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DFX or DFO + DFP therapy is more suitable choice for iron chelation treatment in transfusion-dependent beta-thalassemia patients than DFO therapy in term of comorbidities and quality of life in Bangladesh.

See Original Article Page 11

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Jivaka Komarabhacca, was a famous physician in Ancient India lived in the Magadha capital of Rajagaha during late 5th century BC. He was a personal physician of Indian King Bimbisara and Gautama Buddha. Jivaka was also well versed in Asana and meditation practice. He is also considered the father of Siddha-Veda. Jivaka becoms to be knwn as the Father of medicine in southern part of India and in the Thailand.

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Contents

Secondhand smoking in children : Bangladesh perspective

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Globally, more than 5 million deaths are attributable to direct tobacco use annually, while more than 600,000 nonsmokers die every year from secondhand smoke (SHS).[1] Secondhand tobacco smoke contains more than 7,000 chemicals, 70 of which can cause cancer.[2] SHS exposure may cause the same complications as active smoking. Accordingly, SHS may cause both acute and chronic diseases. Chronic exposure to SHS is suggested to be, on average, 80%90% as harmful as chronic active smoking.[3,4] Scientific evidence has confirmed a doseresponse relationship with no risk-free level of exposure (threshold dose). The harms associated with children's exposure to SHS are now well documented. For the same level of SHS exposure in the environment, children tend to be more susceptible to SHS-related harm than adults.[5] In terms of disability adjusted life years (DALYs) lost, children bear the biggest burden of disease due to SHS exposure than any other age group. Children are particularly vulnerable to the harms caused by this smoke, as their lungs are still developing and they breathe at a faster rate than adults. About 165,000 of the deaths occur in children due to SHS, and most are due to infections specially chest and ear infection.[2]

Worldwide, as many as 40% of children are exposed to second-hand smoking (SHS).[5] In Bangladesh this condition is very worse. Nine out of ten primary school children in Dhaka city, Bangladesh are exposed to SHS.[2] In Bangladesh, 80% - 95% children reporting social visibility of smoking in their surrounding public spaces, it is likely that these children got exposed to SHS in public places as well as or instead of their homes and cars.[2,6]

Most governments have recognised the harms associated with secondhand smoke and have introduced comprehensive smoking bans in enclosed public places and workplaces.[7-8] Bangladesh was among the first 40 countries that signed the Framework Convention on Tobacco Control (FCTC). The Bangladesh Tobacco Control Act (TCA) 2005, which includes enhanced warning labels on tobacco packaging, smoke-free legislation, and advertising and promotion restrictions was implemented in 2006. It was further strengthened in 2012 with an amendment, including comprehensive smoke-free laws and displaying graphic warning labels. Currently, there is complete prohibition to smoke in the majority of indoor public places, workplaces, and public transport in Bangladesh.[9] Healthcare and educational facilities are also covered by the legislation with no provision for any outdoor designated smoking zones In many Western countries, these bans were introduced with widespread public support. There has been an increase in the number of smoke-free homes in many countries, indicating shifting social norms. However, evidence on the positive impact of smoke-free legislation indicating their successful implementation originated mainly from high-income countries (HIC). In contrast, such evidence remains scarce in low- and middle-income countries (LMICs).[10,11] The implementation of smoke-free policies in public places in Bangladesh is very poor. The different surveys[2,6] findings in Bangladesh suggested that, quite clearly, current measures are failing to protect the vast majority of children from secondhand smoke and the risks it poses. Smoking on public places and homes is still commonplace, and there is no restriction on smoking in the home in Bangladesh.

Any death due to secondhand smoke is avoidable, and Bangladeshi children are clearly not benefiting from their country's smoking ban. The authorities clearly need to do more, including properly enforcing the laws on smoking in public places. Public

awareness campaigns are also needed to raise awareness about the harms of secondhand smoke exposure in children. And nongovernmental organisations should support a grassroots movement to change smoking norms in communities. Urgent action on multiple fronts is needed to address this serious public health issue.

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Detection of plasmid profile from MDR *Pseudomonas aeruginosa* isolate from wound infection.

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Abstract

Background: Antibiotic resistance is a major problem in treating infections in hospitals. Wound infections are one of the most common infections in hospitals and *Pseudomonas aeruginosa* is a predominant isolate which is usually multi drug resistant. **Objective:** To find out the antibiotic resistance pattern and plasmid profiling of resistant *Pseudomonas aeruginosa* isolated from wound infection. Methods: A descriptive type of cross sectional study was carried out in the Department of Microbiology of Rajshahi Medical College and the Department of Botany of Rajshahi University, Rajshahi during the period from July 2014 to June 2015. A total of 150 wound swabs were collected from patients admitted in surgery department and its allied branches and cultured on appropriate bacteriological culture media. **Results:** Culture had yielded growth in 131(87.33%) cases and *Pseudomonas aeruginosa* was 27(18%), *Staphylococcus aureus* was 22(14.66%), *Escherichia coli* was 56(37.33%), *Proteus spp.* was 19(12.67%), *Klebsiella spp.* was 7(4.67%) respectively. Antibiogram test was done on *Pseudomonas aeruginosa* with 7 different groups of antibiotics and found 4(14.81%) were resistant to 3 groups of antibiotics, 2(7.41%) were on 4 groups, 5(18.52%) were on 5 groups, 10(37.04%) were on 6 groups and 6(22.22%) were on 7 groups. A total of 27(18%) isolates were resistant to 3-7 groups of antibiotics. These isolates were further tested for plasmid detection and plasmid was responsible in 19(70.37%) resistant cases. Conclusion: All wound infections should be treated after performing antibiogram with adequate doses and duration.

Key words: Wound infection, *Pseudomonas aeruginosa,* Multidrug resistance, Plasmids, Hospital infection.

Introduction

Wound infections (WI) include skin and soft tissue infection. Intact skin protects the underlying tissue against colonization and invasion by bacteria. But loss of integrity of the skin provides a moist, warm and nutritive environment for bacterial colonization, proliferation leading to wound infection[1].Wound infections may be hospital acquired (nosocomial) or community acquired. Hospital acquired infections is about 5% to 34% in both developed and developing countries [2] and it may cause by both aerobic and anaerobic bacteria and fungus. Clinically WI may be traumatic, burn, surgical and bed sore (in diabetic). Whatever the clinical nature of infections, *Pseudomonas aerugenosa* is considered as a major hospital problem. This bacterium is frequently found in the hospital utility solutions, tap water, sink, mops, detergents, respiratory and physiotherapy equipments etc.[3] Hospital personnel may transmit this bacteria from patient to patient while handling patients. It also causes septicemia, urinary tract, respiratory tract and great variety of systemic infections Reported incidences of nosocomial pneumonia was 16%, urinary tract infection was 12%, wound infection was 17-26% and septicemia was 10% [4]. *Pseudomonas* is an opportunist pathogen with resistance to â-lactams, quinolones, chloramphenicol and tetracyclines. It also develops resistance due to the production of enzymes (cephalosporinase), active efflux, very low permeability and poor affinity for the target [5]. These resistant is due to mutation in chromosomal genes, acquisition of resistant genes from same or different species of bacteria via plasmids or transposons genes by conjugation and transduction [6]. All these mechanisms make *Pseudomonas* most difficult bacteria to treat. A large number of acquired resistance genes for â-lactamases, extended-spectrum â-lactamases and

metallo-â-lactamases have been detected in *Pseudomonas* [7]. In recent years, increase prevalence of multidrug resistance in *P. aeruginosa* has been noticed. A limited number of antibacterial agents such as ticarcillin, piperacillin, cephalosporins, carbapenems and fluoroquinolones are effective against *P. aeruginosa*. Aminoglycosides are also used as a part of combination therapy [8]. So the present study has been carried out to determine pathogens responsible for wound infection, antibacterial resistance pattern of *P. aeruginosa* and detection of plasmids for resistant.

Table 1: Antibacterial resistance of *Pseudomonas aeruginosa*. N=27

Antibacterial groups .	Isolated number of *Pseudomonas aeruginosa*																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Cephalosporins: Ceftriaxone(30µg)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R
Carbapenem: Meropenem(10µg)	S	S	S	S	S	S	S	S	R	S	S	S	S	R	R	R	R	R	R	R	S	S	S	S	R	S	S
Aminoglycoside: Gentamicin (10µg)	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Monobactam: Aztreonam (30µg)	R	R	R	R	S	S	R	S	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	R	S	S	R
Tetracycline: Tigecycline (15µg)	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Fluoroquinolone: Ciprofloxacin(5µg)	R	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Penicillin: Ticarcillin(75µg)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
No. of resistant.	6	6	6	6	4	3	3	3	5	6	6	6	5	7	6	7	7	7	7	7	6	5	5	4	3	5	6

Note: R= Resistant; S= Sensitive

Materials and Methods

This study was conducted in the Department of Microbiology of Rajshahi Medical College, Rajshahi during the period from July 2014 to June 2015. A total of 150 wound swabs were collected from patients admitted in Surgery Department and its allied branches. The samples were cultured on blood agar, nutrient agar and MacConkey's agar. *Pseudomonas aeruginosa* was identified by its colony morphology, microscopy, motility, pigment production, fruity odour and oxidase positivity. Antimicrobial susceptibility test was performed on Mueller-Hinton agar media with 7 different groups of commercially available antibiotics by disc diffusion method. Tested antibiotic discs were meropenem (10µg), ciprofloxacin (5µg), ceftriaxone (30µg), aztreonam (30µg), gentamicin (10µg), tigecycline (15µg) and ticarcillin (75µg). The result was reported as sensitive or resistant according to CLSI, 2012 recommendation. The strains which showed resistance to more than 3 different groups of

Table 2: Multidrug resistance patterns of *Pseudomonas aeruginosa*. N= 27

Resistant *Pseudomonas aeruginosa.*	No. of antibacterial drugs in group.				
	3 N(%)	4 N(%)	5 N(%)	6 N(%)	7 N(%)
	4(14.81)	2(7.41)	5(18.52)	10(37.04)	6(22.22)

antibiotics were considered as multidrug resistant (MDR). The MDR strains were tested for plasmid detection.

