KHARTOUM
MEDICAL JOURNAL
The Official Journal of the Faculty of Medicine, University of Khartoum
Published every four months

Address
P.O. Box 102, Khartoum, 11111 Sudan
E-mail:khartoummedicalj@gmail.com
E-mail:kmj@meduofk.net
Tel.: +249155171858
ISSN 1858-5345

Ethical policies and procedures
- Any material submitted for publication in KMJ must conform to the ethical norms as defined by the Faculty of Medicine, University of Khartoum, Research and Ethical Committees.
- Research papers must be the result of original work and should not be submitted for publication elsewhere.
- Any related previously published work must be referred to by the author(s).
- Authors and co-authors are equally and completely responsible for their manuscripts and should all be aware of contents and have substantial contribution to the work done.
- Authors should accept full legal, moral, scientific and professional responsibility for their articles.
- Authors should include an acknowledgement of data, material or assistance they obtained and used that may otherwise lead to conflicts with other papers.
- Reviewers and readers are expected to report any duplication or fraud they recognize in a manuscript to the Editor-in-Chief. The Editorial Board will investigate the matter and take the appropriate action.
- KMJ reserves the right to accept or reject any article submitted for publication.

Khartoum Medical Journal Objectives
1. Provide a forum for scientific and clinical medicine publications.
2. Serve the medical community in Sudan and the region in the field of continuing medical education.
3. Offer opportunities for the publication of service-oriented research and disseminate information aimed at the promotion of health services.
4. Encourage the development of medical and allied sciences research.
5. Provide opportunities for development of expertise in medical and allied sciences education.
6. Act as a platform for the expression of professional and scientific opinion and exchange of information.
7. Provide a forum for the exchange of ideas and experiences in the field of education and training in the medical and health professions.

Contents

Original Articles

Case report

Original Articles

Erosive tooth wear potential of some traditional plant drinks and cola based drinks on human extracted teeth

Sanhouri. N. M[1], Ziada. H[2], Yahia. N. A[1*], Kamis. A. H[3]

[1] *Faculty of Dentistry; University of Khartoum*

[2] *Faculty of Dentistry; Kuwait University, Department of Surgical Sciences*

[3] *Hamdan Bin Mohammed College of Dental Medicine,UAE*

Abstract

Background: This study aimed at investigating the pH, titratable acid and erosive tooth wear potential of some local beverages in Sudan, *Adansonia digitata* (baobab), *Tamarindus indica* (tamarind), *Hibiscus sabdariffa* (hibiscus)) and cola-based drinks.

Methods: Twenty extracted premolar teeth were sectioned and treated with tested drinks for 10, 20 and 30 minutes representing three consumption frequencies. The pH and titratable acid of drinks were measured using a pH meter. Calcium and phosphorus release were recorded as measures for teeth demineralization. Calcium release was determined photometrically, using atomic absorption spectrophotometry, and phosphorus release using the phosphomolybdate-malachite green procedure.

Results: Cola- based drinks recorded the lowest pH (1.87) while *Hibiscus sabdariffa*, the second lowest pH (1.93) and the highest titratable acid (60.12 mmolOH^-/L). The local beverages (*Adansonia digitata, Tamarindus indica,* and *hibiscus sabdariffa)* showed high levels of calcium as compared to the cola-based drinks. There was a correlation between the calcium and phosphorus contents of the drinks and calcium and phosphorus release over different times. The pH of drinks and calcium release correlated significantly. There was no significant association between pH of drinks and phosphorus release and with TA and calcium and phosphorus release.

Conclusion: Despite their high nutritive values, local drinks tended to alter the ionic concentration of teeth when contacting them indicating an erosive potential. Work needs to be supplemented by in-vivo trials investigating factors such as salivary parameters and modes of drinking influencing their erosive potential.

***Corresponding author:** *Sanhouri N. M. E-mail Address: nadasanhouri@gmail.com,*

Introduction:

Erosive tooth wear seems to be one of the most concerning problems in current dentistry. One of the predisposing factors to erosive tooth wear is dietary acids [1-3]. Organic acids, in particular maleic, tartaric and citric acid are strongly erosive to dental tissues, due to their ability to form chelate complexes with calcium released from teeth [4]. The erosive potential of food and beverages is not only dependent on their pH, but also influenced by their titratable acidity and mineral contents, mainly calcium and phosphorus [4, 5].

In hot tropical countries there is increased consumption of locally produced drinks, containing one or more organic acids as well as consumption of cola-based drinks. In Sudan the most commonly consumed local drinks are: baobab (*Adansonia digitata),* locally known as gongolaise or tabaldi which is rich in ascorbic, tartaric, maleic and citric acids [6, 7].

Another local drink is tamarind (*Tamarindus indica)*, known locally as aradieb. In Sudan and other African countries, it is either consumed as a drink or the pulp is sucked and then eaten. The pulp contains tartaric acid, citric, succinic and maleic acids as well as sugars, proteins and also rich in polyphenols [8, 9] also known for their antibacterial properties [10] .

The third commonly consumed refreshing beverage is karkade (*Hibiscus sabdariffa).* It contains citric, maleic, tartaric, and ascorbic acids [11, 12]. The tooth surface erosive potential of these frequently consumed drinks has been reported in school children in Khartoum [13].

The aim of this study was to evaluate the pH and titratable acid of local drinks as well as cola- based drink and the diet version of the cola- based drink, and to investigate the erosive potential of these drinks on human extracted teeth.

Materials and Methods

Tested drinks:

The test drinks included a cola- based drink, Pepsi and the same version named Diet Pepsi (Arrak Company for Food Industry; Pepsi Co Ink, Borchas, New York) together with samples of the local drinks (baobab, tamarind and hibiscus).

The local drinks were prepared according to conventional local steps by immersing 50g of the plants in 50 mls of water for half an hour then sieving the solution to separate the contents. Fifty mls of each of the local drinks were used. After drinks preparation, 10 mls of each drink were utilized for the experiment.

The pH and titratable acidity measurements of tested drinks:

The pH of each drink was measured at room 35-38°C using a standard pH meter with two decimals accuracy (Metrohm Biotrode, 6.0224.100, Switzerland). The titratable acidity (TA) was measured as the volume (in ml) of 1 M NaOH (Titrisol) required to raise the pH of 50 ml of the drink to neutral pH. For the TA, a standard titrator and auto-burette were used [14]. Both pH and TA were recorded three times and the mean values were taken. The initial pH as well as after 10 min, 20 min, and 30 min intervals was measured in each drink.

Erosive tooth wear potential of tested drinks:

Twenty premolar teeth extracted as part of orthodontic management used for testing erosive tooth wear potential, were disinfected and stored in sodium hypochlorite immediately after extraction. They were examined under the stereo microscope to exclude teeth defects. The teeth crowns were then sectioned into two halves, rinsed with distilled water and dried. A random selection was conducted for the sections to be immersed in the tested drinks. Each section was immersed in 10 ml of the respective drink, and the drinks were tested for calcium and phosphorus presence before and after 10, 20, and 30 minutes of immersion.

The ability of the solutions to demineralize the teeth sections leading to changes in the calcium and phosphorus contents of the tested solutions was taken as a measure of the degree of erosive wear. The erosive potential was measured using the chemical analysis test, where the differences in calcium and phosphorus concentrations of the drinks were recorded before and after immersion of teeth sections [15].

Calcium and phosphorus content of tested drinks:

The calcium was measured using the spectrophotometry procedure (Atomic Absorption Spectrophotometer, AA-6800, Shimadzu). The phosphorus was measured by the phosphomolybdate-malachite green procedure (6505uv/vis, Spectrophotometer, Jenway). The calcium and phosphorus contents of the tested drinks were measured prior to teeth sections' immersion. After immersion of the teeth sections for the different times, the calcium and phosphorus contents of each tested drink were measured. The measurements were made in duplicates and a mean value was calculated for each tested drink according

to the standardization of the device. The mean concentration of calcium and phosphorus contents of the tested drinks was taken as a measure of ionic movements between drinks and teeth sections per solution.

Statistical analysis

The student's t-test, ANOVA and Pearson's correlation coefficient tests were used to analyze the data with the p-value set at 0.05.

Results

The pH and titratable acidity (TA):

The initial pH values of drinks and their corresponding TA are shown in Figure 1. The initial pH of drinks tested were; 2.52, 2.13, 1.93, 1.87 and 2.39 for baobab, tamarind, hibiscus, cola- based and its diet version respectively. The titratable acidity showed that hibiscus had the highest (TA) of 60.12 mmolOH^- /L and pepsi-diet drink had the lowest (TA) of 41 mmolOH^- /L. The changes in drinks' pH during the first 10 minutes of exposure were seen mostly with the baobab showing an increase of 0.14 in pH value whereas the other drinks had minimal changes. However, none or minimal changes in pH were noted after 20 and 30 minutes intervals as shown in Figure 2.

Chemical analysis of the drinks:

Local drinks have high levels of calcium compared to the cola-based drinks. Hibiscus had the highest level of calcium with a mean concentration of 291.5ppm, followed by baobab (89.82 ppm) and tamarind (48.97 ppm). The cola- based and its diet version had a mean calcium concentrations of 3.42 ppm and 3.06 ppm respectively. There was a gradual increase in the mean level of calcium of baobab drink detected over the three immersion times.

The mean calcium level of tamarind drink dropped in the first 10 minutes of immersion and increased after the 20 minutes, and dropped again after 30 minutes immersion.

The mean level of calcium in hibiscus dropped after 10 minutes of immersion and increased considerably after 20 minutes and dropped again after 30 minutes, to a level still higher than the initial calcium concentration.

The cola-based drink showed a drop in the mean calcium level after 10 minutes but increased considerably after the 20 and 30 minutes immersion solutions. Its Diet version showed considerable reduction in the mean calcium level during the 10 and 20 minute immersion times but increased dramatically after the 30 minutes immersion time as shown in Figure 3.

The highest phosphorus level was detected in hibiscus with a concentration of 0.163 ppm followed by tamarind, cola-based drink and its diet version with concentrations of 0.074, 0.095 and 0.044 ppm respectively. No phosphorus was detected in baobab.

The phosphorus contents of the tested solutions increased between 4 to 6 times for tamarind, cola-based and diet cola-based drinks and almost 2 times for hibiscus, with very minimal increase for baobab after 10 minutes of immersion. After the 20 minutes immersion time, there was a considerable drop in phosphorus concentrations in all solutions. After the 30 minutes immersion times phosphorus presence was not detected except for the diet version of the cola based drink, Figure 4.

There was a significant correlation between the calcium and phosphorus contents for the different drink and calcium and phosphorus release over the different times (r_2=0.697; $p<0.001$ and r_2=0.950; $p<0.001$) respectively. There was also a significant association between the pH of the drinks and calcium release ($p=0.03$), whereas there was no significant association between the pH of drinks and phosphorus release or between TA of drinks and calcium and phosphorus release.

Discussion

Drinks and beverages prepared from plants in many African and Asian countries are widely consumed due to their low cost as compared to cola-based and similarly manufactured drinks. Their nutritive values have been well documented [7, 8, 16]

The chemical process that leads to erosive wear is a complex process. The erosive solution has to contact the tooth surface to interact with the mineralized portion of the tooth which is a carbonated and calcium deficient hydroxyapatite [17, 18]. The acid in the solution with its hydrogen ions or chelating properties will dissolve the apatite crystals releasing its minerals which are the calcium and phosphorus. The outflow of ions will continue until no further chelating agents or acids are provided. The pH, titratable acid, calcium and phosphorus concentrations are the primary determinants of the erosive potential of a drink, since they determine the degree of saturation with respect to tooth minerals [18]. In this study, an efflux and influx of calcium and phosphorus ions was recorded in the solutions over the different time intervals.

This study recorded a significant association between pH of tested drinks and calcium release. This result is in harmony with an in-vitro study by Jensdottir et al [19]. However, there was no significant association between calcium and phosphorus release and the titratable acid of the drinks, as reported by Hannig et al [20].

The calcium and phosphate loss from human teeth sections, utilized as an indicator of erosive potential, is considered a sensitive and reliable method [20, 21]

The progression of erosion differs according to the type of enamel. This study took this into consideration and hence human teeth were used to give a true representation of the process, unlike other studies that utilized bovine teeth [22, 23].

