS:*** *Among the 50 cases 27 cases showed undetectable (group Ia) HbA_{1c} value by HPLC method but mean (±SD) value of HbA_{1c} by TINIA method was 8.81±2.56. In group Ib subjects HbA_{1c} values obtained by HPLC was 3.30± 0.47 and TINIA method was 8.17± 1.76, two values showed significant difference $p<0.0001$.Fasting blood glucose in group Ia and group Ib were strongly correlated with HbA_{1c} value obtain by TINIA method ($r=0.70$, $p<0.0001$ and $r= 0.87$, $p<0.0001$ respectively) but does not correlate with HbA_{1c} value by HPLC method in group Ib($r=0.10$, $p=0.64$). Alkaline electrophoresis showed Hb E trait in case group but not in control caes.* ***CONCLUSION:*** *For the measurement of HbA_{1c} level, all laboratories should use alternative forms of testing, such as Turbidimetric Inhibition Immunoassay, for accurate determination of glycemic control in these individuals. Again proper knowledge of hemoglobin variants affecting the measurement of HbA_{1c} level is essential to the physician in order to avoid mismanagement of diabetic patients.*

KEYWORDS: Hemoglobin A_{1c}, Hemoglobin Variant, Hemoglobin E, Turbidimetric Inbibition Immunoassay, High Performance Liquid Chromatography.

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INTRODUCTION

HbA_{1c} is considered the "gold standard" to evaluate the degree of glycemic control in patients with diabetes[1,2].The American Diabetes Association (ADA) recommended an HbA_{1c} goal of less than 7%, while the American Association of Clinical Endocrinology recommended less than 6.5%[3,4]. HbA_{1c} stands for an accurate and objective measure of the ambient blood glucose concentration over a period of 8-12 weeks [5]. Accordingly it is advised to be done at 3 months interval, which has more prognostic value than diagnostic value. A value of less than 6% indicates good glycemic control over last the three months, but

more than 9% indicates poor control[5].

Hemoglobin E (HbE) is mainly found in the eastern half of Indian subcontinent and throughout South East Asia, where in some areas, carrier rates are as high as 60% of the population [6]. It is the second most prevalent hemoglobin variant worldwide [7]. HbE arises from the substitution of lysine for glutamic acid at position 26 of the β-globin chain [8].

There are more than 20 different assay methods are being used to measure the level of the HbA_{1c} in clinical Laboratories. These methods are on different analytical principles such as immune turbidimetry, cation exchange chromatography, HPLC[9,10]. Approximately 30% use a Cation-Exchange or Ion Eexchange HPLC (High Performance Liquid Chromatography) method. Nearly 15% use immunoassay, whereas less than 5% use electrophoretic methods. However, presently, Cation Exchange performed by HPLC is the most widely used assay method[11]. Another method that continues to be used for HbA_{1c} measurement in the clinical lab is Turbidimetric Inhibition Immunoassay (TINIA) method. This method quantifies HbA_{1c} using antibody-mediated inhibition of latex agglutination. Antibodies (Abs) used in this methods recognize the N-terminal glycated amino acid in the context of the first 4 to 10 amino acids of the Hb β-chain[12]. The antibody is able to detect hemoglobin A, A1, A2, S, C, O and E that have been glycosylated. This method is accurate in hemoglobin variants C, E and O because the terminal β- globin chains are identical to hemoglobin A[13]. In Hb E the amino acid substitution at position 26 was far from the N-terminus of the β-globin chain where HbA_{1c} glycation and antibody binding took place [8]. As the mutation falls outside regions of recognition of most antibodies, the mutation has little effect on immunoassay methods[14]. More than 1000 hemoglobin variants have been identified with many of them being clinically silent. Therefore, a falsely high or low HbA_{1c} value caused by the presence of a clinically silent hemoglobin variant may lead to over or under treatment of diabetic patients[10]. Our study was to compare the HbA_{1c} values measured on High HPLC and TINIA in diabetic patients with variant hemoglobin.

MATERIALS AND METHODS

The study was carried out on adult stable male and female type 2 diabetic patients receiving treatment at BIRDEM General Hospital within December 2013 to January 2014. 50 samples showed presence of variant window on the HPLC chromatogram, so they were included in our study. HbA_{1c} measurements were performed on EDTA blood samples. 50 Samples were first analyzed by HPLC method using VARIANT-II TURBO- Bio Rad Laboratories (USA) according to the manufacturer's instructions. Samples that showed undetectable or below 4% HbA_{1c} levels by HPLC method as well as a variant window on the HPLC chromatogram were selected for comparison with TINIA method using Dade Behring autoanlyzer (USA) according to the manufacturer's instructions. Fasting blood glucose was correlated with HbA_{1c} values obtain by both HPLC and TINIA method. Hemoglobin electrophoresis was done for each subject by alkaline method on agarose gel to detect Hb variant.

Among the 50 study subjects 27 showed undetectable HbA_{1c} value by HPLC and they were included in Group Ia and 23 showed below 4% HbA_{1c} value by HPLC and they were included in Group Ib. All showed variant window on HPLC chromatogram. 20 age matched healthy volunteer without diabetes were included as controls (Group II). Comparison of HPLC and TINIA method was performed on a total of 70 subjects.

Data was expressed as Mean±SD, number (percent) as applicable. Student't' test, Pearson's correlation was performed by using statistical package for social science (SPSS) for windows version 11.5 and MedCalc Statistical

software respectively. $P < 0.05$ was taken as statistical significant level.

RESULTS

In group Ia mean HbA_{1c} value was 8.81±2.56 by TINIA where HbA_{1c} value could not be detected by HPLC. In group Ib subjects HbA_{1c} values obtained by HPLC was 3.30± 0.47 and TINIA method was 8.17± 1.76, two values showed statistically significant difference p<0.0001. Whereas in controls HbA_{1c} measured by TINIA and HPLC did not show significant difference (>0.05) (Table I).

Table I: HbA_{1c} values estimated by HPLC and TINIA method in different groups.

Groups	HPLC method	TINIA method	P value
Group Ia (n = 27)	#	8.81±2.56	NA
Group b (n = 23)	3.30± 0.47	8.17± 1.76	<0.0001
Group I (n = 20)	5.80 ±0.46	6.00±0.35	>0.05

Results expressed as Mean ± SD; SD: Standard deviation; student't' test was done for appropriate significant test; n: Number of subjects;
Group Ia: Undetected HbA_{1C} by HPLC;
Group Ib: having Less than 4% HbA_{1c} by HPLC;
Group II: Controls;NA: Not applicable. # = Undetectable.
P< 0.05 was taken as level of significance.

The relationship of Fasting blood glucose with the mean HbA_{1c} values obtained by TINIA method in group Ia and in group Ib were significantly positive (r=0.70, p<0.0001 and r= 0.87, P<0.0001 respectively). But HbA_{1c} values obtained by HPLC method in group Ib did not show significant correlation with fasting blood glucose (r=0.10, p>0.05) (Table II).