Plasmid DNA Extraction and Gel electrophoresis

Plasmid extraction was carried out by using the alkaline lysis method. Plasmids were then electrophoresed on 1% agarose gel in a horizontal tank at a constant voltage of 100V for 60 minutes. After electrophoresis, plasmid DNA bands were viewed under UV transillumination and photographed using a digital camera. The DNA bands were compared with those for the lambda DNA *Hind*III digest molecular weight marker (Promega Corporation) which ranged in size from 250bp to 10000bp and results recorded.

Results

In this study 27(18%) *Pseudomonas aeruginosa* was isolated from 150 wound swabs. Among 27 isolates, ceftriaxone was resistant to 25(92.59%), meropenem was 9(33.33%), gentamicin was 22(81.48%), aztreonam was 18(66.67%), tigecycline was 25(92.59%), ciprofloxacine was 22(81.48%) and ticarcillin was resistant to 26(96.3%) isolates (Table-I). Among 27 *Pseudomonas aeruginosa*, 4(14.81%) were resistant to 3 groups of drug, 2(7.41%) were 4 groups, 5(18.52%) were 5 groups, 10(37.04%) were 6 groups and 6(22.22%) were resistant to 7 groups of drug (Table-II). The plasmid was detected in 19(70.37%) isolates of which one in 3 groups , another one in 4 groups, four in 5 groups, seven in 6 groups and six in 7 groups of drugs (Table-III). swabs were collected from patients admitted in Surgery Department and its allied branches. The samples were cultured on blood agar, nutrient agar and MacConkey's agar. *Pseudomonas aeruginosa* was identified by its colony morphology, microscopy, motility, pigment production, fruity odour and oxidase positivity. Antimicrobial susceptibility test was performed on Mueller-Hinton agar media with 7 different groups of commercially available antibiotics by disc diffusion method. Tested antibiotic discs were meropenem (10µg), ciprofloxacin (5µg),

Table 3 : Correlation of multidrug resistant strains of *Pseudomonas aeruginosa* with detected plasmid. N=27

No. of antibiotic group N	No. of MDR isolates N (%)	Proportion of MDR isolates which carried plasmid % (N/n)
3	4(14.8)	25.0 (1/4)
4	2(7.4)	50.0 (1/2)
5	5(18.5)	80.0 (4/5)
6	10(37.0)	70.0 (7/10)
7	6(22.2)	100.0 (6/6)
Total	27(100.0)	70.4 (19/27)

Discussion

In this study seven groups of antibiotics were studied for sensitivity test. The groups were cephalosporin, carbapenem, aminoglycoside, monobactam, tetracycline, fluoroquinolone and penicillin. Among cephalosporins, ceftriaxone was 92.59% resistant which is similar with the study of Rostamzadeh *et al.* (2016) in Iran[9] and Mahmoud *et al.* (2013) in Egypt[10] where ceftriaxone resistant was 94.37% and 87.7%. But dissimilarity was found with the study of Garba *et al.* (2012)[11] and Mohammed *et al.*

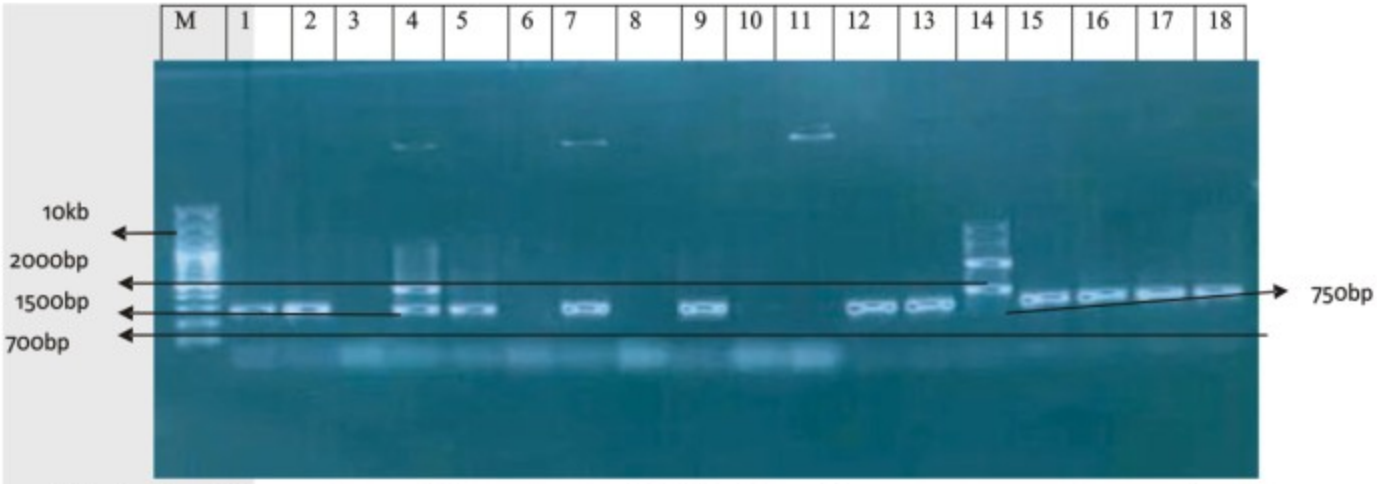

Plate 1: Plasmid profile of the MDR *Pseudomonas aeruginosa* isolates: 1,2,5,7,9,12,13,15,16,17,18(700bp), 4(700bp,1500bp),14(750bp,2000bp) is the clinical isolates. Lane M, 10kb DNA ladder.

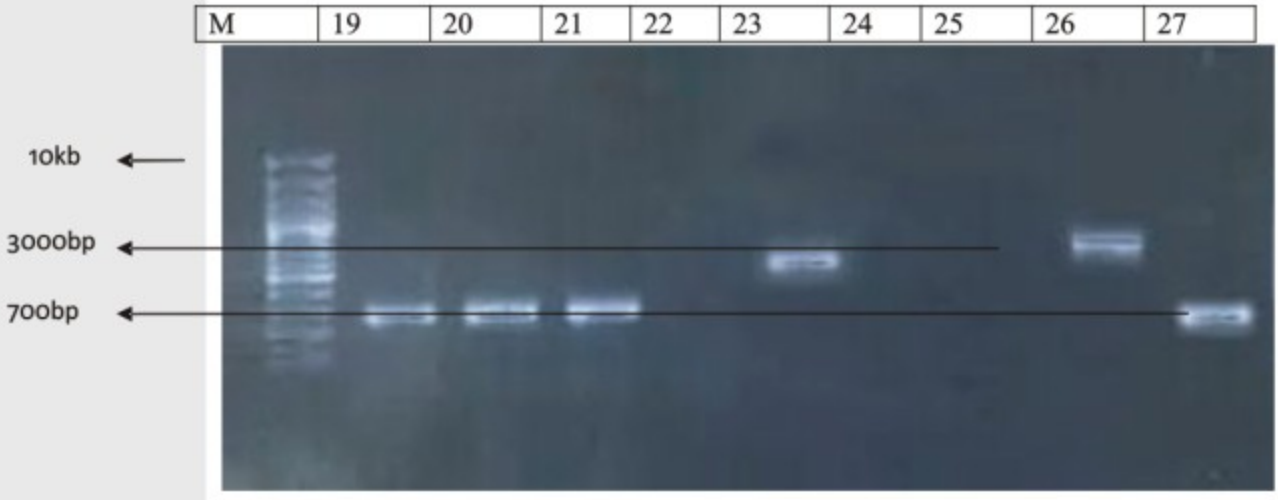

Plate 2: Plasmid profile of the MDR *Pseudomonas aeruginosa* isolates: 19,20,21,27(700bp), 23 and 26(3000bp) is the clinical isolates. Lane M, 10kb DNA ladder.

(2013)[12] both were in Nigeria and showed ceftriaxone resistant was 45.4% and 46%. Among the carbapenems, meropenem was 33.33% resistant which is similar with the study of Joseph *et al.* (2013) in India[13] and Khan *et al.* (2014) in Pakistan[14] where meropenem resistant was 34.8% and 30.4%. The dissimilarity noted by Yasemin *et al.* (2013) in Turkey[15] and Biswal *et al.* (2014) in India[16] where rates were 19% and 13.79%. Gentamicin was 81.48% resistant which is similar to the study of Rajput *et al.* (2015)[17] and Biswal *et al.* (2014)[16] both were in India where gentamicin resistant was 81% and 81.03%. Dissimilarity with our study was reported by Shah *et al.* (2015) in Pakistan[18] and Yasemin *et al.* (2013) in Turkey[15] where resistant was 35.3% and 36%. Aztreonam was 66.67% resistant which is nearly similar to the study of Nazli *et al.* (2015) in Turkey[19] where aztreonam resistant was 56.7% and dissimilar with the study of Khan *et al.* (2014) in Nepal[20] and Mahmoud *et al.* (2013) in Egypt[10] where resistant was 31.96% and 82.5%. Tetracycline was 92.59% resistant which is similar with the study of Smith *et al.* (2012) in Nigeria[21] and Mohiuddin *et al.*

(2010) in Dhaka, Bangladesh[22] where resistant was 95% and 91.17%. But our study is dissimilar with the study of Akingbade *et al.* (2012) in Nigeria[23] and Masood & Zahra (2014) in Iran[24] where resistant rates were 70.9% and 72%. Ciprofloxacin was 81.48% resistant which is nearly similar to the study of Mohiuddin *et al.* (2010) in Dhaka, Bangladesh[22] and Khan *et al.* (2014) in Pakistan[14] where ciprofloxacin resistant were 92% and 75%. But dissimilarity with our study was reported by Mahmoud *et al.* (2013) in Egypt[10] and Golshani *et al.* (2012) in Iran[25] were 56.1% & 58%. Ticarcillin was 96.3% resistant which is similar to the study of Shahini *et al.* (2012)[26] and Ranjbar *et al.* (2011)[27] both in Iran where resistant rates were 100% and 93%. But dissimilarity was reported by Sarwat *et al.* (2015) in India[28] & Masood and Zahra (2014) in Iran[24] was 58.46% and 5%.