The use of 10ml solutions almost mimic the amount of fluid left following swallowing and thus simulate the process of drinking in the mouth where such small amounts remain contacting the teeth surfaces. The 10, 20 and 30 minutes time resemble drinking juices once, twice and three times per day.

Baobab recorded low pH and a high titratable acid (2.52, 47 mmolOH^- /L respectively) and high level of calcium 89.818ppm. The high levels of calcium do not usually result in a high degree of erosiveness. However, a continuous rise in the drink's ions, indicate a continuous outflow of tooth minerals and hence a high erosive challenge. This could be explained by the fact that Baobab has a low pH, high titratable acid and a number of organic acids such as citric, maleic, tartaric, and ascorbic acids, known for their strong chelating and hence potent erosive characters[20].

Hibiscus and Tamarind had similar patterns of calcium and phosphorus ions movement during the first 10 minutes solutions and seemed to lose ions, indicating an inward flow and hence mineralization of tooth surface which is reversed during the 20 minutes immersion time indicating an outflow of ions and hence erosion of the teeth surfaces followed by an inward flow during the 30 minutes immersion time. These inward-outward movements of ions could be explained by the fact that the volume of solutions is comparatively small and when becoming supersaturated compared to teeth hydroxyapatite, an inward movement of ions will occur mineralizing teeth surfaces; but when becoming under-saturated, due to mineral loss, ions will flow outwards eroding teeth surfaces. These differences in behavior compared to Baobab could be due to Baobab phosphorus deficiency indicating higher erosiveness.

The cola-based drink showed an inward movement of calcium ions during the 10 minutes immersion time followed by a recognizable outward flow of ions during the 20 and 30 minutes immersion times indicating great erosive potential. On the other hand, its diet version, showed an inward movement of calcium ions during the 10 and 20 minutes immersion times followed by a dramatic outflow of ions during the 30 minutes immersion time. This behavior indicates that the diet cola-based drink tested in this study was less erosive than the non -diet version, concurring with the findings of Attin et al [24].

The tested local drinks Baobab, Tamarind and Hibiscus, are of high nutritive significance [7, 12]. On contact with the teeth, they tend to alter the ionic concentration of hydroxyapatite indicating a potential erosive challenge to the teeth. Their

potential erosiveness need to be further investigated since they contain more than one organic acid with different structures so various behaviors are expected when interacting with the hydroxyapatite of the teeth.

Behavioral factors such as food type, eating and drinking habits, oral hygiene methods; biological factors such as saliva and soft tissues; together with the chemical properties of saliva [14] may all affect the erosive potential of the drinks.

The nutritional values of these local drinks cannot be overlooked .However, public awareness of their erosive effect on teeth and the consequent complicated oral rehabilitation needed for severe cases must be addressed.

Taking the drinks in a chilled form is better than drinking them hot or at room temperature. The use of straws directed to the oral cavity, as oppose to direct contact on teeth surfaces, will minimize the erosive potential of these drinks [(25)].

In addition, and as a result of the increase in dental awareness, many people tend to brush their teeth immediately after drinking acidic juices. This misbehavior will aggravate the condition. So, it is recommended to postpone the brushing of teeth for an hour and to use fluoridated pastes if available.

The current study supported the findings of other investigators regarding the erosive potential of Cola-based drinks in relation to ordinary and light or diet versions [(23, 24)]

Further in-situ studies are required to test their actual erosive effect under biological conditions.

Acknowledgement

This work is funded by a grant from the Ministry of Higher Education and Research in Sudan.

*This work is dedicated to the soul of our ex-dean Dr. Nadia Ahmed Yahia . May her soul rest in peace.

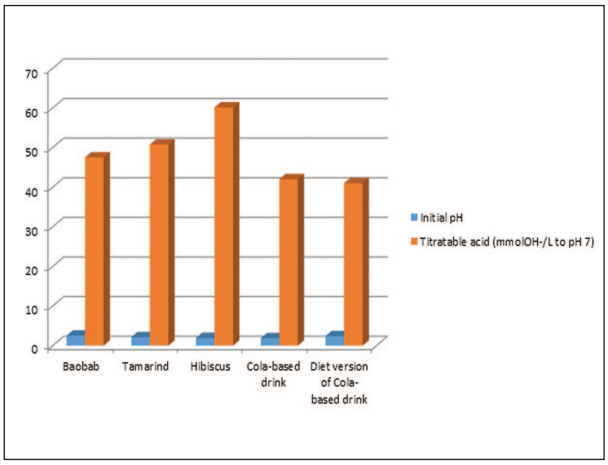


Figure 1: Initial pH and titratable (mmol OH-/L to pH 7) acids of tested drinks before teeth sections' immersion

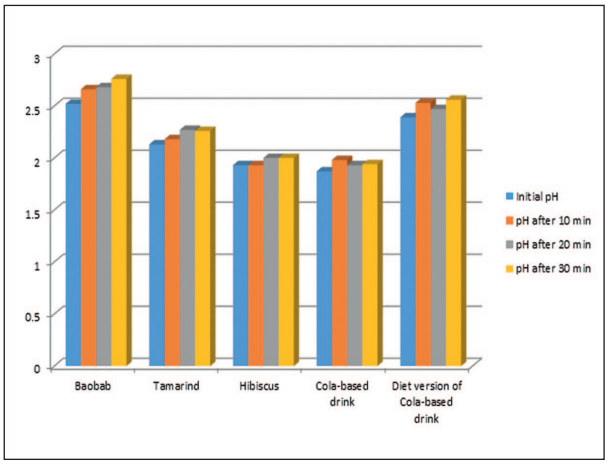


Figure 2: Initial pH of drinks and their pH after teeth sections' immersion for 10, 20 and 30 min

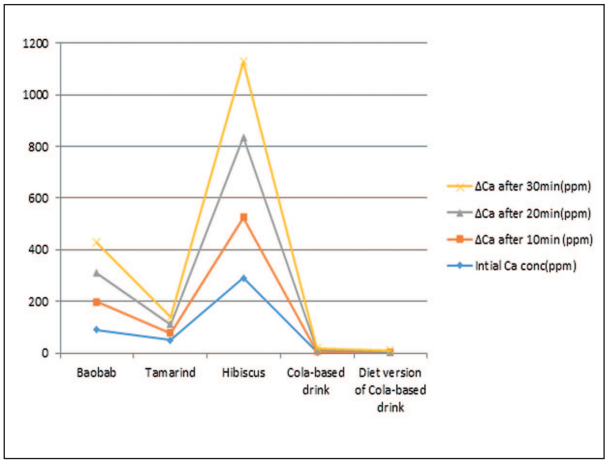


Figure 3: Initial calcium concentration & calcium concentration of tested drinks after 10, 20 & 30 min time

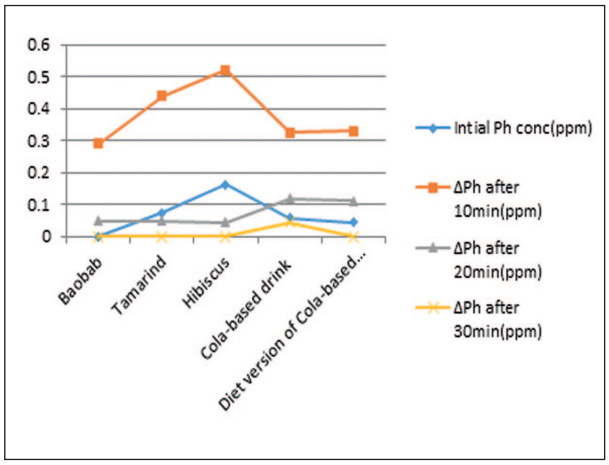


Figure 4: Initial phosphorus concentration & phosphorus concentration of tested drinks after 10, 20 & 30 min time

References

1. Bartlett DW. The role of erosion in tooth wear: aetiology, prevention and management. *Int Dent J*. 2005;55:277-84.

2. Wang YL, Chang CC, Chi CW, Chang HH, Chiang YC, Chuang YC, et al. Erosive potential of soft drinks on human enamel: an in vitro study. *J Formos Med Assoc*. 2014;113:850-6.

3. Lussi A, Jaeggi T. Erosion--diagnosis and risk factors. *Clin Oral Investig*. 2008;12:S5-13.

4. Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. *Monogr Oral Sci.* 2006;20:66-76.

5. Shellis RP, Barbour ME, Jesani A, Lussi A. Effects of buffering properties and undissociated acid concentration on dissolution of dental enamel in relation to pH and acid type. *Caries Res*. 2013;47:601-11.

6. Ghoneim MA, Hassan AI, Mahmoud MG, Asker MS. Protective Effect of Adansonia digitata against Isoproterenol-Induced Myocardial Injury in Rats. *Anim Biotechnol*. 2016;27:84-95.

7. Chadare FJ, et al. . Baobab food products: a review on their composition and nutritional value. . *Crit Rev Food Sci Nutr.* 2009;49:254-74.

8. Glew RS, Vanderjagt DJ, Chuang LT, Huang YS, Millson M, Glew RH. Nutrient content of four edible wild plants from west Africa. *Plant Foods Hum Nutr*. 2005;60:187-93.

9. Paula FS, Kabeya LM, Kanashiro A, et al. Modulation of human neutrophil oxidative metabolism and degranulation by extract of Tamarindus indica L. fruit pulp. *Food Chem Toxicol.* 2009;47:163-70.

10. Djeussi DE, Noumedem JA, Seukep JA, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC *Complement Altern Med*. 2013;13:164.

11. Zizka A, Thiombiano A, Dressler S, et al. Traditional plant use in Burkina Faso (West Africa): a national-scale analysis with focus on traditional medicine. *J Ethnobiol Ethnomed*. 2015;11:9.

12. Sena LP, Vanderjagt DJ, Rivera C, et al. Analysis of nutritional components of eight famine foods of the Republic of Niger. *Plant Foods Hum Nutr*. 1998;52:17-30.

13. Sanhouri NM ZH, Ahmed GI, Kamis AH. Tooth surface loss, prevalence and associated risk factors among 12-14 years school children in Khartoum State, Sudan. *Community Dent Health*. 2010; 27:206-12.

14. Hooper SM, Hughes J.A, Newcombe R.G, Addy M, West N.X. A methodology for testing the erosive potential of sports drinks. *J Dent*. 2005;33:343-8.

15. Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. *Arch Oral Biol.* 2005;50:541-52.

16. Ochani PC, D'Mello P. Antioxidant and antihyperlipidemic activity of Hibiscus sabdariffa Linn. leaves and calyces extracts in rats. *Indian J Exp Biol*. 2009;47:276-82.

17. Barac R, Gasic J, Trutic N, et al. Erosive Effect

of Different Soft Drinks on Enamel Surface in vitro: Application of Stylus Profilometry. *Med Princ Pract.* 2015;24:451-7.

18. Karda B, Jindal R, Mahajan S, Sandhu S, Sharma S, Kaur R. Analysis of the Erosive Potential of Commercially Available Drinks on Dental Enamel and Various Tooth Coloured Restorative Materials - An In-vitro Study. *J Clin Diagn Res*. 2016;10: 117-21.

19. Jensdottir T, Bardow A, Holbrook P. Properties and modification of soft drinks in relation to their erosive potential in vitro. *J Dent*. 2005;33:569-75.

20. Hannig C, Hamkens A, Becker K, Attin R, Attin T. Erosive effects of different acids on bovine enamel: release of calcium and phosphate in vitro. *Arch Oral Biol.* 2005;50:541-52.

21. Eisenburger M, Addy M, Rossbach A. Acidic solubility of luting cements. *J Dent.* 2003;31:137-42.

22. Amaechi BT, Higham SM, Edgar WM, Milosevic A. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. *J Dent Res*. 1999;78:1821-8.

23. Zimmer S, Kirchner G, Bizhang M, Benedix M. Influence of various acidic beverages on tooth erosion. Evaluation by a new method. *PLoS One*. 2015;10:e0129462.

24. Attin T, Weiss K, Becker K, Buchalla W, Wiegand A. Impact of modified acidic soft drinks on enamel erosion. *Oral Dis*. 2005;11:7-12.

25. Eisenburger M, Addy M. Influence of liquid temperature and flow rate on enamel erosion and surface softening. *J Oral Rehabil*. 2003;30:1076-80.