Table II: Correlation between mean FBG and HbA_{1c} values in study groups

Groups	Methods of HbA1c estimation	r	p value
Ia	HPLC	NA	NA
	TINIA	0.70	<0.0001
Ib	HPLC	0.10	>0.05
	TINIA	0.87	<0.0001

Results expressed as Mean ± SD, SD: Standard deviation
Correlation coefficient: r ; FBG: Fasting blood glucose;
HbA1c: Glycosylated hemoglobin;
Group Ib: < 4% HbA1c by HPLC.
Group Ia:Undetected HbA1C by HPLC
P< 0.05 was taken as level of significance.

DISCUSSION

In this study the patients with HbE showed low HbA1c value by HPLC method when compared to TINIA method. Similar findings were demonstrated by Little RR et. al. [8]. In their study they had demonstrated that with HbE trait HbA1c estimated by HPLC was low compared to the immunoassay. The significant difference between HbA1c as estimated by TINIA and HPLC method was supported by another study [7]. However, Pravatmuang P et. al.[15] found no statistically significant difference of HbA1c results in Hb E trait patients using HPLC (Bio-Rad Variant) and immunoassay (Tina-quant / Hitachi 912).

Reports suggested that erythrocyte lifespan is normal in individuals with HbE which might be the reason that results from TINIA method are not affected by this certain type of hemoglobinopathy[16, 17].Again, Cation-exchange HPLC separates hemoglobin species based on charge differences. Inaccurate HbA1c values can occur when hemoglobin variants or its glycated derivatives cannot be separated from HbA or HbA1c. Co-elution of the hemoglobin variant with HbA_{1c} will cause gross overestimation of HbA_{1c}, while co-elution of

the hemoglobin variant with HbA, with resolution of the glycated hemoglobin variant from HbA_{1c}, will underestimate the HbA_{1c} results[11].

Moreover, HbE1c frequently elutes as a shoulder to HbA_{1c} in most HPLC or Cation-Exchange Chromatographic methods[14]. Unless corrected, these methods lead to inaccurate determinations of HbA1c that may be spuriously increased or decreased, depending on the method used[18,19]. HbE arises from the substitution of lysine for glutamic acid at position 26 of the β-globin chain[8]. In TINIA method Antibodies are used to recognize the N-terminal glycated amino acid in the context of the first 4 to 10 amino acids of the Hb β-chain[12]. As the mutation falls outside regions of recognition of most antibodies, the mutation has little effect on immunoassay methods although one study reported that a correction was required to determine accurate glycated Hb values with the immunoassay[14].

CONCLUSION

HbA_{1c} is considered as the "gold standard" to evaluate the degree of glycemic control in patients with diabetes. But variant hemoglobin can affect the value of HbA_{1c}. Knowledge and awareness of hemoglobin variants affecting HbA_{1c} measurements is essential, especially in areas with a high prevalence of hemoglobinopathy. Though HPLC is reference method for HbA_{1c} analysis, in presence of Hb E trait HPLC cannot give accurate result. So, an alternate method such as TINIA can be used in the laboratories for cross check the result. Moreover, HPLC may be a convenient tool for screening of hemoglobinopathies especially among diabetic population in Bangladesh as it is less expensive.

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Original article

Single Versus Multiple Dose Regimen of Prophylactic Antibiotic in Cesarean Section.

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ABSTRACT

BACKGROUND: *Postoperative infection is common in Caesarean section. Antibiotic prophylaxis may have significant impact in reduction of infections and thus the need to study its role in sepsis prevention systematically.* ***OBJECTIVE:*** *The aim of this study is to compare the efficacy of single dose versus multiple doses of a third generation cephalosporin ceftriaxone, to reduce postoperative infectious morbidity in elective caesarean section.* ***METHODS:*** *Prospective randomized trial. One hundred and fifty pregnant women who underwent elective and emergency cesarean section were randomly assigned into 2 groups. In group A (n=75) patients received 1 gm of ceftriaxone intravenously after delivery of the baby and umbilical cord clamping. Patients in group B(n=75) received 1 gm of ceftriaxone intravenously after delivery of the baby and umbilical cord clamping and additional dose of injection ceftriaxone 1 gm intravenously daily for 3 days. Both groups were observed regarding maternal febrile morbidity, endometritis, wound infection, and urinary tract infection (UTI).* ***RESULTS:*** *Both groups were comparable regarding maternal age, body weight, gestational age, parity and indications of cesarean section. The number of cases who had febrile morbidity, endometritis, wound infection, and UTI were almost similar, these differences did not reach statistical significance (p= 0.97, 0.65, 0.47 and 0.89 respectively). The overall infectious morbidity in group A was 11(14.67%) and 9(12%) in group B and the difference between two groups was not statistically significant (p>0.05).* ***CONCLUSION:*** *A single prophylactic dose of ceftriaxone is effective as multiple postoperative doses of ceftriaxone in preventing postoperative infectious morbidity in caesarean section when given post-umbilical cord clamping.*

KEY WORDS: Antibiotic prophylaxis, cesarean section, ceftriaxone.

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INTRODUCTION

Cesarean section is the most commonly performed major surgical procedure [1]. Infectious morbidity is the most common complication following cesarean section with reported rates ranging from 18-83%.[2-5] Women undergoing cesarean delivery have significant incidence of many infectious complications; including fever, wound infection, endometritis, urinary tract infections and pelvic abscess [6]. Antibiotic prophylaxis for cesarean section has been a general practice because it significantly reduces postoperative infections[7]. Cephalosporin second or third generation has been evaluated as prophylactic antibiotic in cesarean section with emerging results[8]. Ceftriaxone, a third generation cephalosporine has shown an excellent profile against surgical

infecting organisms[9].

In developed countries prophylactic antibiotics are not used for more than 24 hours because studies have proved that prophylaxis is more efficacious when given in preoperative period and is maintained during surgery and there is no added benefit of multiple dose prophylaxis given after wound closure[10,11]. In developing countries like Bangladesh, prolonged antibiotic therapy is still used considering that facilities for operation theatre sterilization are not ideal[12]. We expect probably prolonged antibiotic therapy will be more effective to control infectious morbidity, although this results in economic burden on health system as well as emergence of resistant organisms[13,14].

This current study was conducted to investigate the impact of single dose ceftriaxone versus three dose ceftriaxone as prophylactic antibiotic for cesarean section on maternal infectious morbidity.

MATERIALS AND METHODS

This prospective study was performed at Combined Military Hospital, Rangpur in one calendar year from July 2013 to June 2014. After departmental approval and obtaining informed written concepts from the patients, 150 patients who were scheduled for emergency or elective cesarean section were enrolled in the study. Women were excluded from the study if they had absent membranes for more than 4 hours, severe anaemia, diabetes mellitus, impaired glucose tolerance test, received antibiotics within two weeks prior to the operation, visible infection at any site, fever at the time of operation, known case of suppressed immunity or unwilling to participate in the study. Women were randomly distributed in two groups of 75 each. In group A patients received single dose of 1 gm of ceftriaxone intravenously during delivery of the baby and umbilical cord clamping. Patients in group B received 1 gm of ceftriaxone intravenously after delivery of the baby and umbilical cord clamping and additional dose of inj. ceftriaxone 1 gm intravenously daily for 3 days. All cesarean section were done by standard technique. Each patient was examined daily and post-operative infectious morbidity noted till the date of discharge from the hospital. A complete blood count and urine analysis were performed if necessary on third post-operative day. The primary outcomes were the postpartum complications.