The resistant pattern of *Pseudomonas aeruginosa* in our study is different with the studies of others may be due to the random use of 3rd generation cephalosporins and carbapenem without doing culture and sensitivity which lead to the emergence of resistance and their dissemination throughout the hospital. This dissemination is due to inadequate sanitation of hospital, improper use of antibiotics, inadequate antibiogram of empirical antibiotics, inadequate dose and duration, may be insufficient ingredients as mention by the pharmaceutical company and inaccuracy of culture and sensitivity test.

In our study 14.81% *Pseudomonas aeruginosa* was resistant to 3 antibiotics which is similar to the study of Gobedo *et al.* (2013) in Ethiopia[29] where they found 14.9%. Dissimilarity with our study was reported by Biswal *et al.* (2014)[16] in India where resistant isolates were 10.34%.

7.41% isolates were resistant to 4 antibiotics which are similar to the study of Yakha *et al.* (2014) in Nepal[30] and Odumosu *et al.* (2013) in Nigeria[31] where resistant isolates of both were 6.45%. Dissimilarity was reported by Biswal *et al.* (2014) in India[16] were 3.45%. In this study 18.52% isolates were resistant to 5 antibiotics which are similar to the study of Mehdi *et al.* (2014) in Iran[32] and Yakha *et al.* (2014) in Nepal[30] where resistant isolates were 17.8% and 19.35%. Dissimilarity was reported by Gobedo *et al.* (2013) in Ethiopia[29] were 4.1%. In this study 37.04% isolates were resistant to 6 antibiotics which are similar to the study of Mehdi *et al.* (2014) in Iran[32] were 38.4%. But dissimilarity was reported by Odumosu *et al.* (2013) in Nigeria[31] were 9.68%. In our study 22.22% isolates were resistant to 7 antibiotics which are dissimilar with the study of Gobedo *et al.* (2013) in Ethiopia[29] where resistant isolates were 5.4%.

The dissimilarities of the multidrug (3-7 drugs) resistant isolates may be due to use of antibiotics in our study is different from others study, different therapeutic dose and route; patients may have different pH in their stomach which may differ the activity of orally administered drugs like ciprofloxacin; food can interfere the absorption of drug e.g. tetracycline; milk, antacid, sucralfate and iron salt may reduce the absorption of certain drugs e.g. tetracycline, fluoroquinolone etc. Dissimilarities may also be due to achlorhydia, partial gastrectomy, tropical sprue where absorption of drug reduce and cannot reach at optimum serum concentration. In oral administration as only 20-40% drug reaches the systemic circulation while 100% in parenteral administration, metabolism may also alter the efficacy and half-life of drug. Besides these oral formulation of a drug from different manufacturers or different batches from the same manufacturer with same amount of drug may not yield the same blood levels. Mutation may occur in bacteria if optimum blood level is not attained by orally administered drug that also causes antibiotic resistance.

In this study, plasmid was detected in 19 (70.37%) isolates out of 27 MDR strains which is nearly similar with the study of Daini *et al.* (2008)[33], Smith *et al.*

(2012)[21] and Daini & Onyeaghala (2012)[34] all were from Nigeria where detected plasmid were 66.67%, 80% and 81.48%. Dissimilarity with our study was also reported by Akingbade *et al.* (2012) in Nigeria[23] and Afrin. (2015) in Bangladesh[35] where detected plasmid was 36.4%, and 50%.

The dissimilarities of plasmid detection may be due to inter species dispersion of plasmid, the presence of transmissible and non-transmissible plasmids. It has been seen in last two decades that bacterial resistance to a large number of antibiotics may be transfer by plasmids (Hasan *et al.* 2007)[36]. In our study, no plasmid had been detected in 8(29.63%) isolates and it is nearly similar with the finding reported by Quashem (1987) in Bangladesh[37] who reported plasmid in 30.59% isolates. There is a possibility that some of the plasmids were lost due to 6 months storage of the MDR samples at 20^0C before test. Loss of plasmid due to storage has been reported also by many workers (Watanabe *et al.*, 1964)[38]. The failure of plasmid detection were also might be the cause of that the resistance determinants were either carried by chromosomes or by small molecular weight plasmids.

It can be concluded that though a proportion of multidrug resistance can occur due to mutation in chromosomal DNA but plasmid bearer always play an important role in antibiotic resistance. From this study, it has found that *Pseudomonas aeruginosa* isolates are resistant to commonly used antibiotics and its resistance to antimicrobials are gradually increases day by day. Therefore the rational use of antibiotics must be a priority. Public health policy on appropriate prescribing and antibiotics should be used only after performing antibiogram with adequate dose and duration.

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Comparative effects of iron chelators on the transfusion-dependent Beta-Thalassemia Patients

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Abstract

Background: Treatment of patients with thalassemia major consists of regular blood transfusions and iron chelation therapy, which is vital to prevent excess iron buildup in the body. In Bangladesh there are three iron chelating agents available: deferoxamine (DFO, Desferal), an iron chelator given by infusion, and two oral chelators deferiprone (DFP, Ferriprox) and deferasirox (DFX, Exjade). **Objective:** To compare the disease characteristics, comorbidities and quality of life of the patients with transfusion-dependent beta-thalassemia receiving three different chelation treatments. **Methods:** This was a cross-sectional descriptive type of study conducted at the Bangladesh Thalassemia Centre and Haematology department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. A total of 175 attending transfusion-dependent beta-thalassemia patients between February 2012 and February 2013 were enrolled in this study, among them 135 (77.1%) patients were responded. Data were collected by self-administered questionnaires: SF-36 questionnaire and a personal questionnaire. Statistical analyses were performed with SPSS version 18.0 for Windows 7. Chi square test and univariate regression analysis were performed. **Results:** A total of 135 patients, 75(55.6%) patients were receiving DFX, 39(28.9%) were receiving combination therapy of DFO + DFP and the rest 21(15.5%) patients were receiving DFO alone. Mean hemoglobin level prior to transfusion (gm/dl) in DFX therapy recipients was significantly higher than the other two groups (p=0.0208). Highest percentage (92.3%) of the patients in DFO+DFP therapy garoup were moderately or highly physically active than the patients in DFO and DFX therapy groups. The patients receiving DFO had significantly higher percentages of myocardial dysfunction (33.3%), hepatic dysfunction (38.1%), splenectomy (71.4%) and allergies (14.3%) than the other two groups. A higher percentage of patients receiving DFO felt that their treatment negatively influenced their body and skin appearance and limited their ability to work, attend school, and perform daily tasks (P=.0.0066). **Conclusion:** DFX or DFO + DFP therapy is more suitable choice for iron chelation treatment in transfusion-dependent beta-thalassemia patients than DFO therapy in term of comorbidities and quality of life in Bangladesh.

Key Words: Iron Chelators, Beta Thalassemia, Blood Transfusion

Introduction

Beta-thalassemia is a genetically inherited disorder characterized by reduced synthesis of the beta-hemoglobin chain which in turn results in reduced synthesis of hemoglobin A (HbA). To date more than 1,000 mutations are known that influence the structure or synthesis of the alpha- and beta-globin chains that make up HbA and which are listed in the HbVar database 5(HbVar), a database of all the mutations related to thalassemia and the variations of hemoglobin[1,2,3].

Treatment of patients with thalassemia major consists of regular blood transfusions and iron chelation therapy, which is vital to prevent excess iron buildup in the body. In Bangladesh there are three iron chelating agents available: deferoxamine (DFO, Desferal), an iron chelator given by infusion, and two oral chelators deferiprone (DFP, Ferriprox) and deferasirox (DFX, Exjade). Treatment with iron chelators has significantly increased the life expectancy of affected individuals into the third to fifth decade[4], while simultaneously decreasing the comorbidities of the disease[5].

Despite advancements in care, patients with transfusion-dependent beta-thalassemia still present complications and often suffer from psychological problems due to their lifestyle[6]. While the effectiveness of iron

chelation therapies has been thoroughly investigated, there is limited comparative information about the benefits of the therapies on the quality of life and self-esteem of the patients. Furthermore, the quality of life of patients presenting with this disease and the effect of the type of iron chelation treatment on the patient's quality of life have not been evaluated. Thus, the objective of the present study was to compare the quality of life, self-esteem, and satisfaction and adherence to treatment of patients with transfusion-dependent beta-thalassemia in Bangladeshi population receiving three different chelation treatments and to identify parameters affecting their quality of life. The SF-36 questionnaire was used in order to evaluate the quality of life in the 135 patients of the study. Three other questionnaires were administered which provided important information on factors varying among patients receiving different types of iron chelation therapy.