Perinatal outcome of multiple pregnancy in a tertiary maternity hospital in Sudan

Abdelmoneim E M Kheir[1*], Nada M. Mohammed[2]

[1]* *Department of Paediatrics and Child Health, Faculty of Medicine*

[2]*Department of Neonatology, Soba University Hospital, Khartoum, Sudan*

Abstract

Background: The incidence of multiple pregnancies, including high-order multiple pregnancies, has been increasing recently.

Objectives: The objectives of this research were to determine the risk factors and early neonatal outcome of multiple pregnancy.

Methods: This was a descriptive, cross- sectional, hospital-based study, that was conducted in Omdurman Maternity Hospital, during the period January 2015 to August 2015. Two hundred ladies with multiple pregnancy and their respective 428 babies were included in the study. Data was collected using a specifically designed questionnaire and data was analyzed using Statistical Package for Social Sciences (SPSS) program version16. Frequency analysis was used to present the socio-demographic data. Chi square test was used to study the association between the risk factors and type of multiple births.

Results: The result revealed that most of the mothers were between 21-40 years of age and more than half had parity between 2-5; 86% of the mothers had positive family history of multiple pregnancy. The study showed that 87.5% of the deliveries were twins, 11% were triplets and 1.5% were quadruplets. More than two thirds of the deliveries were pre-term and 82% of the babies had low birth weight. Adverse peri-natal outcome included: low Apgar score; neonatal sepsis; respiratory distress syndrome; and hyperbilirubinaemia. The overall mortality rate was 150 per 1000 birth.

Conclusion: Multiple births were found to have an elevated risk of prematurity, low birth weight, and small-for- gestational age. There is need to identify these cases early in order to provide good antenatal care and deliver them in hospitals with facilities for neonatal intensive care.

***Corresponding author:** Department of Paediatrics and Child Health, Faculty of Medicine, University of Khartoum and Soba University Hospital, P.O. Box 102, Khartoum, Sudan. Email: moneimkheir62@hotmail.com*

Introduction

The incidence of multiple pregnancies, includin ghigh-order multiple pregnancies, has been increasing recently. This dramatic rise is due to more frequent use of ovulation-inducing agents, assisted reproduction techniques and a shift towards bearing children at older maternal ages.[1, 2]

Twin pregnancy has a variable incidence worldwide. Japan has the lowest incidence of 4/1000, whereas African countries have higher incidence of 54/1000 births as reported from Nigeria. [3]

The natural incidence of triplet pregnancies was reported to range from 1 per 6400 to 1 per 9520 pregnancies and that of quadruplet pregnancies, to range from 1 per 537 to 1 per 600 000.[4]

Multiple pregnancy is considered unfavourable due to the poor neonatal outcome, maternal complications, possible long-term developmental problems and high costs involved.[5]

Multiple pregnancies in low income countries like Sudan pose higher foeto-maternal risks due to scarcity of human and material resources, which is in turn reflected into inappropriate care during pregnancy and delivery.[6] As a result, multiple pregnancies in these countries expose mothers and infants to extremely high risks. There is limited information about maternal and foetal complications of multiple pregnancy in Sudan. In a retrospective study done in the central part of Sudan during the period 1985 to 1999, that study included a follow-up of 597 twin pregnancies and 30 triplet pregnancies, had revealed significant maternal and peri-natal mortality with a peri-natal mortality rate of 115/1000 for twin and 223/1000 for triplet pregnancy.[7]

The objectives of this research were to determine the risk factors and early neonatal outcome of multiple pregnancy, and to find out if there is any significant correlation between the risk factors and the type of multiple pregnancy (either twins, triplets or higher order).

Materials and methods

This was a descriptive, cross-sectional, hospital-based study, that was conducted in Omdurman Maternity Hospital which is the largest maternity hospital in Sudan, during the period January 2015 to August 2015.All women with more than, or equal to, 24 completed weeks of gestation, having multiple pregnancy (twins / triplets or higher order) and admitted to the labour ward during the study period, were included.

Those excluded from the study were those who refused to participate. Two hundred ladies with multiple pregnancies and their respective 428 babies were included in the study. Sampling technique was total coverage during the study period.

Data was collected using a specifically designed questionnaire filled-out by the researchers containing maternal and neonatal data. Clinical examination of the newborns was done and they were followed-up for 7 days, to determine early neonatal outcome.

The case definition of multiple births was: twins, triplets or higher – order birth. Independent variables of interest included: maternal factors such as age; level of education; and origin. Obstetric history such as:

antenatal care(ANC) and mode of delivery. Other variables included: parity and mode of conception

(spontaneous; induction of ovulation by using drugs; assisted reproductive technique such as in vitro fertilization (IVF) or intra-cytoplasmic sperm injection(ICSI).

Neonatal outcomes consisted of: pre-term birth (less than 37 weeks of gestation). Low birth weight (less than 2,500 g); very low birth weight (1000- <1,500 g); extremely low birth weight (less than 1000gm).

Small-for-gestation age (determined by birth weight less than the third percentile of expected for gestational age). Birth asphyxia (indexed by Apgar score less than7 at 5 minutes).Suspected neonatal

sepsis (used for septicemia / meningitis and pneumonia). Also, development of respiratory distress syndrome (RDS) which was radiologically confirmed; and hyperbilirubinaemia requiring phototherapy,were included.

Additionally, admission to the Special Care Baby Unit (SCBU) aimed at capturing any other undocumented and undiagnosed morbidities. Neonatal deaths and the cause of death were studied.

Data was analyzed using Statistical Package for Social Sciences (SPSS) program version 16. Frequency analysis was used to present the sociodemographic data . Chi square test was used to study the association between the risk factors and type of multiple births. P value was set on an alpha level at 0.05 level of significance.

Ethical approval for conducting this research was granted by the Ehical Committee of Sudan Medical

Specialization Board as well as Omdurman Maternity Hospital. Prior informed consent was obtained from individual subjects with full explanation of the study.

Results

During the study period, a total of 200 multiple births were enrolled out of a total number of deliveries of 16000 during the study period giving an overall prevalence of multiple pregnancy of 1.25%. The age group distribution of the mothers revealed that 6(3%) were more than 40 years; 95 (47.4%) were between 31-40 years; 95(47.5%) were between 21-30 years and 4 (2%) were less than 20 years old. Regarding the level of education of mothers: those who were university graduates were 50 (25%); those who received secondary school education were 58(29.0%), primary school education in 40 (20.0 %) and 52(26%) were illiterate.

When the parity of the mothers was studied: 21% were primiparous; 26% were multiparous; and 53% had parity between 2-5. Regarding family history of multiple pregnancy: there was family history in 86 % and no family history in 14 % of the mothers. The present study revealed that 94.5% of the mothers were on regular ANC; 3.5% were on irregular ANC; and 2% had no ANC at all. Our data indicated that 175 (87.5%) of the deliveries were twins; 22 (11%) were triplets; and 3 (1.5%) were quadruplets. The mode of pregnancy was: spontaneous in 176 (88.0%) women; induction of ovulation with drugs in 18 (9.0%) ; IVF in 5 (2.5%); and ICSI in 1 (0.5%) woman.

Regarding the mode of delivery: it was spontaneous vaginal delivery in 73 (36.5%); emergency caesarean section in 92 (46%); elective caesarean section in 32 (16.0%); and assisted vaginal delivery in 3 (1.5%).

The gestational age at which the mothers with multiple pregnancy presented to labour was found to be less than 28 weeks in 5 (2.5%), 28 – 34 weeks in 71 (35.5%), 34+1 - 36+6 in 51 (25.5%) and equal to or more than 37 weeks in 73 (36.5%). Figure 1

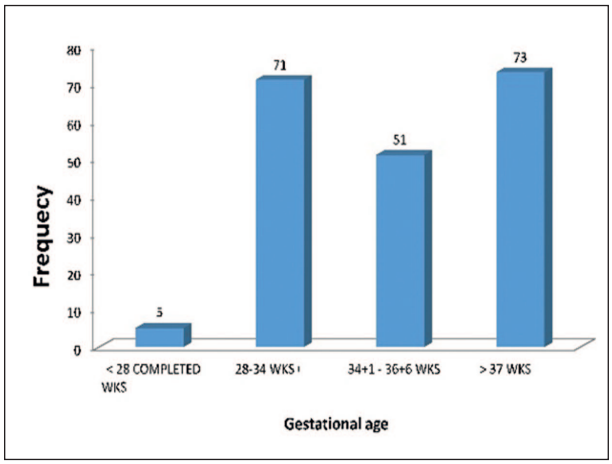


Figure 1:Distribution of mothers with multiple pregnancy according to gestational age

Out of 428 babies, low birth weight was found in 351 (82%). Table (1) illustrates the distribution of babies according to birth weight.

Table 1.Distribution of the babies according to birth weight

Table 1.Distribution of the babies according to birth weight

Birth Weight	1st baby	2nd baby	3rd baby	4th baby
< 1 kg	11 (5.5%)	9(4.5%)	1 (4%)	1 (33.3%)
1 - 1.5 kg	48 (24%)	51 (25.5%)	9 (36%)	1 (33.3%)
1.6 - 2 kg	56 (28%)	54 (27%)	14 (56%)	1 (33.3%)
2.1 - 2.5 kg	47 (23.5%)	50 (25%)	1 (4%)	0
> 2.5 kg	38 (19%)	36 (18%)	0 (0.0%)	0
Total	200 (100%)	200 (100%)	25 (100%)	3 (100%)

Small-for-gestational age was found in 128 (29.9%). Birth asphyxia (Apgar score less than 7 at 5 min)

occured in 17 (8.5%) of first babies; 15 (7.5%) of second baby; and in 3 (12%) of third babies. Regarding neonatal admission to the SCBU, there were 124 (62.0%) of first babies admitted to the SCBU; 119 (59.5%) of second babies; 23 (92.0%) of third babies and all of the quadruplets (100%) were admitted to the SCBU. When the neonatal complications were studied, RDS developed in 77(38.5%) of first babies; 73(36.5%) of second babies; 14(56%) of third babies; and all fourth babies (100%).Neonatal sepsis was reported in 38 (19.0%) of first babies; 41(20.5%) of second babies; and 3(12%) of third babies.

Hyperbilirubinaemia requiring phototherapy was observed in 16 (8.0%) of first babies; 19 (9.5%) of second babies; and 3 (12%) of third babies; and all of the quadruplets (100%).

Among multiple birth neonates, there was no deaths reported in the quadruplets, but it was reported in

29(14.5%) of first babies; 31(15.5%) of second babies; and 4(16%) of third babies. The overall mortality rate was 150 per 1000 birth. With regards to the cause of death, in the first, second and third babies RDS (as a cause of death) was reported in 17(58.6%); 17(54.8%); and 2(50%) respectively; while neonatal sepsis was reported in 12(41.4%); 11(35.5%); and one (25%) respectively. Disseminated intravascular coagulation (DIC) was reported in second babies group only and it occurred in one case (3.2%). Stillbirth was reported in one case in second babies only (3.2%). Table 2

Table 2. Distribution of multiple birth babies according to cause of death

Cause of Death	1st baby	2nd baby	3rd baby	4th baby
Respiratory distress syndrome	17 (58.6%)	17(54.8%)	2 (50%)	0 (0.0)
Neonatal Sepsis	12 (41.4%)	12 (38.7%)	1 (25%)	0 (0.0)
stillbirth	0 (0.0%)	1 (3.2%)	0 (0.0)	0 (0.0)
Others	0 (0.0%)	1 (3.2%)	1 (25%)	0 (0.0)
Total	29 (100%)	31 (100%)	4 (100%)	0 (100%)

Our study revealed significant association between mode of pregnancy and type of multiple pregnancy

(P value = 0.00). However, the study didn't find any significant association between family history of multiple births and type of multiple pregnancy (P value = 0.778). Also correlation between age of the mother and type of multiple pregnancy was insignificant (P value = 0.948). The present study didn't reveal any significant association between type of multiple pregnancy and parity (P value = 0.961).

Discussion

Multiple births are much more common today than they were in the past. The present study is an attempt

to determine the risk factors and early neonatal outcome of multiple pregnancy in a tertiary maternity hospital in Sudan. Our data revealed a prevalence rate of multiple pregnancy of 1.25% which is comparable to the figures from other developing countries[8,9]. This could be mainly due to referral of multiple pregnancies to this tertiary hospital for better neonatal care of low birth weight and prematurity.