Febrile morbidity: Oral temperature above 38°C on two or more occasions at four hour apart excluding first 24 hours after cesarean section.

Endometritis: Fever, uterine tenderness and purulent lochia.

Wound infection: Cellulites, fever and exudates.

Urinary tract infection (UTI): Fever and positive urine analysis.

These infectious morbidities were treated according to their respective protocol.

All results were expressed in mean $\pm$ SD or percentage as applicable. Statistical analyses were carried out using Statistical Package for Social Science (SPSS) for Windows Version 17.0. Results were considered statistically significant if $p < 0.05$.

RESULTS

The demographic data of all the subjects are shown in Table I. Two groups were similar and fairly comparable with respect to age, body weight, parity and gestational age and differences were statistically not significant ($p > 0.05$).

Table I: Demographic characteristics of the study subjects.

Variables	Group A (n=75)	Group B (n=75)	p value
Age (years)	26.71 ± 3.98	27.23 ± 4.21	0.47[ns]
Weight (kg)	65.18 ± 5.87	64.43 ± 6.23	0.39 [ns]
Parity	1.64 ±1.2	1.7 ±1.31	0.13 [ns]
Gestational Age (weeks)	38.03 ± 0.91	38.87± 1.2	0.97 [ns]

Values are expressed in Mean + SD
Students unpaired 't' test was done for comparison;
n= number of subjects; ns= not significant.

Various indications of cesarean section are shown in Table II. There were no significant (p>0.05) differences regarding indications of cesarean section in both groups.

Table II: Distribution of the subjects according to Indications for cesarean section.

Indication	Group A (n=75)	Group B (n=75)	Chi-square	P value
Previous cesarean section	32(42.67%)	34(45.33%)		
Foetal distress	13(17.34%)	11(14.67%)		
Failure to progress labour	10(13.34%)	9(12%)	0.507	0.99186[NS]
Breech presentation	9(12%)	8(10.67%)		
Cephalopelvic disproportion	6(8%)	7(9.33%)		
Others	5(6%)	6(8%)		

Figures in parenthesis indicates Percentage. chi square test was done for comparison.
n= number of subjects; NS= not significant.

The frequencies of maternal infectious morbidity are shown in Table III. Incidences of febrile morbidity was 4(5.33%) in group A and 3(4%) in group B and difference was statistically not significant (p>0.05). Endometritis was found 3(4%) in group A and 2(2.67%) in group B and difference was statistically not significant (p>0.05). Wound infection was observed 2(2.67%) in group A and 3(4%) in group B and difference was statistically not significant (p>0.05). There was no statistically significant (p>0.05) difference in the incidence of UTI between two groups, 2(2.67%) in group A and 1(1.33%) in group B.

Table III: Distribution of the subjects according to maternal infectious morbidity

Outcome	Group A (n=75)	Group B (n=75)	Chi - square	P value
Febrile morbidity	4(5.33%)	3(4%)		
Endometritis	3(4%)	2(2.67%)	0.683	0.87719[NS]
Wound infection	2(2.67%)	3(4%)		
UTI	2(2.67%)	1(1.33%)		

Values in parenthesis are expressed in Percentage. chi square test was done for comparison.

N= number of subjects; NS= not significant at <0.05.

DISCUSSION

In all surgical procedure there is potential for postoperative infection. This is particularly the case for cesarean section, due to the direct anatomical connection of the vagina with the operation site, allowing normal vaginal and bowel flora and pathogens to ascend intra and post-operatively and colonize both the placental site and the wound site[15]. Advances in surgery and sophisticated life-saving procedures make it essential to pay particular attention to the prevention of infection. The concept of antibiotic prophylaxis was introduced in 1960s which has remarkably reduced the rate of postoperative infections[16,17]. Overuse of prophylactic antibiotics has resulted in emergence of resistant organism and increased economic burden on health system[18,19]. In developing countries like Bangladesh there is a general trend of 5 to 7 days of antibiotic prophylaxis. Reasons for this prolong administration of antibiotics is probably the fear of increased rate of infections due to substandard theater environment in busy hospitals having limited resources, overcrowding, malnutrition and environmental pollution.

In this study, both emergency and elective caesarean sections have been included. Single

dose ceftriaxone was compared with three dose ceftriaxone as prophylactic antibiotic for cesarean section on maternal infectious morbidity. The present study shows no statistically significant difference as regards demographic characteristics, maternal characteristics in obstetrical history, and about indications of cesarean section. Concerning the indications of cesarean section, the present study findings revealed that, the previous cesarean section were found 32(42.67%)in group A and 34(45.33%)in group B. This is agreed nearly with Gulfareen et. al.[20], who showed that, repeated cesarean sections were primary indication.

Regarding postoperative infectious morbidity; febrile morbidity was observed in 4(5.33%) patients in group A with 24 hours antibiotic prophylaxis and 3(4%) patients in group B with 3 days antibiotic prophylaxis. The difference between two groups regarding febrile morbidity was statistically not significant. This finding is comparable to other studies done by Bagratee et. al.[5] Rouzi et. al.[21], Dimitrov et. al.[22] and Jacobi et. al.[23] Another indicator of postoperative maternal infection is endometritis. In this study endometritis was found 3(4%) in group A and 2(2.67%) in goup B and difference is statistically not significant. In a prospective study of 122 patients studied two dose of amoxicillin-clavulanic acid versus three doses of the same had 0% incidence of endometritis in the two doses and 1.6% in the three dose group [24].

In the study by Noyes et. al., 293 patients received single dose of one of the three drugs cefazoline, ampicillin-sulbactum or cefotan; the incidence of endometritis with cefazoline regimen was 14.3%, with ampicillin-sulbactum was 7.4% and cefotan was 11.1% [25]. Wound infection is one of the most common complications occurring following any surgery. The incidence of wound infection following cesarean delivery ranges from 3% to 15% with an average of 6% [26]. In this study wound infection had found 2(2.67%) patients in group A with 24 hours antibiotic prophylaxis and 3(4%) patients in group B with 3 days antibiotic prophylaxis. This finding is comparable to finding of study conducted by William et al[(27)] and Shetty et. al.[(28)] Kahyihura et. al.[(29)] did a study in Mozambique comparing single dose versus existing policy of administering multiple dose regimen for seven days. However, that study showed 5.8% of wound infection with no statistically significant difference.

Urinary tract infection (UTI) is common infectious morbidity following cesarean delivery. In this study UTI found 2(2.67%) patients in group A and 1(1.33%) patients in group B. This finding is comparable to study of William et. al., [27] Shetty et. al.[28] and Shakya et. al.[30] in which incidence of urinary tract infections were 3.5%, 2% and 2.4% respectively.

In the present study, we did not note a difference between the two groups in rates of endometritis, wound infection, or other infectious morbidity. Over all total number of infectious morbidity was 11(14.67%) patients in group A with 24 hours antibiotic prophylaxis and 9(12%) and results agree with a previous study by Chittacharoen et. al. [31].

The limitations of the study were small sample size, cost was not investigated and there was no other type of antibiotic used as control.