Methods

This was a cross-sectional descriptive type of study conducted at the Bangladesh Thalassemia Centre and Haematology department of BSMMU (Bangabandhu Sheikh Mujib Medical University) Dhaka. All the transfusion-dependent beta-thalassemia patients attending at the aforementioned centre and department constituted the study population. A total of 175 attending patients between February 2012 and February 2013 were briefly informed about the study and invited them to participate in this study providing the self administered questionnaire and written informed consent. Among them 135 patients returned the questionnaires with their answers and written consent. The scientific committee of the study and the local ethics committees of the participating hospitals approved the study.

Data were collected by self-administered questionnaires, SF-36 questionnaire and a personal questionnaire. The SF-36 questionnaire was used as a measurement of the quality of life of the patients. This questionnaire consists of eight scales (1) physical functioning, (2) role limitations because of physical health problems, (3) bodily pain, (4) general health perceptions, (5) vitality (energy/fatigue), (6) social functioning, (7) role limitations due to emotional problems, and (8) general mental health (psychological distress and psychological wellbeing). Scores for all dimensions are expressed on a scale 0-100, where higher scores indicate better health and well-being. The scores were calculated for respondents completing 50% or more of the items within a scale. The personal questionnaire was designed to record the personal and disease characteristics of the patients. The personal characteristics included age, gender, marital status, parent hood, physical activity, sports, smoking status and employment status. The disease characteristics include age of onset of disease, age of starting the treatment, frequency of transmission per month, hemoglobin level gm/dl prior to transfusion, ferritin level ng/ml upon enrollment, myocardial dysfunction, hepatic dysfunction, thyroid diseases, hypogonadism, slenectomy and allergies. Patients took help from the attending data collectors and doctors if needed to give the answers of these questionnaires.

Statistical analyses were performed with SPSS version 18.0 for Windows 7. All continuous variables are expressed as the mean ± standard deviation (SD). The categorical (nominal) variables are expressed as percentages of the total population. Comparisons of the categorical variables between the three therapies were performed by the chi square test. In order to investigate if chelation treatment is associated with patients' quality of life, univariate regression analysis was performed in which the eight scales and the two components of the SF-36 were set as dependent variables and chelation treatment was set as the independent variable.

Results

A total of 135 patients, 75(55.6%) patients were receiving deferasirox (DFX; Exjade,

Novartis), 39(28.9%) were receiving combination therapy of deferoxamine (DFO; Desferal, Novartis) + deferiprone (DFP; Ferriprox, Demo S.A.) and the rest 21(15.5%) patients were receiving DFO alone.

The mean age of the patients was 37.3(±10.1) years for the DFO group, 34.3 (±7.4) years for the DFX group, and 37.8 (±8.3) years for the DFO + DFP group. The differences of mean ages among the groups were not statistically significant. Males were predominant in all therapy groups, but not statistically significant. In DFO group, 57.1% patients were single. It was 66.7% in DFX and 71.8% in combination (DFO+DFP) therapy group. More than 61% of the patients receiving DFO were moderately or highly physically active.

The percentages of moderately or highly physically active patients in DFX and DFO+DFP therapy group were 74.7% and 92.3% respectively The majority of the patients receiving DFO (90.5%, 19/21) and DFO + DFP (84.6%, 33/39) were not involved in sports, while 48.0% (36/75) of DFX patients were involved in sports (P<0.0001). In DFO therapy group, 14.3% of the patients were smokers. The percentages of smokers in DFX and DFO + DFP therapy groups were 20.0% and 25.6% respectively. But in DFO group, more than 57.0% of of the respondents did not mentioned about their smoking/tobacco consumption status, More than 71.0% of the patients in all the groups were employed (Table 1).

Table 1: Patient characteristics and category of therapy received.

	DFO (n=21)		DFX (n=75)		DFO + DFP (n=39)		*P* value
	N	%	n	%	n	%	
Gender							
Male	15	71.4	40	53.3	21	53.8	NS
Female	06	28.6	35	46.7	18	46.2	
Marital Status							
Single	12	57.1	50	66.7	28	71.8	NS
Married	08	38.1	21	28.0	11	28.2	
Divorce	01	04.8	04	05.3	00	00.0	
Parent hood							
Yes	06	28.6	15	20.0	11	28.2	NS
No	15	71.4	60	80.0	28	71.8	
Physical activity							
None/Low	08	38.1	19	25.3	03	07.7	NS
Moderate/high	13	61.9	56	74.7	36	92.3	
Sports							
Yes	02	09.5	36	48.0	06	15.4	<0.0001
No	19	90.5	39	52.0	33	84.6	
Smoking/tobacco consumption status	03	14.3	15	20.0	10	25.6	NS
Smoker	16	28.6	36	48.0	10	25.6	
Non-smoker	12	57.1	24	32.0	19	48.8	
Did not answer							
Employment status							
Employed	16	76.2	60	80.0	28	71.8	NS
Unemployed	05	23.8	15	20.0	11	28.2	

NS: not significant

Table 2: Disease characteristics and category of therapy received.

	DFO Mean±SD	DFX Mean±SD	DFO + DFP Mean±SD	*P* value
Age of diagnosis (years)	2.1 ± 2.4	2.8 ± 4.5	2.3 ± 4.1	NS
Starting age of DFO treatment (years)	13.1 ± 11.1	9.0 ± 9.6	11.1 ± 11.6	NS
Frequency of transfusion per month	2.2 ± 0.6	1.9 ± 0.5	2.1 ± 0.7	NS
Hemoglobin level prior to transfusion gm/dl	9.5 ± 0.9	10.1 ± 3.4	9.7 ± 0.4	0.0208
Ferritin levels upon enrollment ng/ml	1559.2 ± 1778.1	1738.0 ± 1636.9	1023.1 ± 944.3	NS

NS: not significant

Table 3: Frequency of comorbidities or prior splenectomy in different drug recipient groups.

Comorbidity/splenectomy	DFO %	DFX %	DFO + DFP %	*P* value
Myocardial dysfunction	33.3	6.7	15.4	0.0058
Hepatic dysfunction	38.1	6.7	2.6	<0.0001
Thyroid disease	28.6	58.7	53.8	0.0499
Hypogonadism	14.3	10.7	10.3	NS
Splenectomy	71.4	38.7	48.7	0.0319
Allergies	14.3	9.3	2.6	0.0487

NS: not significant

Table 4: Association between SF-36 scales and chelation treatment.

Components of SF-36 scale	Chelation treatment	Number N	Estimated mean score	95% CI for estimated mean score	P value
Physical Functioning	DFX	72	80.3	75.7 - 84.8	
	DFO+DFP	19	80.9	74.2 - 87.6	0.048
	DFO	33	68.4	59.6 – 77.3	
Role limitations due to physical health	DFX	71	79.9	71.5 – 88.3	
	DFO+DFP	17	76.5	64.2 – 88.8	0.021
	DFO	33	52.9	35.8 – 70.1	
Bodily pain	DFX	71	80.3	74.4 – 86.3	
	DFO+DFP	17	73.6	64.9 – 82.3	0.015
	DFO	33	60.7	48.6 – 72.8	
General health perceptions	DFX	70	51.6	47.4 – 55.9	
	DFO+DFP	17	53.1	46.9 – 59.3	0.111
	DFO	33	42.3	33.6 – 50.3	
Vitality	DFX	71	61.8	57.6 – 65.9	
	DFO+DFP	17	68.5	62.4 – 74.6	<0.001
	DFO	33	46.2	37.7 – 54.7	
Social Functioning	DFX	71	76.4	71.2 – 81.6	
	DFO+DFP	17	77.3	69.7 – 85.0	0.845
	DFO	33	73.5	62.9 – 84.1	
Role limitations due to emotional problems	DFX	71	77.9	69.6 – 86.3	
	DFO+DFP	17	71.4	59.2 – 83.6	0.338
	DFO	33	64.7	47.7 – 81.7	
Mental Health	DFX	71	65.4	61.1 – 69.6	
	DFO+DFP	17	65.3	59.1 – 71.6	0.001
	DFO	33	46.8	38.1 – 55.5	

The type of chelation treatment was proven to be statistically significantly associated with physical functioning (P=0.048), role limitations due to physical health problems (P=0.021), bodily pain (P=0.015), vitality (P<0.001), and mental health (P=0.001) (Table 4). Pairwise comparisons performed in the aforementioned scales in order to ascertain differences among the treatments revealed that those who received DFX or DFO + DFP demonstrated significantly higher mean scores (better quality of life) than patients who received DFO alone, in all scales tested, apart from the bodily pain scale. In the bodily pain scale, only treatment with DFX resulted in a significantly higher mean score than treatment with DFO alone.

Discussion

One hundred and thirty-five adult beta-thalassemia transfusion-dependent patients took part in this study. The majority of the patients were single without children, in agreement with previous reports[9]. One-fifth of the patients were unemployed, a not very high percentage.

The DFO + DFP combination therapy offers a better control of serum ferritin levels, thus requiring less frequent DFO infusions[10]. It was thus not surprising that we found a decreased frequency of transfusions in the DFO + DFP combination group (P<0.0001; Table 2). A higher percentage of DFO patients had comorbidities compared to the other two groups, except for thyroid disease, which was more prevalent in DFX patients. The presence of hepatic dysfunction in patients with homozygous beta-thalassemia has been correlated with iron overload in the liver as

well as to chronic hepatitis[11]. It is also notable that patients receiving DFX had the lowest prevalence of myocardiopathy which is in accordance with reports on the ability of DFX to prevent iron overload in the myocardium[12].