The present study also showed that 95% of the mothers were between the age group 21-40 years. This is contrary to what is reported in the literature as advanced maternal age is a risk factor for multiple pregnancy, which operates through the hypothalamic-pituitary-ovarian axis and the associated obstetric complications, such as hypertension.[10, 11] More than half of the mothers in our study were either high secondary school or university graduates. This increased risk among this group may reflect their higher economic capacity to access infertility treatments with a greater possibility of multiple gestations.Our data didn't demonstrate any significant association between family history of multiple birth and type of multiple pregnancy. Family history is a well known risk factor for multiple gestation[12]. However this difference in our series may be due to local genetic and environmental factors. Moreover, our data showed that 88% of women conceived spontaneously, which clearly demonstrates that assisted reproductive technique is still not widely-embraced as an option for many women in developing countries for economic and cultural reasons.[13]

More than one third of the mothers in our series delivered prematurely i.e. between 28-34 weeks gestation. Over the last two decades there has been a steady increase in the incidence of preterm births worldwide with a range of 7-13%.[14] One of the reasons attributed to this increase has been the widespread use of assisted reproductive techniques leading to pregnancies with multiple gestations. [15] The higher rate of prematurity in multiple pregnancy can be explained by uterine over-distension and associated complications that require early intervention for termination of the pregnancy.

The present data indicated that 82% of the babies had low birth weight and 29.9% were small-for-gestational age which is in line with other similar studies.[16, 17] Low birth weight and intrauterine growth restriction occur more often in multiple pregnancies. This is due to the higher chance of placental insufficiency as the number of foetuses increase. Growth restriction might also be related to other antenatal complications such as gestational hypertension, which occurs more frequently in multiple pregnancy.[+]

The present study indicated that multiple pregnancies were associated with a range of adverse perinatal outcomes such as RDS; neonatal sepsis; and birth asphyxia(indexed by low 5 minute Apgar score) which is consistent with the findings of other similar studies.[10,18] The overall mortality rate was 150 per 1000 birth. Other studies showed different mortality rates. One study from Hong Kong quoted a mortality rate of 113 per 1000 birth [4]. Another study from Pakistan showed a higher mortality rate of 172 per 1000 birth [5]. One study quoted a lower perinatal mortality rate, of 50 to 60 per 1000 birth [19]. This difference in mortality is due to early diagnosis; better antenatal care; early detection of complications; and facilities for neonatal intensive care.

Conclusion

Multiple births are much more common today than

they were in the past. Multiple births were found to have an elevated risk of prematurity; low birth weight; and small-for-gestational age. These factors significantly contributed to other adverse perinatal outcomes, such as: low five-minute Apgar scores; neonatal sepsis; RDS; and hyperbilirubinaemia. There is need to identify these cases early in order to provide good antenatal care and deliver them in hospitals with facilities for neonatal intensive care.

Acknowledgements

The authors express their sincere appreciation to the Administration of Omdurman Maternity Hospital for giving their approval and assistance in conducting this research. In addition, the authors are grateful to the mothers who participated willingly in this study.

References

1. Cruikshank D. P. Intrapartum management of twin gestations. *Obstet. Gynecol*. 2007; 109:1167-76.
2. Chittacharoen A. Pregnancy outcome of twin pregnancy in Ramathibodi Hospital. *J Med Assoc Thai* 2006; 89:576-80.
3. Fisk, N. M. Multiple pregnancy,In: Edmond D.K,editor. Dewhurst's textbook of Obstetrics &Gynaecology.7th ed. Massacheusetts: Blackwell Publishing 2007; pp 166-76.
4. Lam H, Ho PC. A comparison of pregnancy outcome between high-order multiple and twin pregnancies: matched-pair retrospective study. *Hong Kong Med J*.1999; 5:16-20.
5. Qazi G. Obstetric and Perinatal Outcome of Multiple Pregnancy,*Journal of the College of Physicians and Surgeons Pakistan* 2011; 21 : 142-145
6. Smits, J., Monden, C.Twinning across the Developing World. *Plos One* 2011; 6: e25239.
7. Dafallah, S. E., Yousif, E. M. A comparative study oftwin and triplet pregnancy. *Saudi Med J* 2004; 25 :502-6.
8. Bortolus R, Parazzini F, Chatenoud L, Benzi G, BianchiMM, Marini A. The epidemiology of multiplebirths. *Hum Reprod Update* 1999;5:179-87
9. Guo G, Grummer-Strawn L. Child mortality amongtwins in less developed countries. *Popul Stud* 1993;47:495-510.
10. Olusanya BO. Perinatal outcomes of multiple births in southwest Nigeria. *J Health PopulNutr.* 2011;29:639-47.
11. Harrison KA, Rossiter GE. Multiple pregnancy. *Br J ObstetGynaecol*1985;92(Suppl 5):49-60.
12. Parazzini F, Tozzi L, Bocciolone L, Molteni E, Moreschi C, Fedele L. Risk factors for multiple births. *ActaObstetGynecol Scand.* 1993; 72 :177-80.
13. Ombelet W, Cooke I, Dyer S, Serour G, Devroey P.Infertility and the provision of infertility medicalservices in developing countries. Hum Reprod Update 2008;14:605-21.
14. Hamilton BE, Martin JA, Ventura SJ: Births: preliminary data for 2005. *Natl Vital Stat Rep* 2006, 55:1-18.
15. Tough SC, Greene CA, Svenson LW, Belik J: Effects of in vitro fertilization on low birth weight, preterm delivery, and multiple birth. *J Pediatr* 2000; 136:618-622.
16. Umeora O U J, AneziOkoro E A, Egwuatu V E. Higher-order multiple births in Abakaliki, Southeast Nigeria, *Singapore Med J* 2011; 52 : 163
17. Sassoon DA, Castro LC, Davis JL, Hobel CJ. Perinatal outcome in triplet versus twin gestations. *ObstetGynaecol 1990*; 75: 817-20.
18. Refuerzo JS, Momirova V, Peaceman AM, SciscioneA, Rouse DJ, Caritis SN et al. Neonatal outcomes intwin pregnancies delivered moderately preterm, latepreterm, and term. *Am J Perinatol* 2010; 27:537-42.
19. Newman RB, Hamer C, Miller MC. Outpatient triplet management:a contemporary review. Am J ObstetGynecol 1989;161:547-55.

Pattern and Outcome of abdominal gunshot wounds in El-Fashir Teaching Hospital

ElhajI.Tibin[1], Alfatih M. Alnajib[1], *Mohamed E.Ahmed[2].

[1]El-Fashir Teaching Hospital. [2]Department of Surgery, Faculty of Medicine, University of Khartoum.

Abstract

Background: Abdominal gunshot wounds (GSWs) are important cause of morbidity and mortality worldwide in both military and civilian settings. They are commonly encountered in the western states of Sudan where the civil war and tribal conflicts are prevalent.

Objectives: To describe the pattern of presentation and outcome of abdominal gunshot wounds and to assess the factors that predict morbidity and mortality at El-Fsahir Teaching Hospital, Northern Darfur.

Patients and Methods: It is a retrospective, analytical, hospital- based study. Data were collected by retrospective review of hospital records of patients with abdominal gunshot wounds who presented between January 2006 and December 2012.

Results: A total number of seventy four patients were included. Male to female ratio was 5:1. The majority of patients 67 (90%) were below the age of 40 years. More than half of the patients 39 (52.7%) had a delayed presentation to the hospital (after eight hours since the time of injury). About one-fourth of patients 18 (24.3%) were shocked at the initial presentation. Surgical exploration was performed in all patients. The most commonly injured organs were: the small intestine 43 (57.1%), the colon 33(44.6%), the stomach and the liver 9(12.2% each). Negative exploration was encountered in six (8.1%) patients. Associated extra-abdominal injuries were found in 24 (33.1%) patients. They were mainly in the extremities 17 (23%), the chest seven (9.5%) and the pelvis four (5.4%).The overall morbidity was 44.6% (n=33). They were mainly: surgical site infections (SSI)followed by septicemia. Colonic injuries are associated with a higher risk of SSI and septicemia. The mortality rate was 21.6% (n=15). The commonest causes of death were septicemia and hypovolemic shock.

Conclusion: There was a high incidence of morbidity (44.6%) and mortality (21.6%) among patients with abdominal gunshot wounds in El-Fashir Hospital in comparison to the literature. This can be explained by various reasons including: the lack of appropriate pre-hospital trauma management; the delayed presentation to the hospital (as a result of insufficient ambulance services and lack of adequate security) and the shortage of well-trained staff. Presence of colonic injuries, increasing number of injured intra-abdominal organs and associated extra-abdominal injuries are predictors of poor outcome in patients with abdominal gunshot wounds in El-Fashir hospital.

***Corresponding author:** Professor of Surgery, Faculty of Medicine, University of Khartoum. Email: rasheid@usa.net*

Introduction

Trauma continues to be a significant cause of morbidity and mortality worldwide[(1)]. Gunshot wounds are now more than in the past and result in a profound morbidity and a significant mortality[(1)]. The causes of gunshot injuries in Africa and developing world include communal clashes, military violence, armed robbery and political conflicts [(2)]. Gunshot wounds (GSWs) to the abdomen are still classically treated with surgical exploration. This approach of management can be explained by a relatively higher

incidence of intra-abdominal injuries and a lower rate of negative exploration when they compared to stab injuries to the abdomen [3]. It was found that negative laparotomy ranged from 23 to 53% for patients with stab wounds to the abdomen, in contrast to a range of 5.3 to 27% for those with abdominal GSWs [3].

Patients and Methods

This is a retrospective, analytical, hospital- based study. Data were collected by a retrospective reviewing of medical records of patients who presented to El-Fashir Teaching hospital, Northern Darfur State, with abdominal gunshot wounds in the period 2006- 2012.The study was approved by the Ethical Committee of Sudan Medical Specialization Board (SMSB).Demographic data were obtained, the time elapsed between the injury and arrival to hospital was recorded. Associated extra-abdominal injuries were reported.

All patients were examined using the standard techniques of examining the abdomen. Haemodynamic stability and signs of peritoneal irritation were evaluated. There were no specific protocols for applying investigations and diagnostic techniques and only plain X-rays were used.

All patients underwent an exploratory laparotomy after adequate resuscitation through a midline incision under general anesthesia. Preoperative antibiotics were given to all patients. Number of injured intra-abdominal organs was recorded. Diverting colostomy was performed in patients with colonic and rectal injuries. Non-suction drains were inserted in all patients and removed after three to ten days postoperatively. The hospital stay for all patients was measured. Morbidity and mortality were recorded.Morbidity encountered during hospital stay included :surgical site infection (SSI), septicemia, hemorrhage and wound dehiscence. SSI was defined as any infection that involved the skin or subcutaneous tissues at the site of incision with the following signs: local swelling and redness, pain or tenderness or purulent discharge. Wound swabs for culture and sensitivity were not taken in all cases due to financial constraints and clinical diagnosis was made in the majority of cases. Septicemia was defined as presence of systemic inflammatory response (SIR) signs (pulse rate is >90 bpm, respiratory rate >20 breathes per minute, fever >38 or hypothermia <36 and total WBCs of more than >12,000/mm³ or less than 4,000/mm³) in the presence of an infection site. Due to abovementioned constrains, the blood cultures were not taken in all suspected patients.

Data were analyzed using the SPSS software package (version 21 Windows). To determine the statistical significance of differences the Pearson test was used and probability test (*P*. value) with $P < 0.05$ was considered significant at 95% confidence interval.

Results

Seventy four patients with abdominal gunshot wounds (GSWs) were included. There were 62(83.6%) males and 12 females (16.4%) with a sex ratio of 5: 1. Sixty seven patients (90.5%) were below the age of 40 years. Thirty nine (52.7%) patients were from El-Fashir City, others were from other cities and towns in Northern Darfur State. The peak incidence of patients was seen in the years 2008 and 2009 (16) patients (21.6% in each).More than the half of patients 39 (52.7%) had a delayed presentation to the hospital (after eight hours since the time of injury), while 35 (47.3%) had presented within the first eight hours of the injury. About one fourth 18 (24.3%) of patients were shocked at the initial presentation.