CONCLUSION

Single dose regimen of ceftriaxone as a prophylaxis during cesarean section versus multiple dose regimen of ceftriaxone for three days was equally effective in preventing surgical infectious morbidities during cesarean section. Therefore there is a need of change in our practice and thereby development of a hospital protocol with emphasis on single dose of prophylaxis in cesarean delivery.

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Original article

Study on Serum total Iron in Urban and Rural School Going Children.

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ABSTRACT

Background: *Undernutrition is one of the major health problem in Bangladesh. Urban and rural school going children are usually affected by micronutrient deficiency.* ***Objectives:*** *To determine serum total iron level in selected urban and rural school going children and compare the values among the groups.* ***Methods:*** *This cross sectional study was conducted from July 2013 to June 2014 in the Department of Physiology, Rangpur Medical College, Rangpur. For this, 50 urban school going children were included in group A and 50 rural school going children were included in group B. The children were selected from both primary and secondary section of urban and rural area of Rangpur district. Serum total iron was estimated by Bichromatic endpoint technique. For statistical analysis independent sample "t" test was performed by computer based software SPSS- 17.0 version for windows.* ***Results:*** *Serum total iron was significantly decreased ($P<0.05$) in urban school going children compared to rural group.* ***Conclusion:*** *Urban school going children have decreased level of serum total iron.*

KEY WORDS: Serum total iron ,Urban school going children, Rural school going children.

INTRODUCTION

Micronutrients are of minerals & vitamins[1]. The minerals are classified as principal elements & trace elements. The microminerals (trace elements) are required in amounts less than 100 mg/day. Essential trace elements are iron, copper, iodine, magnesium, manganese, zinc, molybdenum, cobalt, fluorine, selenium and chromium. Minerals perform several vital functions which are absolutely essential for the very existence of living organisms [2]. Minerals play an important role in the promotion of health & prevention of diseases [3]. Approximately 2 billion children in the world suffer from micronutrient malnutrition & 85% of them resides in developing countries [4].

Iron is of great importance in human nutrition. It is necessary for many functions in the body including formation of hemoglobin, development of brain and it's function, muscle activity and catecholamine metabolism [1]. School going age is a period of life characterized by rapid increase of physical growth [5]. At this period children are at risk of iron deficiency because of an expanding muscle mass and increased red cell production [6]. Iron deficiency is one of the most common causes of anaemia. It is a world-wide problem that is highly prevalent in developing countries with the highest incidence reported in Asia. Again, high prevalence of anaemia is observed in children and its causes are multifactorial. It has been estimated that about 40% of the worlds population (more than 2 billion individuals) suffer from anaemia with a prevalence of 48% incase of school aged children [7]. Socio-economic factors like low income,

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illiteracy & more numbers of family member are the common factors of causing iron deficiency anaemia in rural children [8]. Moreover, urban children usually passes more time in school. They lacked organized feeding programs [9] Poor bio-availability of iron from diet and rising trend of consumption of 'empty calorie' foods were suggested to be the main causes of low serum total iron level in urban school going children[10].

Bangladesh is one of the most densely populated country in the world. The country has a population of about 160 million, with a corresponding population density of more than 920 persons per square kilometer [11]. The population in Bangladesh is predominantly rural, with almost 80% of the population living in the rural areas [12]. Many of them live in remote areas that lack services such as education & health care [13]. Hookworm infestation & under nutrition are also leading causes of anaemia of rural children in Bangladesh because they have lack of knowledge about sanitation, nutrition [14]. But, now a days, rates of malnutrition are currently increasing faster in urban than in rural areas of developing countries, which may be due to alteration of dietary habit and life style modification in urban children [15].

The relationship between health and education is very important where healthy child can be human resource in future. In order to prepare them physically, mentally and socially for entry into adulthood as well as step towards achieving the goal of 'Health for All', it is necessary to provide comprehensive health care that is promotive, preventive, curative and rehabilitative, to school students [16]. In this context our present work has been designed to study the serum total iron levels in urban and rural school going children in Bangladesh.

MATERIALS AND METHOD

The present cross-sectional analytical study was carried out in the Department of Physiology, Rangpur Medical College, Rangpur from July 2013 to June 2014. The protocol of the study was approved by the Rangpur Medical College Ethical committee and thesis protocol committee. For this study a total number of 100 primary and secondary school going children both boys and girls (6-16 years) were selected. Study subjects were divided into two groups. 50 urban school going children were included in group A and 50 rural school going children were included in group B . After selection of subjects, the purpose of the study was explained to each subjects. When they agreed for participation, then Informed written consents were taken from the subjects. Detailed family history and medical history were taken. Five (5) ml of venous blood was collected from antecubital vein from each subject under all aseptic precaution by a disposable syringe. Then blood was taken for haemoglobin estimation and the needle detached from the nozzle and blood was immediately transferred into a deionized test tube with a gentle push to avoid haemolysis. Test tubes were kept in standing position till formation of clot. Serum was separated by centrifuging the blood at 3000 rmp for 5 minutes. The clear supernatant serum was taken and kept in an ependorf tube. Tests were carried out as early as possible. All data were recorded systematically in a preformed history sheet and all statistical analysis was done by computer using the software SPSS 17.0 version for windows. Comparison of total serum total iron levels in urban and rural school-going children were done by unpaired 't' test. In the interpretation of results, level of probability (P) < 0.05 was accepted as significance.

RESULTS

The mean ± SD serum total iron levels were 66.17 ± 38.865 µgm/dl in group A and 84.59 ± 46.838 µgm/dl in group B. Serum total iron is significantly decreased ($P<0.05$) in urban school going children(group A) compared to rural group (group B) (Table I).

Table I: Mean serum total iron level in urban and rural

Groups	Serum total iron level (µgm/dl)	't' value
Group A (n= 50)	66.17 ± 38.865 (50 - 120)	2.052*
Group B (n= 50)	84.59 ± 46.838 (53 - 122)	

figures in parenthesis ranges. Statistical analysis done by Unpaired students't' test.
n= Number of subjects. *= $P < 0.05$.
#= Normal range of serum total iron level is 50 to 175 µgm/dl. [17]

DISCUSSION

The present work was undertaken to study the serum total iron levels in age matched apparently healthy urban and rural school going children. In the present study serum total iron levels in healthy urban and rural school going children were within normal ranges.