The highest rate of patient adherence to treatment was observed in the DFX patients. Adherence to therapy is the most important parameter for successful therapy. In fact low adherence of patients receiving DFO has been linked to the absence of clinical benefit[5]. In a previous study, low adherence to DFO was linked to smoking/tobacco consumption and to difficulties with self-administering the infusion[13].

Our results about satisfaction and ease of receiving their therapy matched those of previous studies, in which DFX was associated with increased satisfaction to treatment. Importantly, it was shown that switching chelators resulted in increased adherence, regardless of whether the patients switched from the oral to the intravenous chelator or vice versa, although the switch from DFO to DFP occurred more often[14].

According to previous studies, patients receiving DFO were more likely to suffer from depression, fatigue, dyspnea, and decreased physical functioning [15]. The majority of patients felt that they could participate in more activities if they were not receiving DFO[16] in accordance with the results of this study indicating that DFO limited the ability of patients to participate in sports and perform daily functions. Furthermore, the results of our study indicate that patients receiving DFO had lower self-esteem and worse PCS scores. These observations are in agreement with the results of the ITHACA study, in which the PCS score was low for patients receiving DFO[17] and with the study of Abetz et al., in which patients with DFO suffered from low self-esteem[15].

Of the specific components of the SF-36 questionnaire, the type of chelation treatment was proven to be statistically significantly associated with physical functioning, role limitations due to physical health problems, bodily pain, vitality, and mental health. Importantly, the results of our multivariate analysis indicate that the dependence of the PCS score on the type of chelation treatment was not confounded by anthropometric variables, such as gender, marital status, level of physical activity, presence of comorbidities, or smoking status. The importance of the SF-36 questionnaire and the results of the individual scales on the multidisciplinary actions that should be taken for patients with beta-thalassemia have been reported[18].

This study possesses a number of methodological limitations that must be taken into consideration. First, sample size was small. Second, data were collected retrospectively. Third, study subjects in different groups were unequal. A well designed cohort study with large sample size would be needed to ascertain the cause and effect relationship between quality of life and type of iron chelation therapy.

The results of this study have certain implication in clinical practice however. The study findings suggest that DFX or DFO + DFP combination therapy is the drug of choice for iron chelation of the transfusion-dependent beta-thalassemia patients rather than DFO therapy in Bangladesh.

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Detection of diarrheagenic strains of E.*coli* from pediatric diarrheal infection in a tertiary hospital, Bangladesh

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Abstract

Background: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in Bangladesh and E.coli is an important agent of childhood diarrhea. Objectives: To determine the prevalence of diarrheagenic Escherichia coli (DEC) and its pathotypes in pediatric population with diarrheal infection using polymerase chain reaction (PCR). Methods: This was a descriptive type of cross sectional study conducted among the pediatric population with diarrhea admitted in Rajshahi Medical College hospital, Rajshahi, Bangladesh from July 2014 to June 2015. In this study, 268 children with diarrheal infection aged from 3 to 12 years were included. Stool samples were collected and identified as E. coli isolates by culture on MacConkeys agar media, microscopy and standard biochemical tests. Diarrheagenic strains of E.coli (DEC) were identified by multiplex PCR assays using six primer pairs by detecting the genes of enterotoxigenic E.coli (lt,st), Enteropathogenic E.coli (eae,bfp), Enteroaggregative E.coli (aat) and Enteroinvasive E.coli (iPah). Results: Among 268 stool samples,166 E. coli were isolated. Of the total 166 isolated E.Coli, 68(38%) were DEC identified by multiplex PCR. Among DEC, most frequently isolated pathotypes was EPEC (38, 44.7%), followed by ETEC (26, 30.5%), EAggEC (20, 23.5%) and EIEC (1, 1.1%). Conclusion: This study shows that DEC is an important pathogen causing diarrhea in pediatric group but yet there is no data available of strains responsible in the study area. Strain identification is essential for E. coli diarrhea and by using multiplex PCR assay, the simultaneous detection of strains in one PCR reaction can be done that makes a conclusive diagnosis of diarrhea.

Key words: diarrhea, DEC, ETEC, EPEC, EAggEC, EIEC, PCR.

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Introduction

Diarrhea is a major public health problem throughout the world and still continues to be one of the most important causes of morbidity and mortality among infants and children in developing countries .[1] Diarrheal disease is the second leading cause of death and causes 1.3 million deaths every year in children of under five years.[2] The causes of diarrhea include a wide range of bacteria, viruses and parasites .[3] In developing countries, 50-60% of cases are caused by bacteria and the peak incidence occur during the summer months. Bacterial causes include, *E.coli, Campylobacter jejuni, Campylobacter coli, Vibrio cholerae, Salmonella spp, Shigellaspp, Aeromonas, Plesiomonas, Bacterioidsfragilis*.In Bangladesh, *E. coli* was responsible for 68% cases followed by *Campylobacter* spp. (13%), *Pseudomonas* spp. (11%), *Klebsiella*spp. (5%), *Salmonella* spp. (2%) and *Shigella*spp. (2%).[4] In rural area, children suffer on an average of 4.6 episodes of diarrhea, of which about 2.3 million die every year.[5] Diarrheagenic strains of *E .coli* (DEC) is responsible for 41.33% cases of acute childhood diarrhea.[6]

The genome of *E. coli* consist of a single circular chromosome of about 4 to 5 million base pairs (bp) and multiple plasmids. Besides that phage genes and transposons are also present. These genes are encoded for various virulence factors of *E. coli*.[7] Diarrhagenic stains of *E. coli* are divided into six groups on the basis of their virulence properties, such as enterotoxigenic *E.coli* (ETEC) ,enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), entero-haemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAggEC) and diffusely adherent *E.coli* (DAEC).[8]

Identification of diarrheagenic strains of *E.coli* is limited in many developing countries because conventional microbiological method is unable to distinguish between normal flora and pathogenic strains of *E.coli*. Serotyping is the traditional method for detection of DEC. But it has limited sensitivity and specificity, it is tedious and expensive and is performed correctly only in few reference laboratories. So, it may therefore be insufficient and unreliable to define an isolate as truly pathogenic by using serotyping alone. Thus, detection of diarrheagenic strains of *E.coli* has focused increasingly on the identification and characterization of genome.[9]

Now a days PCR is considered as one of the molecular methods which is most reliable, rapid and sensitive technique for identification of diarrheagenic strains of *E. coli*.[10] The present study was designed to detect the diarrheagenic strains of *E. coli* using multiplex PCR. It was hoped that this study will be very helpful for the clinician for proper diagnosis and attract the researchers to do further study in this field.

Methods

A descriptive type of cross sectional study was carried out in the Departments of Microbiology of Rajshahi Medical College and of Dhaka Medical College and Department of Pediatrics, Rajshahi Medical College Hospital during the period from July 2014 to June 2015. A total of 268 stool samples were obtained from children of 3 to 12 years with diarrhea, who were not on antibiotic therapy and cultured on MacConkey agar media for isolation of *E.coli*. Suspected lactose fermenting colonies were further subcultured onto motility-indole-urease and Simmons citrate agar media and identified by colony morphology, Gram staining and standard biochemical tests.

Multiplex PCR

After confirmation, *E.coli* isolates were subcultured onto Muller Hinton agar media, then again inoculated into sterile ependorf tube containing tryptic soya broth. The tryptic soy broth containing bacterial growth was centrifuged at 4000 rpm for 10 minutes for formation of pellet. Then the pellets were re suspended with 300 microliter of sterile deionized water, boiled at 100^{0}C for 10 minutes and immediately kept on ice.It was again centrifuged at 14000 rpm for 10 minutes .The supernatant was used as DNA template for multiplex PCR. Six primer pairs were used listed as follows:[11,12,13]

Primers were diluted by mixing with different volume of Tris EDTA buffer according to manufactures instruction. Three different primers can be used at a time for multiplex PCR. A total of 25 µl of mixture was prepared with 8 µl of master mix, 3 µl of forward

Primer sequence for PCR:

Primer	Primer sequence	Amplicon size(bp)
Lt	5-TCTCTATGTGCATACGGAGC-3 5-CCATACTGATTGCCGCAAT-3	322
St	5-GCTAAACCAGTAGAGGTCTTCAAAA-3 5-CCCGGTACAGAGCAGGATTACAACA-3	147
Eae	5CCCGAATTCGGCACAAGCATAAGC 3 5CCCGGATCCGTCTCGCCAGTATTCG 3	881
Bfp	5-TTCTTGGTGCTTGCGTGTCTTTT-3 5-TTTTGTTTGTTGTATCTTTGTAA-3	367
Aat	5-CTGGCGAAAGACTGTATCAT-3 5-CAATGTATAGAAATCCGCTGTT-3	630
ipaH	GCTGGAAAAACTCAGTGCCT CCAGTCCGTAAATTCATTCT	424

primer (1 µl for each), 3 µl of reverse primer (1 µl for each), 3 µl of extracted DNA template and 8 µl of nuclease free water in a PCR tube. After a brief vortex, the tube was centrifuged in a microcentrifuge machine for few seconds. In this way 25 µl of mixture was prepared for amplification.

The PCR run for detection of ETEC, EPEC, EAggEC strains comprises of preheat at 94°C for 10 minutes, then 36 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 45 seconds, extension at 72°C for 2 minutes with final extension at 72°C for 10 minutes. For EIEC strains, each PCR run comprises of preheat at 94°C for 10 minutes, then 30 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 2 minutes, extension at 72°C for 1 minute with final extension at 72°C for 7 minutes.