The most commonly injured organs were: the small intestine 43 (57.1%), the colon 33 (44.6%), the stomach and the liver 9 (12.2% each). About 23(17%) had multi-organ injuries. Negative exploration was encountered in six (8.1%) patients**(Table 1).**More than two-thirds 78.5% (n=11) of those who had postoperative SSI had an associated colonic injury and the majority of deaths (82%) (n=12) had occurred in those with colonic injuries as a result of septicemia. Associated extra-abdominal injuries were encountered in 33.1% (n=24). They were mainly in the extremities 17 (23%), the chest seven (9.5%) and the pelvis four (5.4%).

The mean duration of the hospital stay was 10.2 days. About 33 (45%) patients stayed for more than 10 days.The overall morbidity in our series was 44.6% (n=33). The commonest morbidities were: surgical site infection 14 (43%) and septicemia 11 (33%) **(Table 2).**More than two-thirds 11 (78.2%) of those who had postoperative SSI had an associated colonic injury.The overall mortality rate was 21.6% (n=15). The common causes of death were septicemia in12 patients and hypovolemic shock in three patients. The number of injured intra-abdominal organs is significantly associated with a higher mortality rate, as 73.3% (n=11) of mortality was observed in those who had multi-organ injuries. Furthermore, all of those who died had associated extra-abdominal injuries. There were no associations between age and sex and mortality rate in our series (P values in both were >0.05).

Table 1.Findings of exploratory laparotomy performed for the patients with abdominal gunshot wounds (GSWs) at Al-Fashir Teaching Hospital

Injured intra-abdominal organ*	Number and Frequency (%)
Small intestine	39 (52.7)
Colon	33 (44.6)
Stomach	9 (12.2)
Liver	9 (12.2)
Mesentery	5(6.8)
Duodenum	4 (5.3)
Diaphragm	3 (4.1)
Gallbladder	3 (4.1)
Kidney	2 (2.7)
Spleen	2(2.7)
Rectum	1 (1.4)
No injury	6 (8.1)

***23 (17%) had multi-organ injuries.**

Table 2. Morbidities encountered in patients with abdominal gunshot wounds at Al-Fashir Teaching Hospital (n=33).

The morbidity	Frequency and Percentage (%)
Surgical site infection	14 (43%)
Septicemia	11 (33%)
Hemorrhage	6 (18%)
Wound dehiscence	2 (6%)
Total	33 (100%)

Discussion:

Gunshot injuries constitute major problems worldwide from both medical and economic perspectives[4]. The sex distribution with male predominance is similar to what is reported in other series [5, 6]. In fact, this pattern is typical to other types of trauma[5].

In this series, the majority of the patients 67 (90.5%) were below the age of forty years. This proportion of under-forty patients is slightly higher than that reported by Ogunlusi[7] in a Nigerian Hospital who found about 78.9% were below the age of forty years[7]. This remarkably high incidence among younger age group implies an enormous premature loss of a productive workforce and would have negative socio-economic impacts on both local and national communities. It is interesting to note that the age of the patient with trauma is an important determinant factor in overall outcome, as it was found that the mortality rate increases significantly among the patients who are over 50 years [8]. However, we did not find this association in our series.

The delayed presentation to the hospital (after eight hours since the time of injury) was reported in 39 patients (52.7%) in this series. About one-fourth of patients 18 (24.3%) were shocked at the initial presentation;nearly more than the half of them(ten patients) had a delayed presentation. The delayed presentation is associated with a prolonged time of shock,which would have a negative impact on the

overall outcome of abdominal trauma patients[8]. Availability of well-established trauma services would hopefully minimize the time interval between injury and admission to the hospital and could allow initial resuscitation to be implemented earlier on during the reversible phase of the hypovolemic shock. Britt et al[9] stated that irreversible hypovolemic shock contributes significantly to the mortality and morbidity in patients with abdominal trauma and it's occurrence should be prevented to avoid multi-organ dysfunction syndrome[9].

All patients underwent an exploratory laparotomy. By adopting this policy, we had a proportion of 8.1% (n=6) of negative explorations. This percentage is in the range of the literature that reported a negative exploration rate of 5.3 to 27%[3]. It is fairly similar to a study done by A. A. Kandil[10]at Al-Shifa Hospital in Gaza strip, Palestine, who performed 15 negative explorations out of 230 patients (6.5%) [10]. In fact, the question of whether to explore all patients with penetrating abdominal GSWs or not has been a subject of a great debate in various studies [11]. One study concluded that conservative management should be strictly followed in a selected group of patients, who are heamodynamically stable and have no signs of peritoneal irritation,with availability of advanced diagnostic tools (abdominal CT scan) that have a high sensitivity and specificity to detect minor solid visceral, hollow visceral and retro-peritoneal injuries[11].

The majority of authors still recommend surgical exploration for all patients with abdominal GSWs due to the high possibility of visceral and retro-peritoneal injuries that result from transmitted thermal injury which is produced by penetrating bullets [12]. However, this policy may carry a variable range of negative results[3]adding an undeniable cost of morbidities that are closely associated with unnecessary laparotomies(most notably postoperative respiratory complications and fibrous adhesions) [13]. On the other hand, some workers advocate that local wound exploration (LWE) under local anesthesia in the emergency department can be a good technique to establish whether the peritoneum is breached or not and this would subsequently affect the decision to explore [14]. Nevertheless, this technique is rather employed in stab-penetrating abdominal wounds and has a high failure rate even in the best hands [12].

The pattern of intra-abdominal injuries encountered in our series is in keeping with the literature [15-17]. The small intestine, the colon and the liver were the most frequently injured organs in a large series of abdominal gunshot wounds[18]. About 23 (17%) had multi-organ injuries and we noticed a significant association between the number of injured organs and mortality rate, the majority of patients (75%) (n=11)who died had multi-organ injuries, which is in line with similar studies that proved an association between the number of injured organs and the mortality rates [18-21].

The overall morbidity in our series was44.6% (n=33), with the SSI being the most frequent one. This finding is fairly comparable to similar studies[22]. More than two-thirds 78.5% (n=11) of those who had postoperative SSI had an associated colonic injury and the majority of deaths (82%) (n=12) had occurred in those with colonic injuries as a result of septicemia. This finding supports the presumed association between colonic injury and the increased morbidity and mortality [18].

Septicemia was the second common morbidity encountered. In fact, the literature reported septicemia as a complication of abdominal GSWsin 2.4% to 55.7% of all reviewed cases[22, 23]. It was found that the use of the broad-spectrum preoperative antibiotics in combination with irrigation of the peritoneal cavity with warm saline has contributed to a lower incidence of postoperative SSI following penetrating abdominal gunshot wounds[23].

The mortality rate for this series was 21.6% (n=15) which was remarkably higher than that mentioned in other series that reported a mortality rate of nearly 10%[24]. This can be explained by a wide variety of reasons including :the lack of the appropriate pre-hospital trauma management; the delayed presentation to hospitals (as a result of insufficient ambulance services and lack of security) ;and the

shortage of well- trained staff. It was found that establishing sufficient ambulance services; blood banks and regional trauma centers have decreased mortality rates to almost 9.5%[25] .

The number of injured intra-abdominal organs is significantly associated with a higher mortality, as 73.3% (n=11) of mortality was observed in those who had multi-organ injuries, supporting the findings of previous studies that document a direct relationship between the number of injured organs and the mortality rate [19- 21, 24].

The commonest cause of death in this series was septicemia (12) patients, followed by hypovolemic shock (three) patients, in contrast to others that found hypovolemic shock as the main cause of death[12, 15].

Conclusion: There was a high incidence of morbidity (44.5%) and mortality (21.5%) in patients with abdominal gunshot wounds in this series in comparison to international literature. This can be explained by various reasons including: the lack of appropriate pre-hospital trauma management; the delayed presentation to hospitals (as a result of insufficient ambulance services and lack of adequate security) ;and the shortage of well- trained staff. Abdominal exploration for patients with peritoneal penetration is mandatory. Presence of colonic injuries; increasing number of injured intra-abdominal organs; and associated extra-abdominal injuries are predictors of poor outcome.

Acknowledgment

We would like to thank medical officers and nursing staff in the Accidents and Emergency Department at El-Fashir Teaching Hospital for their kind help and participation during data collection.

References

1- Saidi H S, Faya S. Gunshot injuries as seen at the Aga Khan Hospital, Nairobi, Kenya. *East Afr Med J*. 2002;79:188-192.

2- Aderounmu AO, Adesunkanmi AE, Oluwadiya KS. The pattern of gunshot injuriesin a communal clash as seen in two Nigerian teaching hospitals. *J Trauma*. 2003; 55: 626-630.

3- Renz B M. Unnecessary laparotomies for trauma: a prospective study of morbidity. *J Trauma*. 1995;38:350-356.

4- Rainio J S. Fatal gunshot wounds between 1995 and 2001 in a highly populated region in Finland. *Am J Forensic Med Pathol*. 2005; 26: 70-77.

5- Marx J, Isenhour JL. Abdominal trauma. In: Rosen's Emergency Medicine: Concepts and Clinical Practice, 6th, Marx, JA (Eds), Mosby, 2006.

6- Onuba O. Management of civilian gunshot wounds in a Nigerian general hospital. *Arch Emerg Med*. 1987; 4: 73-76.

7- Ogunlusi J D, Olasinde A A. Gunshot Injuries in a Nigerian hospital. *Nig J Orthop Trauma*. 2006; 5:34 - 37.

8- Feliciano D V. Abdominal trauma. 1989: Maingot's abdominal operations v, 1 eNYAaL, pp 457-512.

9- Baker CC. Predicting outcome in multiple trauma patients. *Infect Surg*. 1986; 5: 243–245.

10- Ahmed A. Kandil M. Gunshot Wounds of the Abdomen in Shifa Hospital, Gaza- Palestinian National Authority. *SciMed J ESCME*. 2005; 17: 4-8.

11- Demetriades D, Charalambides D, Lakhoo M, Pantanowitz D. Gunshot wound of the abdomen: role of selective conservative management. *Br J of Surg.* 1991;78:220-222.

12- Adesanya A A, Ekanem E E. Factors affecting mortality and morbidity in patients with abdominal gunshot wounds. *Injury*. 2000; 31: 397–404.

13- Meyer A A. Abdominal trauma. *Surg Clin North Amer*. 1982; 62: 105-111.

14- Moore E E. Penetrating abdominal trauma

wounds: rational for exploratory laparotomy. *JAMA*. 1985; 253: 2705-2708.

15- Eliciano D V, Patrinel VS, Mattox KL, G. L J. Abdominal gunshot wounds: An urban trauma center's experience with 300 consecutive patients. *Ann Surg*. 1988; 208: 362–370.

16- Oymaci E K M, Uçar Y,Ertan H, Özdedeli E TY. The effects of gunshot and shotgun wounds to mortality and morbidity. *Turk J Traum& Emerg Surg*. 1997; 3:132–136.

17- Dabkana TM, Bunu B, Na'aya HU, Tela UM, Adamu AS. Pattern of injuries seen during an insurgency: a 5-year review of 1339 cases from Nigeria. Ann Afr Med. 2015; 14:114-117.

18- Dellinger E P, Wertz MJ, Hamasaki V, E S L. Risk of infection following laparotomy for penetrating abdominal injury. *Arch Surg* 1984;119: 20–27.

19- Tegegne A. Abdominal missile injuries at Gonder Hospital, Northwestern Ethiopia. *Ethiop Med J*. 1991;29: 81–86.

20- Dawidson I M, Litwin MS. Gunshot wounds of the abdomen. *Arch Surg*. 1976; 111: 862–865.

21- OheneYM, Dakubo JC, Naeeder SB. Penetrating abdominal injuries in adults seen at two teaching hospitals in Ghana. *Ghana Med J*. 2010;44: 103–108.

22- Bozorgzadeh A, Barie P S, et al. The duration of antibiotics administration in penetrating abdominal trauma. *Am J Surg*. 1999; 177: 125-131.

23- Ivatury R R, Psarras P, et al. Intra-abdominal abscess after penetrating abdominal trauma. *J Trauma*. 1988; 28:1238- 1243.