The mean serum total iron level of urban school going children was significantly ($P<0.05$) lower than the mean serum total iron level of rural school going children. This finding is similar with those reported by Srihari G et. al.[15], Barugahara EI et. al.[9], Dabone C et. al.[18] and Verma et. al[10]. Srihari G et. al.[15] found significantly decreased serum total iron level in upper and middle classes of urban school going children. Low intake of iron, poor bio-availability of iron from diet and rising trend of consumption of 'empty calorie' foods were suggested to be the main causes of low iron level due to alteration of dietary habit and lifestyle modification in those upper and middle classes of urban school going children. They also found that alteration of dietary habits combined with decreased physical activity lead to an increase in over-weight and obese children have a higher prevalence of iron deficiency. They have also mentioned that vitamin A deficiency, chronic infections and inflammations and hemorrhage may lead to iron deficiency. It has been observed that episodes of diarrhea, cough, cold and fever are common amongst children from middle and high socio-economic status of urban school going children. Again, Helicobacter pylori infection is widely responsible to reduce iron absorption. Psychological and bio-ecologic environment of children also affect the micronutrients status of children. Infections are known to reduce uptake, utilization of micronutrient. Barugahara EI et. al.[9] also found significantly decreased serum total iron level in urban school going children. This might be due to the low iron-riched food. They also found that folate, copper, cobalt, zinc, vitamins A, E, B_2, B_6, B_{12} and ascorbic acid deficiency are related to nutritional anemia, because these nutrients are required in the synthesis of hemoglobin. They also reported that urban school going children usually passes more time in a school but they lacked organized feeding programs and only a few of these children carries packed lunch to school which are qualitatively and quantitatively inadequate. Moreover, school canteens sale foods having low nutritive value and often unhygienic. Verma et. al.[10] found significantly decreased serum total iron level in urban school going children, which may be due to the rising trend of consuming snacks and junk foods.

In our study we have observed significantly decreased serum total iron level in urban school going children than rural school going children. The exact mechanisms involved for the alteration of serum total iron levels between urban and rural school going children couldn't be elucidated from this type of study. The underlying possible mechanisms of anemia in this group of children may be due to low intake of iron-rich food, alteration of dietary habit and modification of lifestyle especially during periods of rapid growth, staying long time in school, lack of organized feeding programs, selling of food having less nutritive value by canteens, consuming snacks and junk foods.

CONCLUSION

The serum total iron level in urban school going children is lower than rural school going children. So, urban school going children should be encouraged to take iron riched food, alter their dietary habit and modification of lifestyle.

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Original article

Prevalence of Extended-Spectrum Beta Lactamase-producing uropathogenic *Enterobacteriaceae and Pseudomonas* in a tertiary level hospital in Dhaka, Bangladesh.

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ABSTRACT

INTRODUCTION: *Extended Spectrum Beta Lactamases (ESBLs) are enzymes produced by Gram negative bacteria become a great challenge for antibiotic treatment, as they are capable of hydrolyzing extended-spectrum cephalosporins, penicillins and monobactam but inactive against cephamycin and imipenem.* ***OBJECTIVE:*** *This study was carried out to detect extended spectrum β-lactamase producing Gram negative bacteria rapidly by using a kit containing chromogenic cephalosporin directly from primary culture and by phenotypic confirmatory method.* ***METHODS:*** *This cross sectional study was conducted in the Department of Microbiology, BSMMU from January to December 2006, at a period of one calendar year. For this total 280 urine samples were collected from suspected cases of urinary tract infection from BSMMU. All urine samples were inoculated on CLED (Cystine lactose electrolyte deficient agar) media by calibrated loop technique. All the plates were incubated overnight at 37°C aerobically. Organisms were identified by their colony morphology, pigment production, staining character, motility and relevant biochemical tests. Antibiogram for all bacterial isolates were done by disc diffusion method using Mueller Hinton agar plates. Detection of ESBLs was done by using a kit containing chromogenic cephalosporin and by double disc diffusion test.* ***RESULTS:*** *Total 150 (53.57%) bacteria were obtained from all patients as causative agents. Among the isolates 133 (88.67%) were Gram negative bacteria (E.coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Enterobactor spp., Acinetobactor spp.) and 17 (11.33%) were Gram positive (Enterococci spp. and CNS). Out of 133 Gram negative bacteria 43 (32.33%) were found to be extended spectrum - lactamases (ESBLs) producer. Highest rate of ESBLs was observed in Klebsiella spp. 04 (36.36%) out of 11, followed by E. coli 29 (34.12%) out of 85, Enterobactor spp. 5 (33.33%) out of 15, Proteus spp. 1 (25%) out of 4, Acinetobactor spp. 3 (23.08%) out of 13, Pseudomonas spp. 1 (20%) out of 05.* ***CONCLUSION:*** *Considerable number of ESBLs producer from UTI cases, indicate that ESBLs will be major threat for future antibiotic therapy.*

KEY WORDS: Extended Spectrum Beta Lactamases (ESBLs), The Cica Beta Test, 1/Chromogenic Cephalosporin Test/ HMRZ-86 Test, Phenotypic confirmatory test

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INTRODUCTION:

Extended Spectrum β-lactamases (ESBLs) are the enzymes produced by the member of the Enterobacteriaceae can confer resistance to all extended spectrum cephalosporins, all penicillins and monobactam [1,2]. Such enzymes are most commonly produced by in *Klebsiella pneumoniae* and *Escherichia coli*, but they have also been detected *in Klebsiella oxytoca, Proteus mirabilis, Salmonella species*, other members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa* [3,4].

The first Beta lactamases was identified in

Escherichia coli prior to the use of penicillin in medical practice [5]. Many genera of Gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase [6]. Being plasmid and transposon mediated have facilitated the spread of these enzymes to other species of bacteria.

In vitro, the organisms with ESBL may appear to be resistant to third generation cephalosporin and susceptible to second-generation cephalosporins. These drugs often found to show efficacy in vitro but to be ineffective in vivo[7].

Genes for ESBL are distributed on large plasmids, which confer multiple drug resistance e.g. aminoglycosides, tetracycline, chloroamphenicol, trimethoprim[8]. They are inhibited by the beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. ESBLs are sensitive to imipenem [2].Till now there is no gold standard test for detection of ESBLs. NCCLS (National Committee for Clinical Laboratory Standards) recommended the phenotypic method as confirmatory test [9].

METHODS:

This cross sectional study was conducted in the Department of Microbiology, BSMMU from January to December 2006, at a period of one calendar year. For this a total 280 samples of urine were collected from in-patient and out-patient department of BSMMU having clinical symptoms of infection with informed verbal consent from the patients or from the attendants. Samples were collected from both sexes and different age groups. Laboratory work was performed in department of Microbiology & Immunology, BSMMU. From 280 urine samples 150 bacteria were isolated.

SPECIMEN: Urine

INCLUSION CRITERIA: Patients with clinical sign/symptoms of urinary tract infection.

EXCLUSION CRITERIA: Pus cell < 5/HPF in a centrifuged urine sample.

Collection of urine:

Total 280 urine samples were collected. Urine sample about 15-20ml was collected in a sterile container by standard technique[10]. The female patients were requested to wash their hands and to clean the area around the urethral opening clean with soap and water and to collect mid stream urine with labia held apart after drying the area.

For male patients- after washing hands, clean glans with soap and water and after drying mid-stream urine was collected.

For patients with urethral catheterisation, urine was collected from catheter by syringe and needle after proper cleaning the catheter. Approximately 20 ml of urine was collected aseptically in a sterile container.

Aseptically about 10ml of well mixed urine was transferred to a labelled conical tube and centrifuged at 2000 rpm for 5 minutes. The supernatant fluid was discarded and one drop of sediment was transferred to a clean glass slide, covered with a clean cover slip and then examined under light microscope using 10× and 40× objectives. On the basis of findings of pus cells ≥5/HPF, urine samples were included in this study.