The detection of ETEC, EPEC and EAggEC involved five primers (lt,st,bfp,eae,aat) and EIEC involved one primer (iPaH). The multiplex PCR was done to detect lt. st and aat genes in one PCR run, eae and bfp genes for second run and iPaH for third reaction. Then this amplified DNA was mixed with loading dye and gel electrophoresis was done. The gel was then removed from the tray and visualized by UV illuminator and photographed for documentation. The size of the amplified DNA was assessed comparing with the bands of supplied DNA ladder (Bio-Rad, USA).

Result

In this study, among 268 cases, 115(43%) were from 3-6 years of age group, 97(36%) were from 7-9 years of age group and rest 56(21%) were from 10-12 years. One hundred thirty (48.5%) cases belonged to the lower income group, 88 (32.8%) belonged to middle income group and 50(18.7%) belonged to higher income group. In our study, the prevalence of diarrhea is more in rural area.

Table 1 : Distribution of the children by age and sex.

Age (Years)	Male N (%)	Female N (%)	Total N (%)
3-6	64 (55.7)	51 (44.3)	115 (42.9)
7-9	50 (51.5)	47 (48.5)	97 (36.2)
10-12	33 (58.9)	23 (41.1)	56 (20.9)
Total, N (%)	147 (54.9)	121 (45.1)	268 (100.0)

Among 268 patients,211 (79%) cases of lactose fermenters, 54(20%) cases of non - lactose fermenters and 3(1%) of no growth of bacteria were found by stool culture. Within the lactose fermenters, *E.coli* was 166 (79%) and other than *E.coli* were 45(21%). Within

Table 2 : Socio-economic status and residence of the children

Residence	Socio-economic condition			
	Lower N(%)	Middle N (%)	Higher N (%)	Total N(%)
Urban	59 (22)	32 (12)	21 (08)	112 (42)
Rural	71 (26)	56 (21)	29 (11)	156 (58)
Total N (%)	130 (48)	88 (33)	50 (19)	268 (100)

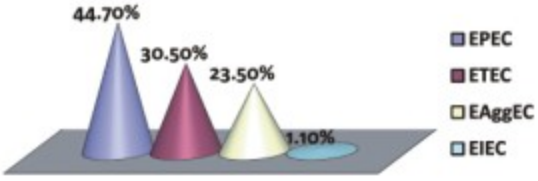

Figure 1:Distribution of detected strains of DEC

A total of 63 DEC, 26(41.2%) were in various combinations. lt +aat and eae+aat was the predominant combination and present in 4(6.34%) of isolates. 3(4.76%) in each were the lt+st and eae+bfp. 2(3.17%) in each were st+bfp, st+eae, st+lt+bfp, st+lt+eae, st+aat and lt+st+bfp+aat combinations. So, among 26 samples ETEC and EPEC each were 3(11.5%) ETEC and EPEC combination were 8(30.7%) followed by ETEC and EAggEC were 6(23%), EPEC and EAggEC were 4(15.3%) and ETEC, EPEC and EAggEC were 2(7.6%) (Table 3).

and 9(35%) were *lt+st* gene. Among EAggEC and EIEC, *aat*gene were 20 and *ipaH* gene was 1 in number. developing countries but also in developed countries. ETEC was listed as the highest priority for vaccine development because of their association with high morbidity and mortality rates.[14] In the present studies, 268 acute diarrhea patient were included. The patient were aged from 3 to 12 years , of them,115(43%) were from 3-6 years of age group, 97(36%) were from 7-9 years of age group and rest 57(21%) were from 9-12 years. Among them, 147 (55%) were male and 121(45%) were female. The ratio of male and female was 1.2:1. The age specific difference suggest that children having immature immune system may be exposed to contaminated formula milk, foods or environment or may have not been protected completely by breast feeding.[15] As the child grows, protective immunity develops and diarrhea is decreased. In Bangladesh, Qadri *et al.* reported in 2007 that the ratio of male to female among the diarrhea patients was 1:1.04[16]. Usually,the number of male and female depends on the availability of patients admitted in hospitals and has no influence on disease process. In Nigeria, Nweze reported in 2010 that sex had no effect on the distribution of diarrheagenic bacteria.[17]

Table 3: Distribution of genes in various combinations among DEC (N=68)

Gene combination	Number (%)	DEC	Number (%)
lt+st	3(4.76)	ETEC	3(11.5)
eae+bfp	3(4.76)	EPEC	3(11.5)
st+bfp	2(3.17)	ETEC and EPEC	8(30.7)
st+eae	2(3.17)		
st+lt+bfp	2(3.17)		
st+lt+eae	2(3.17)		
st+aat	2(3.17)	ETEC and EAggEC	6(23.0)
lt+aat	4(6.34)		
eae+aat	4(6.34)	EPEC and EAggEC	4(15.3)
lt+st+bfp+aat	2(3.17)	ETEC, EPEC and EAggEC	2(7.6)
Total	26(41.2)		26(100)

Discussion

E.coli is an important and unrecognized cause of diarrhea in infancy, not only in the isolated *E.coli,* 68 (38%) DEC were identified, of which 38 (44.7%) was EPEC, 26(30.5%) was ETEC, 20 (23.5%) was EAggEC and 1(1.1%) was EIEC on the basis of multiplex PCR. Among 38 EPEC, 21(55%) contained *eae* gene, 14(37%) contained *bfp*gene and 3(8%) contained *eae+bfp* gene. Among 26 identified ETEC, 6(23%) were *lt*gene, 11(42%) were *st* gene

About 48% pediatric diarrhea belonged to the lower income group and 33% from middle income group. In 2007, WHO reported 90% of all childhood diarrhea occurs in low and middle income groups.[18] Many studies reported among acute diarrheic children, 66% yearly visits to public clinic belongs to low income group and only 8% visit to private pediatrician office from high income group.[16] Diarrhea is more in rural than urban area as rural people are significantly less aware than urban people regarding safe food and drinks, using tube well water for drinking, using latrine. Their knowledge regarding ORS and its uses in diarrhea is very poor. These are may be the causes for more diarrheal patients from rural areas.

Among 268 cases of acute diarrhea, *E.coli* was 166 (79%) and 68(38%) isolates were identified as DEC by multiplex PCR. In previous studies in Bangladesh, 41.33% and 46.78% DEC were identified among diarrheic patients. Similarly in Iraq and Tanzania detection rate of DEC were 38% and 37.5%. However, the frequency of DEC varied in different countries, in Italy it was 6.3% and in Taiwan 5.74%. The prevalence and epidemiology of this pathogen as causative agent of diarrhea vary in the world from region to region and even between and within countries in the same geographical area.[19,20]

In this study, 38(44.7%) were identified as EPEC which was the most prevalent strain among DEC. EPEC was also the most prevalent strain among DEC in Iran, in a study conducted by Alikhani *et al.,* (2006) who reported 44.5% EPEC among diarrheic children.[21] Our study is dissimilar with the study of Arif and Salih (2010) in Iraq, Galane and Roux, (2001) in South Africa and Roy *et al.,* (2014) in Bangladesh.[22,23,4] This dissimilarity may be due to contaminated water, overcrowding and poor sanitation. Among 38(44%) EPEC, 21(55%) contained *eae* gene and 14(37%) contained *bfp* gene and these 92%(55%+37%) were considered as atypical EPEC(as contain only *eae* or *bfp* gene), 3(8%) were typical EPEC as they contained both *bfp* and *eae* genes.

Regarding ETEC, it was 26(30.5%) and the second frequent type of DEC. Similarly, Roy *et al*, in Bangladesh and Arif and Salih, in Iraq reported the isolation rate of ETEC was 35.29% and 26.3% respectively.[4,22] On the contrary,Yang *et al.* observed 66.7% ETEC were associated with diarrhea in Taiwan.[24] The results of a study in Vietnam by Nguyen *et al.*, in Mozambique by Mandomando *et al.,* and in Brazil by Bueris *et al.*, reported 2.2%, 6.8% and 3.7% of ETEC.[11,25,26] Among 26(35.5%) identified ETEC, 11(42%) contained *st* gene, 6(23%) contained lt gene and 9(34.6%) contained both *st*and *lt* genes. In Bangladesh, in a study by Nessa *et al., st* gene was 51.3%, *lt* gene was 25.6% and both *st+lt* genes was 23.1% and in another study by Roy *et al.,*st gene was 37.5%, *lt* gene was 29.17% and *st+lt* genes was 33.33%.[4,6] These findings are consistent with our study.

In the present study, EAggEC was 20(23.5%). Our study is similar with the study of Vilchez *et al.,* (2009) in Nicaragua and Roy *et al.,* (2014) in Bangladesh, they reported 27.8% and 26.4% of EAggEC among diarrheic children.[4,27] Now a days, EAggEC is emerging as an enteric pathogen and responsible for acute and most prominently persistent diarrhea (>14 days) and may cause malnutrition and growth retardation. These malnourished children are more prone to infection like diarrhea and the cycle continues. This strain have been associated with traveler's diarrhea in both developing and developed countries and have been isolated in immune compromised patients.[28]

In the present study, EIEC was 1 (1.1%) among DEC. A study carried out by ICDDR,B, 2002, in which no EIEC strain was detected.[29] Prats *et al.,* detected 0.2% EIEC among DEC positive cases in Spain in 2003.[30] Nguyen *et al.,* detected 2% cases of EIEC in 2005 in Vietnam[11]. The low infection rate of EIEC is nearly similar with our study.