24- Rignault D P. Abdominal trauma in war. *World J Surg*. 1992;16: 940- 946.

25- Fiedler M D, Miller SF, Finley RK. A correlation of response time and the results of abdominal gunshot wounds. *Arch Surg*. 1986;121: 902–904.

Prevalence of Group B streptococcus colonization during labour in Sudanese women

Hala Abdullahi[1*], Suhair Satti[1], Salah K Ali[2], Noreldein M Ali[3],
Duria A Rayis[1], Ishag Adam[1]

[1]*Department of Obstetrics and Gynaecology, University of Khartoum, Sudan*
[2]*University of Science and Technology, College of Medical Laboratory Science*
[3]*Central Public Laboratory – Khartoum State, Sudan*

Abstract

Background: Group B streptococcus (GBS, *Streptococcus agalactiae*) is recognized as a major cause of neonatal sepsis and meningitis. The prevalence of GBS colonization is very variable across the world. There is no published data on the prevalence of GBS among Sudanese women.

Method: A cross-sectional study was conducted at Khartoum Teaching Hospital, Sudan. Obstetric data, low vaginal and rectal swabs were collected during labour.

Results: Two hundred women were included. Only one case of GBS colonization during labour was identified giving a prevalence of 0.5%. Almost 12% of the study population reported use of antibiotics in the last two weeks preceding labour, which may be a factor in the low culture positive rate.

Conclusion: There is a low prevalence of GBS intra-partum colonization among Sudanese women.

***Corresponding author:** Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Khartoum, P O Box 102, Email; halaabdullahi@hotmail.com*

Background and literature review

Group B streptococcus (GBS, *Streptococcus agalactiae*) is recognized as a major cause of neonatal sepsis and meningitis [1-3].

GBS infection acquired from the colonized birth canal during labour or after membrane rupture can lead to miscarriage, stillbirth, prematurity, or invasive neonatal disease [4]. Vaginal colonization with GBS is acquired from the gastrointestinal tract and a large proportion of healthy adults are colonized [5].

The prevalence of GBS colonization is very variable across the world ranging between 1.8% to 26% in various studies [6-14]. Maternal GBS colonization varies by population characteristics such as age, parity, socio-economic status and geographic location [10]. Therefore, a policy of screening of all pregnant women for GBS has been adopted by some countries [2]. Intra-partum antibiotic prophylaxis in cultures-positive women is associated with a 70% reduction in neonatal early-onset sepsis [15].

There is no published data on GBS among Sudanese women; therefore, this study was conducted to determine the prevalence of GBS colonization among parturient women and to verify the risk factors associated with colonization.

Method

A cross-sectional study was conducted at Khartoum Teaching Hospital, Sudan, over a period of five months.

After signing an informed consent, data was collected to gather socio-demographic characteristics, obstetric history and the outcome of labour using a pretested data collection form.

The sample size was calculated using (Kish L. 1965) $n = (Z_{1-\alpha})^2(P(1-P)/D^2)$

where n = sample size,

Z = Z statistic for a level of confidence of 95%, which is conventional, Z1-α value is 1.96

P = expected prevalence, in the absence of local data, was taken as the median prevalence of GBS carriage among pregnant women in developing countries (12%), [16]

d = precision (in proportion of one; if 5%, d = 0.05).

The calculated required sample size was 162

Low vaginal and rectal swabs were taken and sent to the laboratory within one hour for culture.

Swabs were cultured on blood agar and Mc Conky agar. The isolated organism was confirmed as GBS by Lancefield grouping after the observation of complete zone haemolysis. Antimicrobial sensitivity was determined for positive swabs. Sensitivity test was done using Bioanalyse antibiotic kit, in Muller Hinton culture media with the use of Mac Farland 0.5 as a standard.

Ethical approval was obtained from the Ethical Committee of Sudan Medical Specialization Board and the Hospital Research Ethics Committee.

Results

Two hundred women were included in the study. Age ranged from 15 – 41 years with a mean (SD) of 27.0 (5.8) years. Demographic data and previous history are summarised in Table (1).

Table (1) Demographic data and previous history of the study population

	Mean (SD)
Age	27.0 (5.8)
Parity	1.7 (2)
Gestational age	38.5 (2.5)
	Number (%)
Urban residence	149 (74%)
Secondary school education and above	149 (74%)
Regular Ante-natal care	192 (96%)
History of miscarriage	34 (17%)
History of urinary tract infection	108 (54%)
History of vaginal discharge	53 (26.5%)

Three women(1.5 %) had diabetes mellitus. The haemoglobin concentration of the participants ranged from 9 to 14.5 gram/dl with a mean (SD) of 11.6 (1.17) g/dl.

In 50% of the cases, vaginal and rectal swabs were taken after rupture of the membranes. The interval between membrane rupture and delivery was more than 18 hours in 21 women (10.7%).

One hundred eighty six (93%) and 14 (7%) had spontaneous and induced labour, respectively. One hundred seventy four (87%), 9 (4.5%) and 17(8.5%) delivered vaginally, had instrumental delivery and had emergency caesarean section, respectively. Fifteen (7.5%) women had pre-term delivery (< 37 weeks)

Antibiotics were used intra partum in 17women (8.7%), while 23 (11.9 %) reported positive history of using antibiotics in the last two weeks before delivery. The most commonly used antibiotic was the broad spectrum second generation Cephalosporin, Cefuroxime.

The culture was positive in one woman (0.5%) who was a 22 years old, para 2, secondary school graduate, had regular antenatal care and did not use any antibiotics in the two weeks preceding labour. The swabs were taken before rupture of the membranes.

The isolated GBS was sensitive to penicillin, Erythromycin, Cefuroxime, Ceftriaxone, Ceftazidime, Amikacin and Ciprofloxacine but was resistant to Cephalexin.

Table (2) Prevalence of GBS during pregnancy/labour in studies from some countries

Study	Country of study	Prevalence
Steenwinkel et al., 2008 (8)	Mozambique	1.8%
Sharmila et al., 2011(12)	South India	2.3%
Woldu et al., 2014 (26)	Ethiopia	7.2%
Chan et al., 2013 (27)	Bangladesh	7.7%
Puccio et al., 2014 (13)	Italy	8%
Eun Ju Kim et al., 2011 (11)	Seoul Korea	8%
Drowela et al., 2005 (28)	Malawi	16.5%
Hassan et al., 2011(29)	UK	19%
Kraśnianin et al., 2009 (9)	Poland	20%
Mitima et al., 2014 (30)	Democratic Republic of the Congo	20%
Javanmanesh et al., 2013 (7)	Iran	22.8 %
Agricola et al., 2009 (14)	Tanzania	23%
Mavenyengwa et al., 2010 (10)	Zimbabwe	23%
Campbell et al., 2000 (6)	USA	26%

Discussion

The main finding of the current study was the low prevalence (0.5%) of GBS. However, a low prevalence was reported in Seoul Korea (8%) [11], South India (2.3%)[12] and Mozambique (1.8%) [8]. This prevalence is low when compared to a prevalence of around 20-25 % in other countries like Iran [7], Poland [9] and Zimbabwe [10]. Table (2)

In this study, 11.9% had history of antibiotics use in the two weeks preceding labour, which could explain the low prevalence (0.5%) of GBS in this setting. In addition to that, a high room temperature more than (30 ^{0}C) was reported to be associated with loss of positive cultures [17] which is another possible factor as it is usually very hot in this country .

The prevalence of diabetes in this study was 1.5% slightly lower than the reported prevalence of 2-6% in other international and local studies [18-20]. However, GBS positivity during pregnancy was reported not to be associated with diabetes [21]

Intra-partum clinically-suspected chorioamnionitis is known to be present in 4%–10% of women in

labour[22]; In our study antibiotics were administered in 8.7% of the cases due to this suspicion. In spite of the low prevalence of GBS in this population the incidence of pre-term labour is comparable to the international literature of 7-11% [23, 24]

Caesarean section and intact membranes did not seem to prevent the transmission of GBS to a newborn in a Polish study [9]

The most commonly used antibiotic during this study was the second generation Cephalosporin, Cefuroxime, and the isolated GBS was found to be sensitive to it as supported by other studies [25]

Conclusion

There is low prevalence of GBS among the studied parturient Sudanese women (0.5%). Although this low prevalence does not support routine antenatal screening, further studies including a larger population as well as studies looking at the incidence of GBS neonatal infections are recommended to guide establishing the appropriate screening polices.

Conflict of Interest

The authors declare that they do not have competing interests.

Author and Contributors

HA, SS and IA initiated the research idea and drafted the proposal. . SS and DR collected the samples and data. SA and NA performed the laboratory work. HA, DR and IA supervised the research, performed the data analysis. All authors contributed to the writing of the paper and approved the final manuscript.

References

1- Bergeron MG, Ke D, Menard C, Picard FJ, Gagnon M, Bernier M, et al. Rapid detection of group B streptococci in pregnant women at delivery. *N Engl J Med*. 2000 ; 343: 175–179.

2- Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recom Rep. 2002; 51:1–22.

3- Hyde TB, Hilger TM, Reingold A, Farley MM, O'Brien KL, Schuchat A, et al. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. Pediatrics.2002; 110:690–695.

4- Edwards MS, Baker CJ. Group B streptococcal infections. In: Remington JS,Klein JO, editors. Infectious diseases of the fetus and newborn infant. 5th edition. Philadelphia: Saunders; 2001. p.1091–156.

5- Van der Mee-Marquet N, Fourny L, Arnault L, Domelier AS, Salloum M, Lartigue MF, Quentin R. Molecular characterization of human-colonizing Streptococcus agalactiae strains isolated from throat, skin, anal margin, and genital body sites. *J. Clin. Microbiol*. 2008;46:2906-11.

6- Campbell JR, Hillier SL, Krohn MA, Ferrieri P, Zaleznik DF, Baker CJ. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol* 2000 Oct; 96:498--503.

7- Javanmanesh F, Eshraghi N. Prevalence of positive recto-vaginal culture for Group B streptococcus in pregnant women at 35-37 weeks of gestation. *Med J Islam Repub Iran.* 2013 Feb; 27:7-11.

8- Steenwinkel, F.D.O. Tak, H.V. Muller, A.F. Nouwen, J.L. Oostvogel, P.M. Mocumbi, S.M. Low carriage rate of group B streptococcus in pregnant women in Maputo, *Mozambique Tropical Medicine & International Health.* 2008;13: 427-429.

9- Kraśnianin E1, Skret-Magierło J, Witalis J, Barnaś E, Kluz T, Kozieł A, Skret A. The incidence of Streptococcus Group B in 100 parturient women and the transmission of pathogens to the newborn. Ginekol Pol. 2009;80:285-9.

10- Mavenyengwa RT, Afset JE, Schei B, Berg S,

Caspersen T, Bergseng H, Moyo SR. Group B Streptococcus colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstet Gynecol Scand*. 2010; 89:250-5

11- Eun Ju Kim, Kwan Young Oh, Moon Young Kim, Yong Soo Seo, Jung-Hwan Shin, Young Rae Song et al., Risk Factors for Group B Streptococcus Colonization Among Pregnant Women in Korea. *Epidemiol Health*. 2011; 33: e2011010.

12- Sharmila V, Joseph NM, ArunBabu T, Chaturvedula L, SistlaS.Genital tract group B streptococcal colonization in pregnant women: a South Indian perspective. *J Infect DevCtries.* 2011; 5:592-5.

13- Puccio G, Cajozzo C, Canduscio LA, Cino L, Romano A, Schimmenti MG, Giuffrè M, Corsello G. Epidemiology of Toxoplasma and CMV serology and of GBS colonization in pregnancy and neonatal outcome in a Sicilian population. *Ital J Pediatr*. 2014 22; 40:23.

14- Agricola J, Mecky I, Furaha A, Eligius F. Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health* 2009;9:437.

15- Baltimore RS. Consequences of prophylaxis for group B streptococcal infections of the neonate. *Semin Perinatol*. 2007; 31:33–38.

16- Stoll BJ, Schuchat A. Maternal carriage of group B streptococci in developing countries. *Pediatr Infect Dis J*. 1998;17: 499-503.

17- Stoner KA, Rabe LK. Effect of transport time, temperature, and Concentration on the survival of group B Streptococci in Amies transport Medium. *J Clin Microbiol* 2004; 42:5385-7.