Culture

Urine samples were inoculated on CLED (cystine lactose electrolyte deficient) media by calibrated loop technique. All the plates were incubated at 37°C aerobically. After overnight incubation, plates checked for presence of suspected pathogens. Colony count was done by calibrated loop method [11].

Isolation and identification of organisms

All the organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard methods [10,12].

Preservation of isolated organisms:

Organisms grown in appropriate media were inoculated in a nutrient agar slant. When growth is seen, after overnight incubation at 37°C, sterile liquid paraffin oil was added at top of slant. Tube was closed with screw cap, and kept at 2-8°C in refrigerator. Only Gram negative strains (*Enterobacteriaceae, Pseudomonas* spp.) were preserved by this method.

Media for antibiotic sensitivity test

Mueller-Hinton agar medium was used for antimicrobial susceptibility testing for all the bacteria.

Antimicrobial sensitivity test:

All bacterial isolates were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method against different antimicrobial agents [13].

For *E.coli, Klebsiella spp., Enterobactor spp., Acinetobactor spp., Proteus spp.*: Amoxycillin (AMX), Cotrimoxazole (SXT), Gentamicin (CN), Amikacin (Ak), Nalidixic acid (Na), Nitrofurantoin (Nf), Cefradine (CL), Ciprofloxacin (CIP), Mecillinium (Mel), Netilmycin (NET), Ceftriaxone (CRO), Ceftazidime (CAZ) and Imipenem (I) were used.

For *Pseudomonas spp.*: Gentamicin (CN), amikacin (Ak), Ciprofloxacine (CIP), Aztreonom (ATM), Ceftazidime (CAZ), Ceftriaxone (CRO), Carbenicillin (CAR), Pipercillin (Pc), Netilmycine (Net) and Imipenem (I) were used.

Method of sensitivity testing:

Sensitivity test was done by following Kirby-Bauer disk diffusion method.

Interpretation of zone size

Zone of inhibition produced by each was considered into three susceptibility categories namely Sensitive (S), Intermediate and Resistant (R). (NCCLS, 1999) [9].

Isolated gram negative bacteria were subjected to following ESBL tests.

- The Cica Beta Test 1/Chromogenic Cephalosporin Test/ HMRZ-86 Test.
- Phenotypic confirmatory test (NCCLS, 1999) [9].

The Cica Beta Test 1:

All gram negative isolates were tested by using test kit- Cica Beta Test -1/ Chromogenic Cephalosporin. This kit originally designed for rapid detection of ESBLs in gram negative rods directly from isolated colonies.

The kit consists of plastic strip with a paper pad and solution substrate- HMRZ-86 new chromogenic cephalosporin; (7R)-7-[2-(aminothiazol-4-yl)-(Z)-2-(1-carboxy-1-methyl-ethoxyimino) acetamido]-3-(2,4-dinitrostyryl)-3-cephem-4-carboxylic acid trifluoroacetate, E-isomer.

One drop of kit substrate solution was dropped on the filter strip. Single isolated colony of the organism was then rubbed on the pad surface directly from primary culture and was left to stand at room temperature for 15 minutes and the color of the paper pad was observed with the naked eye.

Within 2-15 minutes, a change in color from yellow to red was taken as positive result. If the color remains yellow, the strain was considered as ESBL negative.

Phenotypic confirmatory test (NCCLS, 1999) [9].

Confirmation of ESBLs- producing isolates were done by inhibitor potentiated disc diffusion test according to NCCLS recommendation[9]. Mueller Hinton plates were inoculated with test bacteria (corresponding to 0.5 McFarland tube). Ceftazidime, cefotaxime disc without clavulanic acid was placed on one side of inoculated plate and ceftazidime, cefotaxime disc combined with clavulanic acid

was placed on other side of plate. Then the plates were incubated at 37°C overnight. After overnight incubation inhibition zone diameter was measured with scale. It was observed whether there was an increase in zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid compared to its zone diameter for cefotaxime and ceftazidime tested alone.

Interpretation of phenotypic confirmatory test:

≥ 5 mm increase in a zone diameter for cefotaxime and ceftazidime in combination with clavulanic acid then the zone diameter of cefotaxime and ceftazidime when tested alone, confirms an ESBL producing organism.

Quality control organisms used for ESBL detection:

K. pneumoniae ATCC 700603 (positive control) and *E. coli* ATCC 25922 (negative control) should be used for quality control of ESBL tests[9].

RESULTS

In this study out of 150 isolates 133 (88.67%) were Gram negative bacteria and 17 (11.33%) were Gram positive bacteria and out of 133 Gram negative bacteria 43 (32.33%) were found to be extended spectrum β- lactamases (ESBLs) producer. Highest rate of ESBLs was observed in *Klebsiella* spp. 04 (36.36%) out of 11, followed by *E. coli* 29 (34.12%) out of 85, *Enterobactor* spp. 5 (33.33%) out of 15, *Proteus* spp. 1 (25%) out of 4, *Acinetobactor* spp. 3 (23.08%) out of 13, *Pseudomonas* spp. 1 (20%) out of 05 (Table I).

Table I: ESBLs producer among the Different species of Gram negative bacteria in urine sample.

Sample	Number of Gm -ve Bacteria in urine sample	No of ESBLs +ve Strain (%)
E.coli	85	29 (34.12)
Klebsiella spp.	11	4 (36.36)
Proteus spp.	4	1 (25)
Pseudomonas spp.	5	1 (20)
Enterobactor spp.	15	5 (33.33)
Acinetobactor spp.	13	3 (23.08)
Total	133	43 (32.33)

Figures in parenthesis represent percentage.

Among ESBL producers all the strains were imipenem sensitive(100%) and sensitivity to cephamycin was as follows- *E.coli* 93.1%, *Klebsiella* spp. 100%, *Proteus* spp. 100%, *Pseudomonas* spp. 100% *Enterobactor* spp. 100% and *Acinetobactor* spp. 66.6% (Table II).

Table II: Sensitivity of ESBL producer to Cephamycin and Imipenem:

Name of bacteria (N=43)	Sensitive to Cephamycin (%)	Sensitive to Imipenem (%)
E. coli (n=29)	27 (93.1)	29 (100)
Klebsiella (n=4)	4 (100)	4 (100)
Proteus (n=1)	1 (100)	1 (100)
Pseudomonas (n=1)	1 (100)	1(100)
Enterobactor (n=5)	5 (100)	5 (100)
Acinetobactor (n=3)	2 (66.6)	3 (100)

Figures in parenthesis represent percentage. N=total number of bacteria; n= number of bacteria

Drug resistance among the ESBLs producer, *E.coli* 100% resistant to amoxicillin, cephradine, aztreonam, ceftriaxone, cefotaxime. *Klebsiella* spp. 100% resistant to amoxicillin, cephradine, aztreonam, carbenicillin, ceftriaxone, ceftazidime,

cefotaxime. *Proteus* spp. 100% resistant to amoxicillin, cephradine, aztreonam, ceftriaxone, cefotaxime. *Pseudomonas* spp. 100% resistant to aztreonam, ciprofloxacin, carbenicillin, ceftriaxone, ceftazime, cefotaxime. *Enterobactor* spp. 100% resistant to amoxicillin, cephradine, aztreonam, amikacin, ceftriaxone, ceftazidime, cefotaxime. *Acinetobactor* spp. 100% resistant to amoxicillin, cotrimoxazol, ciprofloxacin, cephradine, aztreonam, ceftriaxone, ceftazidime, cefotaxime (Table III).