EIEC is not frequently detected in developing countries of Africa and Asia and it is associated with occasional food borne or waterborne outbreaks.[31]

In the present study, among 63 DEC positive cases, 26(41.2%) contained more than one pathogenic genes in various combinations . Among co infections, ETEC +EPEC were 8(30.7%), ETEC+EAggEC were 6(23%), EPEC+EAggEC were 4(15.5%), ETEC+EPEC+EAggEC were 2(7.6%). In Bangladesh, Roy *et al.,* (2014) reported among the combination of DEC, EPEC + ETEC was 6 (27.27%), ETEC+EAggEC was 4(18.18%), EPEC+EAggEC and ETEC+EPEC+EAggEC were both in 3(13.6%) which were similar with our study.[4]

The findings of this study showed that DEC is a common cause of childhood diarrhea in Bangladesh. By using multiplex PCR assays, in one PCR reaction DEC can be diagnosed which can be a rapid and reliable diagnosis of diarrhea. If detection of DEC is done by multiplex PCR in tertiary hospitals or in clinical laboratory, it will be a great achievement to treat acute diarrheal infection in paediatric population.

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Study of thyroid hormone status in normal newborn and preterm, low birth weight baby

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Abstract

Background : Screening for thyroid hormones in the newborn baby is extremely important to detect the newborns who are born with hypofunctional state of thyroid gland. This screening program in first few weeks of life is essential to prevent serious complications of hypothyroidism in future such as mental retardation. **Objective :** To assess the thyroid hormone levels in normal newborn and preterm low birth weight (LBW) babies and comparison of thyroid dysfunction between these two groups. **Methods :** This cross-sectional analytical type of study was conducted in the department of physiology and paediatrics of Rajshahi Medical College & Hospital (RMCH) from July 2015 to June 2016. A total of 70 newborn baby were enrolled by systematic sampling of which 40 were normal healthy newborn and 30 were preterm, low birth weight babies. Data was collected from the parents and they were filled up standard questionnaire. Then venous blood was collected from each and every neonate and FT_4 and TSH values were estimated. **Results :** The mean (±SD) serum FT_4 level in term normal and preterm LBW neonates were 14.17±2.14 pg/ml and 12.25±3.16 pg/ml respectively. This FT_4 value is significantly higher in term neonates than preterm neonates ($P< 0.05$). The mean (±SD) serum TSH level in term and preterm neonates were 3.37±2.12 and 4.23±3.23 (μIU/ml) respectively. Statistically there was no significant difference in TSH values between these two groups ($P= 0.05$). **Conclusion :** Screening for serum TSH level among the newborns with low and very low birth-weight should be introduced in Bangladesh. **This can prevent serious complications of hypothyroidism in future.**

Key words : congenital hypothyroidism, **serum thyroid hormone levels, preterm low birth weight babies,**

Introduction

Newborn screening for congenital hypothyroidism is one of the major achievements in preventive medicine. Congenital hypothyroidism (CH) is one of the common causes of irreversible mental and physical disability if undetected in the neonatal period.[1] Congenital hypothyroidism (CH) is the most common congenital endocrine disorder seen in the newborns (1 in 4,000 births). It causes irreversible mental and physical disability if remains undetected or untreated. Diagnosis and treatment of CH before 3 months are mandatory to avoid cretinism.[2] Low birth weight (LBW) babies are those whose birth weight is less than 2.5 kg. It has two types : Preterm baby (Babies which are born before 37th weeks of gestation) and small for gestational age baby. Preterm newborn babies are more likely to develop hypofunctional state of thyroid gland due to immaturity of hypothalamo- pituitary-thyroid axis , immature thyroid hormone synthesis, immature thyroid hormone metabolism and systemic diseases.[3]

Iodine deficiency is the important and easily preventable cause of mental retardation. Globally about 10% population are suffering from iodine deficiency disorder and lack of iodine in mother leads to 30,000 still birth and 1,20,000 Congenital Hypothyroidism in infants.[4]

Bangladesh is known to be one hyperdermic zone for iodine deficiency. Goitre and other iodine deficiency disorder are very common in our country. The national survey for iodine deficiency

disease in 1993 showed that the incidence of congenital hypothyroidism is 0.5 % in our country.[5] But it was thought that the incidence would be much higher and one small study was done at institute of nuclear medicine, Dhaka. It showed the prevalence rate of CH in Bangladesh as 0.9% which is a cause of concern for physicians.[6,7] In Bangladesh, there are few institute based reports on thyroid disorder. In a recent community based study in southern part of Bangladesh revealed that 3.3 % of school going children are suffering from thyroid insufficiency including hypothyroidism and subclinical hypothyroidism.[8] Congenital hypothyroidism identified by newborn screening has favourable outcome but IQ reduction and persistent cognitive deficit are reported in many studies.[9]

In UK screening for congenital hypothyroidism was introduced in 1981 and the program has been successful in identifying infants before irreversible neurological damage has occurred.[10]

The central hypothyroidism may present for short term or long term. However TSH based neonatal screening cannot detect central hypothyroidism.[11]

The thyroxine level of premature babies are low and cause is multifactorial. These are loss of maternal T_4 contribution, immaturity of hypothalamic pituitary axis issue , unresponsiveness of thyroid gland to TSH and immaturity of peripheral tissue deiodination.[12]

A majority of European and Japanese program favors screening by means of primary TSH measurement supplemented by free T_4 determination for both normal newborns and preterm. Neonatal screening program for CH is highly cost effective for a nation because it prevents the mentally retardation of persons.[8] Therefore, screening program has become a routine practice in all developed countries and many developing countries in South East Asia have adopted neonatal screening for CH as an essential part of their health services.[9]

The aim of this study is to measure the thyroid hormone levels in normal newborns and preterm low birth weight babies and comparison of thyroid status between these two groups. It will facilitate the early detection of hypofunctional state of thyroid gland and thus treat accordingly. In this way, this study contributed in reducing infant and childhood morbidity.

Methods

This cross- sectional analytical type of study was carried out in the department of Physiology and department of Paediatrics of Rajshahi Medical College Hospital (RMCH) between the period of July 2015 to June 2016. Seventy (70) newborn babies were selected between the age group 5 to 28 days, among which 40 were normal healthy newborns and 30 were preterm low birth weight newborns. Study subjects were selected by systematic sampling in Rajshahi Medical College Hospital. All the subjects were free from birth asphyxia, meningitis, septicaemia and other serious neonatal diseases. The aim, benefit and procedure of the study were explained to the parents of the newborns and their informed written consents were taken. Data was collected from the parents by face to face interview with the help of a questionnaire. After completion of the interview of the parents, with all aseptic precaution, venous blood was collected from the newborn babies and sent to the laboratory for estimation of thyroid hormone levels. Serum FT_4 and TSH levels were measured by ELISA (Enzyme linked immuno sorbent assay) method. Student's t test was applied to observe the difference of the thyroid hormonal levels between normal and preterm LBW babies.

Results

A total of 40 normal healthy term newborns, 38(95%) has normal FT_4 and TSH level. Only 2(5%) newborns were found to be hypothyroid (low FT_4 and high TSH). On the other hand among 30 preterm low birth weight baby, 6(20%) babies were found to be hypothyroid. The mean (± SD) serum FT_4 level in term and preterm neonates were 14.17±2.14 pg/ml and 12.25±3.16 pg/ml respectively (Table 1). Serum FT_4 level was significantly higher ($P< 0.05$) in term neonates than preterm neonates (Table 2). Mean FT_4 level in <36 weeks, 36-40 weeks and > 40 weeks gestational age group newborn were 12.13, 13.9 and 15.21 pg/ml respectively. The mean(±SD) serum TSH level in term and preterm neonates were 3.37±2.12 µIU/ml and 4.23±3.23 µIU/ml respectively (Table 1). There was no statistically significant difference (P=0.05) of mean serum TSH level between these two groups (Table 2).

Table 1 Mean (±SD) serum FT_4 & TSH level in normal newborn and preterm low birth weight babies.

Group	Mean(±SD) serum FT_4 (pg/ml)	Mean(±SD) serum TSH (µIU/ml)
Term baby	14.17±2.14	3.37±2.12
Preterm low birth weight baby	12.25±3.16	4.23±3.23

Table 2 Comparison of mean serum FT_4 & TSH level between normal newborn and preterm low birth weight babies.

Parameters of thyroid function	Term baby mean(±SD) n= 40	Preterm low birth weight baby mean(±SD) n=30	P value
Serum FT_4 (pg/ml)	4.17±2.14	12.25±3.16	0.003
Serum TSH(µIU/ml)	3.37±2.12	4.23±3.23	0.183

Discussion

Thyroid hormones screening in the neonatal period is essential to detect the hypofunctional state of thyroid gland. Most neonates born with congenital hypothyroidism (CH) have normal appearance and no detectable physical signs. Hypothyroidism in the newborn period is almost always overlooked and delayed diagnosis leads to the most severe outcome of CH, mental retardation, emphasizing the importance of newborn screening. In developed countries, this screening program was initiated in the last century and now it is well established. But in developing country like Bangladesh, this screening program for thyroid status in newborn is a new concept.[9,10]

In this study, total 70 neonates were included out of which 40 were normal, term baby and 30 were preterm, low birth weight baby. In first 2 to 3 days of life, there occurs TSH surge in the newborn baby due to neonatal cooling. It causes raised thyroid hormone level (T_3 & T_4).[3] To exclude this phenomenon, blood was collected from 5th day onwards from the newborn baby.

In this study, the mean(±SD) serum FT_4 values in term and preterm babies were 14.17±2.14 and 12.25±3.16 (pg/ml) respectively. This FT_4 value is significantly higher in term babies than preterm babies ($P< 0.05$). This result is similar to the study performed by Carrascosa et al. in 2004.[15] They measured FT_4 level in 75

preterm, newborn baby and later compared this value with term baby. The FT_4 level was found higher in term babies than preterm counterpart.