18- Mamabolo RL, Alberts M, Levitt NS, Delemarre-van de Waal HA, Steyn NP. Prevalence of gestational diabetes mellitus and the effect of weight on measures of insulin secretion and insulin resistance in third-trimester pregnant rural women residing in the Central Region of Limpopo Province, South Africa. *Diabet Med*. 2007; 24: 233-9.

19- Buckley BS, Harreiter J, Damm P, Corcoy R, Chico A, Simmons D, Vellinga A, Dunne F; DALI Core Investigator Group. Gestational diabetes mellitus in Europe: prevalence, current screening practice and barriers to screening. A review. *Diabet Med.* 2012 ; 29:844-54.

20- Shalayel M H.F, Ahmed Salah A.M., Khattab A. H. & Gwiria M. H. Satti. Frequency of Gestational Diabetes Mellitus and Impaired Glucose Tolerance in Urban Sudanese Pregnant Women in The Third Trimester. *Sudan J.M.S.*2012; 7 :101-104

21- Romanik M, Nowosielski K, Poręba R, Sioma-Markowska U, Martiroisian G, Groborz J.Streptococcus group B serotype distribution in anovaginal isolates of women in term pregnancy. *Neuro Endocrinol Lett. 2*014; 35 :301-5.

22- Martinelli P., Sarno L., Maruotti G.M., Paludetto R. Chorioamnionitis and prematurity: A critical review. *J. Matern. Fetal Neonatal Med*. 2012;2 5:S29–S31.

23- Lawn J.E., Kinney M.V, Belizan J.M, Mason E.M, McDougall L, Larson J et al., Born too soon: Accelerating actions for prevention and care of 15 million newborns born too soon. *Reprod. Health*. 2013;10.

24- Marcos Z. Arriving too early. Lancet Neurol. 2013;12:332–333.

25- Panda B, Iruretagoyena I, Stiller R, Panda A. Antibiotic resistance and penicillin tolerance in ano-vaginal group B streptococci. *J Matern Fetal Neonatal Med.* 2009 Feb; 22 :111-4.

26- Woldu ZL, Teklehaimanot TG, Waji ST[1], Gebremariam MY. The prevalence of Group B Streptococus recto-vaginal colonization and antimicrobial susceptibility pattern in pregnant mothers at two hospitals of Addis Ababa, Ethiopia. Reprod Health. 2014 ;5:11:80.

27- Chan GJ, Modak JK, Mahmud AA, Baqui AH, Black RE, Saha SK. Maternal and neonatal colonization in Bangladesh: Prevalences, etiologies and risk factors. *J Perinatol.* 2013; 33:971-6.

28- Drowela T, Komolafe O, Igbigbi A. Prevalence of group B Streptococcus colonization in an antenatal women at Queen Elizabeth Central Hospital, Blantyre-a preliminary study. *Malawi Med J.* 2005; 17 :97–99.

29- Hassan IA, Onon TS, Weston D, Isalska B, Wall K, Afshar B, Efstratiou A. A quantitative descriptive study of the prevalence of carriage (colonisation) of haemolytic streptococci groups A, B, C and G in pregnancy. *J Obstet Gynaecol.* 2011; 31:207-9.

30- Mitima KT, Ntamako S, Birindwa AM, Mukanire N, Kivukuto JM, Tsongo K, Mubagwa K. Prevalence of colonization by *Streptococcus agalactiae* among pregnant women in Bukavu, Democratic Republic of the Congo. *J Infect Dev Ctries*. 2014; 12; 8:1195-200.

Prevalence of Group B Streptococci vaginal colonization in pregnant women at Saudi Maternal Hospital, Omdurman, Sudan

A E Ibrahim[1*], Mai Masri[2], S M Elsanousi[3]

[1]*Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum*

[2]*Department of Zoology, Faculty of Science, University of Khartoum*

[3]*Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum*

ملخص البحث

الاصابه ببعض الانواع من الباكتيريا تؤثر على النساء الحموامل خاصة فى الاسابيع الاخيرة من الحمل. تعتبر المكورات السبحية من الباكتيريا التى قد تؤدى الى الاجهاض او ربماتنتقل و تصيب الاطفال قبل او اثناء الولادة . اجريت هذه الدراسة على مجموعة من النساء الحموامل فى عمر ٣٥-٣٧ اسبوع من الحمل لعزل وتشخيص الباكتيريا من النوع المكورات السبحية ومدى انتشارها فى المستشفى السعودى للولادة بامدرمان ومدى استجابتها للعلاج بالمضادات الحيوية المعروفة . شملت الدراسة عدد ١٢٥ من المترددات على المستشفى موضوع الدراسة حيث تم اخذ مسحات مهبلية منهن وتم تشخيصها بالاستزراع والتفاعل البيوكيميائى واختبار لانسفيلد ومن ثم تأكيد التشخيص عن طرق التفاعل المتبلمر المتسلسل مع جمع المعلومات عن طريق الاستبان. خلصت الدراسة الى وجود هذا النوع بنسبة تصل الى ٣,٢٪ وسط المترددات على المستشفى. توصى باجراء هذا الفحص كعمل روتينى لجميع النساء الحوامل على نطاق واسع يشمل مستشفيات الولادة لتفادى انتقال العدوى للاطفال حديثى الولادة.

Abstract

Background: Group B Streptococci (GBS) colonization in pregnant women usually has no symptoms, but it is one of the major causes of newborn infection in developing countries.

Objectives: The aim of this study was to determine the prevalence of vaginal GBS colonization in pregnant women at 35 – 37 weeks of gestation at Saudi Maternal Hospital, Omdurman, Sudan and to determine their susceptibility to antibiotics.

Methodology: This is a hospital-based study that surveyed 125 pregnant women who attended Saudi Maternal Hospital at the third trimester (35 – 37 week of gestation) from whom vaginal swabs were collected to investigate GBS streptococcal infection. The data were collected using a questionnaire. The isolated streptococci were identified by biochemical reactions, Lancefield grouping and Polymerase Chain Reaction (PCR).

Result: 3.2 % of the pregnant females attending the Saudi Maternal Hospital had vaginal colonization with GBS.

Conclusion: The prevalence of GBS colonization in pregnant women who attended the Saudi Maternal Hospital during the period of the study was found to be higher than the figure of 1% found on the hospital records. Screening and treatment of pregnant mothers to prevent invasive neonatal GBS disease is recommended.

***Corresponding author:** Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum, E. mail: Abbasesafi @ outlook.com*

Introduction and Literature review

Streptococci are a heterogeneous group of bacteria, including important human pathogens. *S. pneumoniae* is considered a common agent of community-acquired pneumonia, otitis media, and endocarditis. *S. pyogenes* causes a wide array of serious infections, including pharyngitis, soft-

tissue infections, scarlet fever, and toxic shock-like syndromes. *S. agalactiae* is an important cause of serious neonatal infections characterized by sepsis and meningitis. Most other streptococci are members of the normal human floras [(1)].

Streptococci are classified on the basis of colonial morphology, haemolysis, biochemical reactions, and serologic specificity. They are divided into three groups by the type of haemolysis on blood agar: β-haemolytic (clear, complete lyses of red cells), α- haemolytic (incomplete, green haemolysis), and γ-haemolytic (no haemolysis). Serologic grouping is based on antigenic differences in cell wall carbohydrates (groups A to V excluding I and J), in cell wall pili-associated protein, and in the polysaccharide capsule in group B streptococci. Diagnosis is based on cultures from clinical specimens. Group B streptococci typically show hippurate hydrolysis[(1)].

Group B Streptococcus (GBS) is one of the most important bacteria in the majority of maternal and neonatal infections, such as chorioamnionitis, endometritis, bacteremia, sepsis and meningitis. During pregnancy, GBS screening is one of the recommended strategies that are recommended by Center of Disease Control (CDChttps://wwwn.cdc.gov).

In a prospective observational study in Egypt to detect the magnitude of group B streptococcal (GBS) colonization and disease among a sample of pregnant women, 95 pregnant women, 35-37 weeks of gestational age were screened with vagino-rectal swabs by a conventional GBS PCR assay. The study showed that, GBS carriage rate in the study sample was 17.89%. It was concluded that maternal GBS carriage was associated with a significant increase in neonatal infection rate [(2).]

During the last few decades, group B Streptococcus (GBS) has emerged as an important pathogen. The major reservoirs for GBS are the vagina and the peri-anal regions/rectum, and the colonization of these regions is a risk factor for subsequent infection in pregnant women and newborns. A study was performed in India to determine the prevalence of GBS colonization in the vagina and rectum of pregnant women and the antibiotic susceptibility pattern of the isolates. It aimed also to identify risk factors associated with GBS colonization. It showed that GBS colonization rate was 2.3%. It concluded that the GBS colonization rate was low. No resistance to penicillin or clindamycin was seen, while the majority of the isolates were resistant to tetracycline. [(3)]

Group B Streptococci (GBS) are the most frequent cause of severe, life-threatening neonatal infections. Significant infections are also seen in adult women as a complication of pregnancy and in the elderly and immunocompromised hosts. Commonly, 10–40 % of pregnant mothers carry GBS in their genitourinary and gastrointestinal tracts. Maternal colonization with GBS is the predominant risk factor for the development of invasive neonatal GBS disease. GBS carriage screening at weeks 35-37 of gestation and subsequent intrapartum antibiotic prophylaxis (IAP) for culture-positive women is recommended in many countries. [(4)]

A study was conducted in Iran to find out its prevalence among Iranian pregnant women and its vertical transmission to their newborns. It showed that 50% of symptomatic neonates were born from the mothers with positive vaginal culture for group B Streptococcus; whereas para-clinical test was required to detect the infection for the rest of neonates who showed no signs or symptoms[(5)].

The current study is investigating the frequency of Group B streptococci among pregnant women.

Materials and Methods

The study area was the Saudi Maternal Hospital. Lower vaginal swabs were collected in this study. The study population included the pregnant women who attended the study hospital at 35 - 37 week of gestation. All information required from patients were collected by a questionnaire.

Swabs and plastic Petri dishes were purchased readily sterilized. Hydrogen peroxide was prepared

in a concentration of 3% solution and was used for the catalase test.

Defibrinated sheep blood was used in preparing blood agar medium. Blood agar was used in culturing vaginal swabs and it was also used for identification of the type of haemolysis and CAMP reaction.

Bile salt agar was used for detection of growth of the organisms isolated in 40% bile salt agar. Muller Hinton agar was used for sensitivity to antibiotics. Nutrient broth medium (5% serum was added) was used for the catalase test. Hippurate broth was used for the detection of hippurate hydrolysis. All types of media and reagents used were prepared as described by Barrow and Felltham [6].

Samples collection

Samples were collected from un-treated pregnant women who attended the study hospital at 35 - 37 weeks of gestation.

After collection, all samples of vaginal swabs were kept at refrigerator temperature for laboratory investigation

Culturing and Identification of organism

Swabs were streaked directly on blood agar plates containing 5% sheep blood by the use of bacteriological loop. All plates were then incubated in 10% Co2 overnight at 37º C. When no growth was observed, plates were reincubated for further 48 hrs before they were discarded as negative. Haemolysis was observed but it was not used as a stringent identification criterion.

The Gram stain was done from the overnight growth. Organisms that showed Gram-positive reaction were subjected to further examination and organisms that showed Gram – negative reaction were excluded.

All Gram-positive samples were subjected to catalase test which was done by adding few drops of 3% hydrogen peroxide to the tested organism which was previously cultured for overnight at 37 °C in 5 ml nutrient broth to which 5% serum was added. Positive result was detected by immediate production of air bubbles. Streptococci are considered as catalase-negative. Catalase positive samples were excluded.

Further examinations including different biochemical tests were done as described by Barrow and Felltham [8].

These tests included growth on 10% and 40% bile, bile solubility, and hippurate hydrolysis in addition to The CAMP reaction. Sensitivity to antimicrobial drugs was then done by the disc diffusion method.

Lancefield grouping was done by using commercial kits according to the manufacturer instruction (Remel Europe Ltd. Clipper Boulevard Kent, D A2 6 PT UK).