Table III: Rate of antimicrobial drug resistance among the ESBLs.

Antimicrobial Drug	*E.coli* spp. n=29 (%)	*Klebsiella* spp. n=4 (%)	*Proteus* spp. n=1 (%)	*Pseudomo -nas* spp. n=1 (%)	*Enterobac -tor* spp. n=5 (%)	*Acineto- bactor* spp. n=3 (%)
Amoxicillin	29(100)	4(100)	16(100)	-	5(100)	3(100)
Cotrimoxazole	22(75.86)	4(100)	1(100)	-	4(80)	3(100)
Gentamycin	18(62.06)	3(75)	1(100)	1(100)	4(80)	2(66.67)
Nalidixic acid	23(79.31)	3(75)	1(100)	-	4(80)	3(100)
Nitrofurantoin	14(48.27)	2(50)	0(0)	-	3(60)	2(66.67)
Ceprofloxacin	25(86.21)	3(75)	0(0)	1(100)	5(100)	3(100)
Cephradine	29(100)	4(100)	1(100)	-	5(100)	3(100)
Mecillinam	16(55.17)	3(75)	0(0)	-	3(60)	2(66.67)
Aztreonam	29(100)	4(100)	1(100)	1(100)	5(100)	3(100)
Amikacin	24(82.76)	3(75)	1(100)	4(57.14)	5(100)	2(66.67)
Piperacillin	-	-	-	0(0)	-	-
Carbenicillin	-	-	-	1(100)	-	-
Ceftriaxone	29(100)	4(100)	1(100)	1(100)	5(100)	3(100)
Ceftazidime	27(93.1)	4(100)	1(100)	1(100)	5(100)	3(100)
Cefotoxime	29(100)	4(100)	1(100)	1(100)	5(100)	3(100)

Figures in parenthesis represent percentage. - = Not used. n=number of bacteria

DISCUSSION

Bacterial antibiotic resistance has become a major clinical concern worldwide including Bangladesh[14]. Failure to detect these enzymes-ESBLs, AmpC β-lactamases, Metallo-β-lactamases has contributed to their uncontrolled spread and therapeutic failure[15].

In this study out of 280 different samples total 150 (53.57%) bacterial strains were isolated; Isolation rate of different strains among urine sample showed highest rate in *E. coli* 85 (56.67%), followed by *Enterobactor* spp. 15 (10%), *Enterococci* 14 (9.33%), *Acinetobactor* spp. 13 (8.67%), *Klebsiella* spp. 11 (7.33%). A study with urine sample ESBLs positive *Klebsiella* spp 35%, *E.coli* 17.82%, *Proteus* spp 28.57% found in BSMMU by Alim (2005)[16]. A study by Rahman *et al.* in 2004 found that ESBLs producing *E.coli* was 43.2% and *K.pneumoniae* was 39.5% [17]. ESBLs are most commonly recognized in *Klebsiella spp.* and *E.coli* [18].

Detection of ESBL producing bacteria was done using chromogenic cephalosporin (Cica Beta Test 1) and Phenotypic confirmatory method by NCCLS. ESBLs were detected by Cica Beta Test 1 within 15 minutes after primary culture. 18-24 hours was taken for ESBLs detection by phenotypic confirmatory method after primary culture. Phenotypic confirmatory method needed at least over night incubation. In this study Phenotypic confirmatory method was considered as parameter of ESBLs detection test. Cica Beta Test 1 shows 43 positivity and all of the strain showed positivity in Phenotypic confirmatory method.

All the strains were Imipenem sensitive. ESBLs producer show 100% resistance to Amoxicillin, Cephradine, Aztreonum, Ceftriaxone, Cefotoxime, Carbenicillin. High resistance to other antibiotics – Cotrimoxazole, Gentamycin, Nalidixic acid, Nitrofurantoin, Ciprofloxacin, Mecillinam, Aztreonum, Amikacin, Netilmycin, Piperacillin, Ceftazidime were also observed in this study, which implies that ESBL producing organisms are multidrug resistant. Genes that code for ESBL are linked to other resistance genes[19].

In the present study sensitivity of ESBL strains to Cephamycin were 93.1% among *E.coli*, 100% among *Klebsiella* spp., 100% among *Proteus* spp., 100% in *Pseudomonas* spp., 100% among *Enterobacto*r spp. and 66.66% among *Acineterobactor* spp. Sensitivity of ESBL strains to imipenem was 100% in all the strains. ESBLs are defined as enzymes which hydrolyze 3rd generation cephalosporins and aztreonam but sensitive to cephamycin and imipenem[20].

Treatment of ESBL producing organisms can be done by carbapenem eg. imipenem, meropenem, ertapenem. ESBL producing organisms are sensitive to 2nd generation cephalosporins in vitro but not recommended for treatment according to NCCLS as may not be effective in vivo except cephamycin. NCCLS recommends when ESBL production is confirmed, results be reported as resistance to all penicillins, cephalosporins excluding cephamycins [9].

CONCLUSION

A considerable number of ESBL producing bacteria are responsible for urinary tract infection. The routine susceptibility tests done by clinical laboratories fail to detect ESBLs producing strains and many ESBLs producing organism do not appear resistant to newer cephalosporins or aztreonam in routine in-vitro susceptibility tests. Detection of ESBL producing strains will help for appropriate treatment of patients infected by these strains. Knowledge of resistance pattern of bacterial strains will help to guide the appropriate and judicious antibiotic use. Early detection and prompt containment can limit the spread of these multidrug resistant pathogens.

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Case report

Prune Belly Syndrome: A rare disease

Rahman F

ABSTRACT

Prune belly syndrome is a rare congenital anomaly of uncertain etiology almost exclusive to males. On 27 th August 2013 a four month old male baby was admitted in Paediatric Department of Prime medical College & Hospital, Rangpur with history of respiratory distress for about 12 days. On examination baby was Dyspneic. On examination the baby had hypotonia, deficient abdominal muscle, cryptorchidism and palpable kidney. Ultrasound examination of the abdomen revealed bilateral hydronephrosis and megaureter. Provisional diagnosis was Pneumonia with PBS (Prune Belly Syndrome). The patient was treated for his pneumonia and discharged on advice for follow up and adviced for surgical management.

Keywords: Cryptorchidism, hydroneprosis, megaureter, Prune Belly syndrome.

INTRODUCTION

Prune Belly syndrome also called Triad syndrome or Eagle-Barrett syndrome is a rare congenital disorder of unknown aetiology affecting about 1 in 30,000 births [1], of which about 96% are male[2]. The triad syndroms are-deficient abdominal muscle, undescended testes and urinary tract abnormalities. Deficient development of abdominal muscles causes the skin of the abdomen to wrinkle like a prune and abnormalities of the urinary tract such as bilateral gross hydronephrosis, megaureter and megacystitis may occur [2]. The exact etiology of PBS is not known; however, some of the studies revealed the possible association with trisomy 18 and 21[2,3]. The prognosis of babies with PBS is poor , stillbirths and early infant deaths are common.