In my study, mean FT_4 level in < 36 weeks, 36-40 weeks and > 40 weeks gestational age group newborn were 12.13, 13.9 and 15.21 pg/ml respectively. Literature suggests that FT_4 level declines in relation to prematurity.[16] The findings of this study agreed with this.

In this study, mean serum TSH values among newborns having birth weight 2-2.5 kg and > 2.5 kg were 3.12 and 3.69 (μIU/ml) respectively. So it suggested that TSH elevation was attenuated in low birth weight infants. This finding consistent with the study performed by Tylek- Lemanska D, Kopice M & Starzyk J in 2002.[17]

Screening for serum TSH level among the newborns with low and very low birth-weight should be introduced in Bangladesh. This can prevent serious complications of hypothyroidism in future.

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Intoxication due to Datura a case report

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Abstract

Poisoning of a young male caused by the intake of extracts prepared from the plant Datura is presented. Datura is a wild growing plant widely distributed and easily available. It is a broadleaf annual erect herb with spine covered seed capsules. It contains a variety of toxic alkaloids such as atropine, hyoscyamine and scopolamine, cause a series of characteristic classic symptoms of anti-cholinergic poisoning. However ,Datura poisoning is most frequently occurs due to plant abuse by adolescent addicts. Stupefying, Aphrodisiac, Suicidal and homicidal purpose. A high index of suspicion and early management of poison is imperative if a favorable outcome is expected. Here, a non-fatal case of poisoning of Datura in a young male, aged 35 years is presented along with a brief description about the management .Early presentation and the presence of an eye witness contributed to the very good outcome in the case being described by us.

Key words : Datura, imperative, poisoning, abuse.

Introduction

Datura is a wild plant grown all over the country in both urban and rural areas. It is a hallucinogenic wildly growing plant. The common names include Jimson weed, Thorn apple, Stink weed, Angels trumpet, Jamestown weed. It belongs to Solanaceae family. The plant is native to Asia, Indian species. There are varieties of Datura fastuosa Datura Niger (purple flower), Datura Alba (white flower), Datura Stramonium, Datura Metal and Datura Atrox.[1] It grows on waste places all over Bangladesh.

Datura mainly contains the tropane alkaloids atropine , hyoscine (scopolamine) and hyoscyamine. Uses of Datura have long histories of hallucinogenic use and have been connected with sorcery, witchcraft, native medicine and magico-religious rites dating back to 1500 BC and Homer's Odyssey.(Homer's use of the plant moly as an antidote to Circe;s poisonous anticholinergic drugs may have been the first recorded use of an anti cholinesterase to reverse central anticholinergic intoxication). Chinese herbal medicines containing tropane alkaloids have been used to treat asthma, chronic bronchitis, pain and flu symptoms. In Mexico, Datura is taken by Yaqui women to lessen pain of childbirth. In Africa ,a common use is to smoke leaves from Datura to relieve asthma and pulmonary problems. Many cultures world wide add plants with tropane alkaloids to alcoholic beverages to increase intoxication .Recently ,Datura has been used as a recreational hallucinogen in the US,resulting in sporadic cases of anticholinergic poisoning and death.[1] The name Datura is taken from Hindi Dhattura "Thorn Apple ultimately from Sanskrit Dhattura white thorn apple. In the Ayurvedic text Sashrata different species of Datura are also referred to as Kanaca and Unmatta. Record of this name in English dates back to 1662. In Maxico its common name is Toloache. It is used in rituals and preyer to Shiva.[2]

Case report

Man aged 35 years ,bread winner of the family while travelling in bus was offered jhalmuri by an unknown person sitting beside him. The man consumed jhalmuri immediately without any thinking or hesitation. After about 1 hour he started complaining giddiness, vomiting and he was in stupor ,drowsy condition. Later he was robbed by this same person. Immediately he was brought in a state of impaired consciousness to the emergency care unit of a hospital. He was accompanied by his friend

and some other person in the bus. Initially ,the medical officer thought it was a case of alcohol but on enquiry with his attendants gave the history of consumption of jhalmuri. Consistent with the history, he presented with typical signs and symptoms of datura poisoning. On examination, he was unconscious , febrile with axillary temperature of 38.7 degree selcious, dry mouth and dilated reactive pupils bilaterally. He had tachycardia with pulse rate 130 beat/min and systolic hypertension with blood pressure of 150/60 mm Hg. There were no neurological signs and other systemic examination findings were normal. The man was diagnosed to have Datura poisoning ,on the basis of eye witness and the clinical manifestations .He gradually improved clinically and survived with gastric lavage and activated charcoal. The man was treated symptomatically with antipyretic, IV fluid and diazepam. After 20 hours of admission he became fully conscious ,co-operative and communicated intelligently. The recovery was uneventful . Then he was discharged home under treatment.

Discussion

Datura is a wild growing herb known as Jimson weed[1].It also has several slang names-The most common is 'sac el ghoul'. The flowers are bell shaped or tubular. Leaves are dark green with pointed margins. The fruit is spherical in shape, green in colour, covered with multiple thorns (thorn-apple) and contains numerous reniform seeds upto 500 yellowish brown seeds . Fatal dose is 100-125 crushed seeds (0.6 1.0 gm). They bear a superficial resemblance to chilly seeds but they are large, brown coloured, kidney shaped ,laterally compressed and double edged at the convex border, surface has numerous small depression, odorless, bitter to taste.[3] On longitudinal section , embryo is curved outward at the hilum. All parts of the plant are poisonous, particularly the foliage and seeds. Seed is the most toxic constituent and contains the following active principles-Hyoscine (scopolamine), Hyoscyamine and Atropine, which are responsible for anticholinergic syndrome resulting from the inhibition of central and peripheral muscarinic neurotransmission by these toxic components. The alkaloids first stimulate the higher centres and finally cause depression and paralysis , specially of the vital centres in the medulla. The respiration is first stimulated , then depressed and the heart centre is stimulated. Peripheral effects are predominant and result from anticholinergic (parasympatholytic) action[2].[4] Datura is used as mydriatic, antispasmotic, pre-anesthetic medication and antidote for organophosphates and carbamates.[3] It is also used as a herbal medicine for asthma, bronchitis, eczema, hemorrhoid treatment, as an ointment against muscle and joint pain . Children are mostly exposed to the poisoning by the plant species from the genous datuura, however the poisoning most frequently occurs due to plant abuse by adolescent addicts. In Europe, the seeds and plant extracts of datura stramonium are used in the treatment of mania, epilepsy, melancholy, rheumatism and convulsions.[5] Intentional poisoning with datura has also been reported. Ingesting datura for its mind altering properties eating and chewing the seeds of the plant in a suicidal attempt may be used.[6]

The clinical features are seen in 30-60 minutes after ingestion and may continue for 24-48 hours.They can be summarized in classic phrase known as Morton's feature'blind as a bat' ,hot as a hare, dry as a bone, red as a beet, mad as a wet hen. The main features are dryness of mouth,nausea, vomiting, dysphagia, dilated pupils, diplopia, photophobia,
temporary blindness, dry hot skin, drunken gait ,dysuria, impaired short term memory, disorientation, delirium with confusion, agitation, hallucination, drowsiness leading to coma and death due to respiratory failure or cardiac arrythmias.[7] Delirium is restless and purposeless in its earliar stage.The patient may be silent but usually he is noisy, tries to run away from the bed, picks at the bed clothes, tries to pull imaginary threads from the tips of the fingers.[4][2], threads imaginary

needles. Consumption of 100-125 crushed seeds can cause death within 24 hours.[6] Children have a special susceptibility to atropine toxicity, even a small amount may produce severe central nervous system manifestations.

Treatment includes emetics ,gastric lavage with tannic acid, 1% potassium permanganate solution. Activated cha xsrcoal can be administered. Physostigmine is the specific antidote. Pilocarpine nitrate is also useful but not actually used in late case. Delirium can be controlled by bromides and short acting barbiturates. Morphine is to be avoided because of the danger of depressing the respiratory centre. Light diet, purgatives and colonic lavage is also recommended. Artificial respiration and O2 inhalation is given when required. Hyperpyrexia is controlled by fluids and other cooling measures. Symptomatic treatment with IV fluids and supportive care are to be given.[5]

Crushed or powdered seeds is used by criminals as stupefying agent and road side poison.Seeds are mixed with food or drink e.g. chapattis, curry, sweet, tea, jhalmuri, chanachur and given to the victim or travellers in railway station, bus stand, etc. More common uses are for criminal purposes like robbery, rape, kidnapping.[8] The drowsy or stupefied victim is robbed off his money or valuable articles.[9] When such a person or victim goes to either the police or the railway authorities to lodge a complaint that his pocket has been picked, nobody is inclined to believe him, mistaking him for a drunk. Such is the advantage of datura as a road poison. Accidental poisoning is common in children. Sometimes it is used as abortifacient agent, aphrodisiac agent and love philter. Cigarettes made from the leaves of the datura plant used to be smoked in former days for the relief of bronchial asthma. These are called stramonium cigarettes and caused bronchodilatation.[10]

Conclusion

Persons must have access to correct and detailed information on poisoning prevention since it is remains one of the effective interventions in solving health and social challenges facing them. Correct and detailed information is essential to prevent misinformation from peers. Parents and children should be counseled about potential poison and poison risks, including dangers associated with substance abuse. One of the most important challenges in Datura poisoning is the delay in making diagnosis. A high index of suspicion and early management of poisoning in children is imperative if a favorable outcome is expected. Early presentation and the presence of an eye witness contributed to the very good outcome in this case.

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