CASE REPORT

Arman 4 months of old male baby of non-consanguineous parents and immunized as per EPI schedule was admitted with the complaints of fever for about 12 days; cough and respiratory difficulty of about same duration.

Child was born by NVD at home without any complications. He is the second issue of the family. No one of family members is suffering from such kind of disease. On the day of admission child was ill looking, afebrile, dyspneic. On Examination respiratory rate was 52/min, body weight was 5.5 kg, height 57 cm and head circumference 42 cm, chest indrawing was present and on auscultation both crepitation and Ronchi were present. Abdomen was distended and there were visible peristalsis present in right and left lumber and para-umbilical region. Rectus muscle and liver, Spleen and kidneys were palpable. Both testes were absent.Above clinical presentation the child was diagnosed as a case of prune belly syndrome and pneumonia. During hospitalization he was treated for pneumonia. After improvement of the general condition he was discharged with advice for follow up and adviced for surgical management.

DISCUSSION

Although PBS is characterized by the classical triad of urinary tract anomalies, deficient

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abdominal musculature, and bilateral cryptorchidism, association with other anomalies including musculoskeletal, cardiovascular, pulmonary and genital malformations have been reported in the literature [2]. The etiology of PBS is unclear and possible familial genetic inheritance was reported in some of the studies [3]. Haeri *et al.* in 2010 [4] have reported the association of PBS with an apparently *de novo* 1.3 megabase interstitial 17q12 microdeletion that includes the hepatocyte nuclear factor-1-beta gene at 17q12, and the authors suggested that haploinsufficiency of hepatocyte nuclear factor-1-beta may be causally related to the production of the PBS phenotype through a mechanism of prostatic and ureteral hypoplasia that results in severe obstructive uropathy with urinary tract and abdominal distension. The massive bladder distension and urinary ascites due to severe obstructive uropathy leads to degeneration of the abdominal wall musculature and failure of testicular descent. The impaired elimination of urine from the bladder leads to oligohydramnios and pulmonary hypoplasia [2]. The higher incidence of this syndrome in male has been explained on the basis of the more complex morphogenesis of the male urethra, possibly resulting in obstructive anomalies at several levels.

Although the primary molecular defect underlying PBS remains unclear, clinical studies had suggested two main pathogenic hypotheses; these are the mesodermal defect hypothesis and the urethral obstruction malformation complex hypothesis. According to mesodermal defect hypothesis aberrant development of the derivatives of the first lumbar myotome between 6 and 10 weeks of gestation leads to a patchy muscular deficiency or hypoplasia of the abdominal wall as well as to urinary tract abnormalities [5]. The urethral obstruction malformation complex hypothesis proposed that pressure atrophy of the abdominal wall muscles occurs when urethral obstruction leads to massive distension of the bladder and ureters [6]. Bladder distension would also interfere with descent of the testes and thus be responsible for the bilateral cryptorchidism. PBS is rare in females, with fewer than 30 cases reported in the literature [7]. After clinical examination, ultrasonography of abdomen, plain X-ray, intravenous pyelography and micturating cystourethrography can confirm the diagnosis.

Our patient's abdomen was distended and loss of abdominal musculature with visible peristalsis (Fig 1 &2).

Fig 1: Distended abdomen with visible peristalsis.

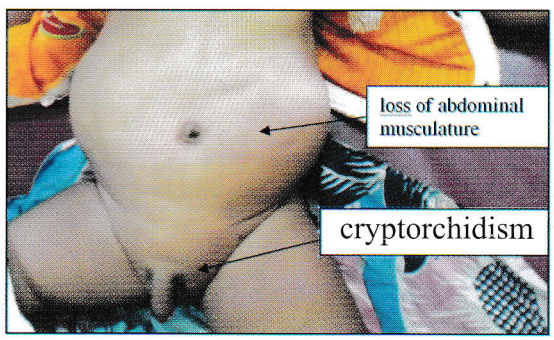
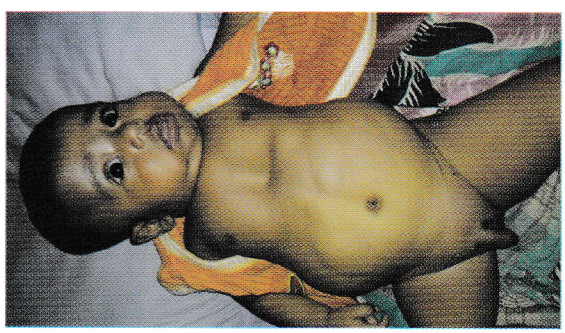


Fig 2: Depressed sternum with empty scrotum.



CONCLUSION

Prune Belly Syndrome has no known prevention other than the routine use of screening for fetal anomalies. It is not so common and it is of great concern to the family and also obstetrician and pediatrician. As there is no appropriate management in our country so routine antenatal care with ultrasonography will help in early detection of the renal anomalies.

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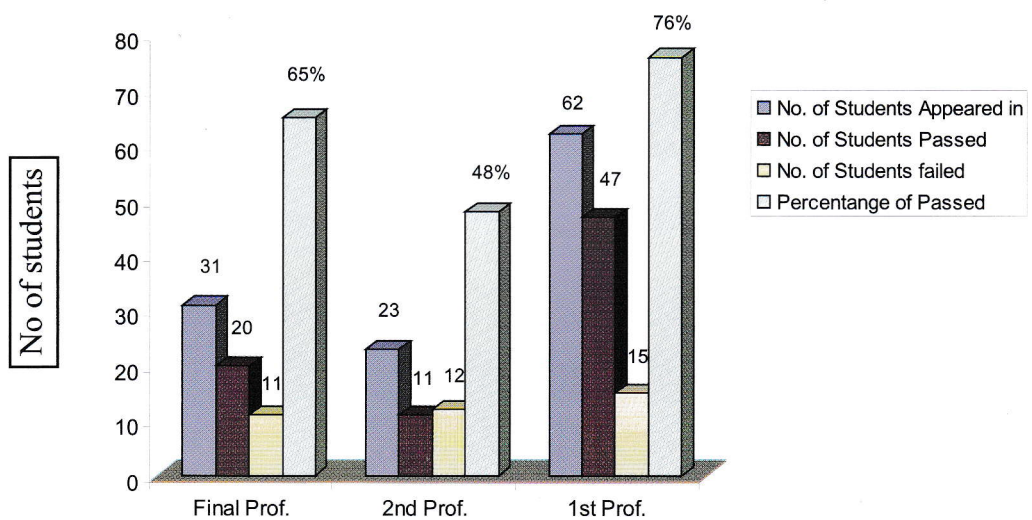
Table I: Result of 1st, 2nd and Final Professional MBBS Examinations in January, 2014.

Exam Year	Exam Name	No. of Students Appeared in	No. of Students Passed	No. of Students failed	Percentage of Passed
	Final Prof.	31	20	11	65%
January' 2014	2nd Prof.	23	11	12	48%
	1st Prof.	62	47	15	76%

Figure 1: Result of 1st, 2nd and Final Professional MBBS Examinations in January, 2014.



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