DNA extraction was done using saturated bacterial culture which was previously kept in a normal saline solution at freezing temperature. The protocol used was a modified protocol; often referred to as plasmid “mini-prep,” which yielded fairly clean DNA quickly and easily. Samples were centrifuged to pellet the bacterial cells then 0.2 ml of ice-cold solution one (50mM Glucose, 25mM Tris HCl and 10 mM EDTA) was added to each pellet and vortexed. Solution two (0.2 N NaOH and 1%SDS) was then added, mixed by inversion and incubated at room temperature for 5 minutes. Ice-cold solution three (5M acetic acid and 3M potassium acetate) was then added. Sample was vigorously shaken to release DNA and kept at room temperature for 10 min. Centrifugation and transfer of supernatant into fresh micro centrifuge tube using clean disposable transfer pipette was done. This fraction step separates the DNA from the cellular debris. The tube was then filled with Ice-cold Isopropanol which effectively precipitates nucleic acids. The tubes were centrifuged to precipitate the milky pallet of the DNA at bottom of the tube and the supernatant was poured off without dumping out the pallet. Then ice-cold 70% ethanol was added, centrifuged and the supernatant was poured off. Samples were dried then dissolved in double distilled water and kept at – 20 ºC.

PCR amplification was done using a genus- specific primer for streptococci (ST R1 5’- GTA CAG TTG

CTT CAG GAC GTA TC-3' and STR2 5'- ACG TTC GAT TTC ATC ACG TTG-3') (Vivantis Technologies). PCR reaction was performed using a touch-down program profile: 94 °C for 2 min as an initial denaturation followed by 14 cycles during which annealing temperature was 63°C, decreasing per cycle for 14 cycles until the annealing temperature 55 °C was reached. The program was then continued using final temperature as annealing step for additional 35 cycles of: 45 s at 94 °C, 45 s of annealing and 1 min of extension at 72 °C. Finally the reaction was ended with 5 min at 72 °C. The mixes contained 1 µl (10 mM) of each forward and reverse primer, 1 µl of 25 mM MgCl2, 1µl of 10 mM dNTPs, 1 unit of Tag DNA polymerase, 2.5µl of 10X PCR buffer (10 mM tris-HCL PH 8.3, 50 mM KCL), ~150 ng of DNA and the volume was completed to 25 µl by double distilled water. PCR products were subjected to gel electrophoreses using 2% agarose.

All bacterial work was conducted at the Department of Microbiology, Faculty of Veterinary Medicine; University of Khartoum. While all molecular part of the study was conducted at the Center of excellence, Department of Zoology, Faculty of Science, University of Khartoum.

Results

Ninety nine samples out of 125 samples showed Gram-positive cocci, but Gram- negative organisms were also present (8 samples). There was no growth at all in 18 samples. All gram-positive organisms showed catalase-positive reaction except four samples that showed catalase-negative reaction. Gram-negative organisms and catalase-positive reaction organisms were excluded.

The four samples that showed gram-positive staining reaction and catalase-negative reaction were subjected to different types of biochemical reactions including the type of haemolysis which was β haemolysis. Growth on 10% and 40% bile were negative. Hippurate hydrolysis was positive in the four samples

According to these results which were specific criteria of group B streptococci only, the four samples were grouped as group B streptococci.

Using Lancefield grouping kit, the organisms were group B streptococci as shown in Table1.

The sensitivity to antibiotics was slightly different. The isolates were found highly sensitive to Penicillin, sensitive to vancomycin, while they showed resistance to chloramphenicol, tetracycline, ciprofloxacin and gentamicinas shown in Table (2).

Table 1.Biochemical reactions of catalase-negative organisms

Type of reaction	No of organisms	Percentage
Growth on blood agar	107	85.6
No growth	18	14.4
Gram- positive	99	92.5
Gram-negative	8	7.5
Catalase negative	4	4.04
Catalase positive	95	95.96
Haemolysis	4 (β haemolysis)	100
CAMP reaction	4 (positive)	100
Growth on 10% bile	zero	zero
Growth on 40% bile	zero	zero
Hippurate	4 (positive)	100
Biochemical grouping	4 (group B)	100
Lancefield grouping	4 (group B)	100
PCR products	4 (positive)	100

Table 2. Sensitivity to antimicrobial drugs presented in crosses

Antibiotic	Sensitive	Resistant
Penicillin	xxxx	-
Vancomycin	xxx	-
Erythromycin	xx	x
Fusidic acid	x	xx
Cloxacillin	x	xx
Chloramphenicol	-	xxx
Tetracycline	-	xxx
Ciprofloxacin	-	xxxx
Gentamicin	-	xxxx

Discussion

In the last few decades Streptococcus agalactiae or Group B Streptococcus (GBS) has gained importance due to its ability to cause serious neonatal infections. In developed countries GBS is a leading cause of sepsis and meningitis in neonates.[4]

In this study we isolated GBS bacteria from 4 pregnant women out of the 125 studied cases (3.2%). Detection rate reached 4% when gram negative samples were excluded. In agreement with the recommendation of the Center of Disease Control (CDC) and Shirazi[5], our results indicated that a screening test for this group of bacteria during pregnancy is recommended. The results of the current study signify a strong need for further biochemical reactions to be done in addition to antimicrobial sensitivity. Detection rates although slightly less but are in agreement with Shirazi[5] who isolated the same type of bacteria with a ratio of 4.8% and Vijayan [3] who found that the colonization rate was 2.3%.

Maternal colonization by GBS was observed to range from 4% to 40% in several studies conducted worldwide[4]. Although the frequency of GBS colonization was less than the rates in nearby countries like Egypt (17%)[2];we recommend that the screening test for this group of bacteria is better done during pregnancy.

The lower rate of GBS colonization might be due to the fact that only lower vaginal swabs were collected instead of rectovaginal swabs (due to a collection mistake).Recto vaginal swabs could have increased the detection rates. GBS should be screened among pregnant women at 35 - 37 weeks of gestation as stated before by Elbaradie [2] and others.

According to the results of anti biogram obtained Penicillin can be used as a drug of choice for treatment of GBS.

Further studies are recommended to investigate the prevalence of GBS in pregnant women at different maternity hospitals by using recto-vaginal swabs as there was no work in Sudan before

Conclusion

As most samples of this study showed significant bacterial growth, other than GBS, there is a strong need for further screening tests including biochemical reactions to identify the type of bacteria other than Streptococcus agalactiae that colonizes or infects the pregnant women in this hospital. We recommend that further identification methods should be done to reach proper diagnosis of bacteria that colonizes the pregnant women at maternal hospitals. Detection methods should be adopted as routine work at this and other hospitals. Follow-up programs of the neonates of infected women or colonizers might be needed as recommended by the Center of Disease Control

Acknowledgement

The authors express their sincere appreciation to the administration and the staff of Saudi Maternal Hospital. Thanks are also extended to the Matron and sisters of the hospital who assisted in collecting the samples. Prof. A. A. G. is also appreciated for helping in the correction of this manuscript.

References

1. Maria Jevitz Patterson. Streptococcus In: Medical Microbiology 4th edition. Edited by Samuel Baron University of Texas Medical branch at Galveston 1996.

2. Elbaradie S. M.Y., Mahmoud M. and Farid M. Maternal and neonatal screening for Group B streptococci by *SCP* B gene based PCR. : A preliminary study. *Indian journal of medical microbiology* 2009; **27**: 17-21.

3. Vijayan S. , Noyal M. J. , Thirunavukkarasu A. B. , Latha C. and Sujatha S. Genital tract group B streptococcal colonization in pregnant women: a South Indian perspective. *J Infect Dev Ctries* 2011; **5**:592-595.

4. Jannati E. M., ArzanlouM., HabibzadehS., Rahimi G. and Shapuri R. Capsular serotype and antibiotic resistance of group B streptococci isolated from pregnant women in Ardabil, Iran. *Iran J Microbiol.*2012; **4**: 130–135.

5. Shirazi M. , Abbariki E. , Hafizi A. , Shahbazi F, Bandari M. and Dastgerdy E. Prevalence of Group B Streptococcus Colonization in Iranian Pregnant Women and Its Subsequent Outcome. *Int J Fertil Steril.* 2014; **7**: 267–270.

6. Barrow, G. I. and Felltham, R. K. A. Cowan and Steel's Manual for identification of medical bacteria, 6thedition. 2003.

Case report

Severe necrotizing pneumonia associated with Sheisha Smoking in a 14 year old child

Mariam Z Mubarak[1*], Hayfa O M Elhassan[1,] Fatma M A Abonora[2], wifag AE Adam[2], Osama O Elamin[2]

[1]*Khartoum University, Department of Pediatrics.*

[2]*Ahmed Gasim Pediatric Specialised Hospital .*

Introduction: Necrotizing pneumonia is a rare complication of bacterial lung infection .Its cause is due to either a virulence factor of the microorganism or predisposing factor of the host. An association between necrotizing pneumonia and Panton –Valentine Leucocidin (PVL) –secreting S.aureus was first recognized in 1999 (1) .Few years later clinical features of the disease was described by Gillet et al (2) .Risk factors were then well -recognized by 2007 (3) The disease causes a devastating complications such as diffuse pulmonary inflammation ,septic shock and respiratory failure .Staphylococcus aureus strains that produce Panton –Valentine leukocidin have been reported to cause rapidly progressive necrosis of the lung tissue in young immunocompitent patients .Furthermore, recent studies have shown the risk of disease progression is associated with underlying medical conditions (4)

Staphylococcus is related to the Micrococcaceae family , *Staphylococcus*- derived from Greek "staphyle" (bunch of grapes). Virulence factors of *S.* aureus includes enzymes : catalase (counters host defences) ;coagulase ,;hyaluronidase ;lipases; B lactasamase (antibiotic resistance).Toxins **:** enterotoxin, TSST and epidermolytic toxin.The effects of smoking on staphylococcus virulence was studied among cigarette smokers. Methicillin-resistant Staphylococcus aureus (MRSA)colonizes the nasopharynx and is thus exposed to inhalants .MRSA exposed to cigarette smoke extract (CSE-MRSA) was more resistant to macrophage killing (4 fold)It also demonstrated a reduced susceptibility to cell lysis and modification of the surface charge of MRSA and eventually impairing the binding of particles with charge similar to that of AMPs by 90% and increased adherence and invasion of epithelial cells (5) .

***Corresponding author:** Dept. of Pediatrics , Khartoum University, Faculty of Medicine. Email :mariam555za@gmail.com.*

Case Report

An adolescent boy aged 14 years from Algazira Province (Halaween) presented to the ER in Ahmed Gasim Pediatric Specialized Hospital in April 2016 with cough , shortness of breathing ,fever and myalgia for 5 days. Cough was productive of whitish sputum occasionally streaked with blood Two days later he developed frank haemoptysis .The shortness of breathing was severe enough to prevent the child from eating and speech ,associated with paroxysmal nocturnal dyspnea .Fever was high grade accompanied with rigors .The patient's general condition worsened to such an extent that he couldn't perform daily activities and moving on wheelchair to bathroom . Systemic review was unrevealing apart from epigastric pain for the last 2 weeks for which he received full treatment for H.pylori in Algazira without improvement .The family denied any sort of active or passive smoking. However, the patient later informed us that he used to smoke sheisha occasionally with his grandfather for one year and then smoked sheisha for few days secretly during a wedding party in Khartoum . The patient is a member of a big loving extended family and was brought to hospital by his grandfather and

the whole family.

He has been healthy prior to admission and had no notable history of allergy or respiratory disease .No mention of contact with patient of chronic cough .He was fully vaccinated .

Examination showed a well thriving adolescent boy with clear consciousness , good physique. He looked sick, in severe respiratory distress ,on oxygen via nasal prongs .Pulse 132/min ,regular ,BP 105/65, RR 72 ,Temp 38.3 .The conjunctivae were pale , the sclerae showed no signs of jaundice and he was not cyanosed .No cervical lymph nodes were palpable . Trachea has been felt normally deviated to the right side . Normally localized apex beat .Chest moved less on right side and an increased tactile vocal fremitus was evident on right middle zone anteriorly . Percussion note was impaired on the same region. Poor air entry was evident on both sides , bronchial breathing auscultated in right upper and middle zones anteriorly and posteriorly and scattered wheezes and crepitations were heard all over the chest.

An enlarged, but not tender, liver of 8 cm below costal margin was palpable .Rest of systemic examination was within normal

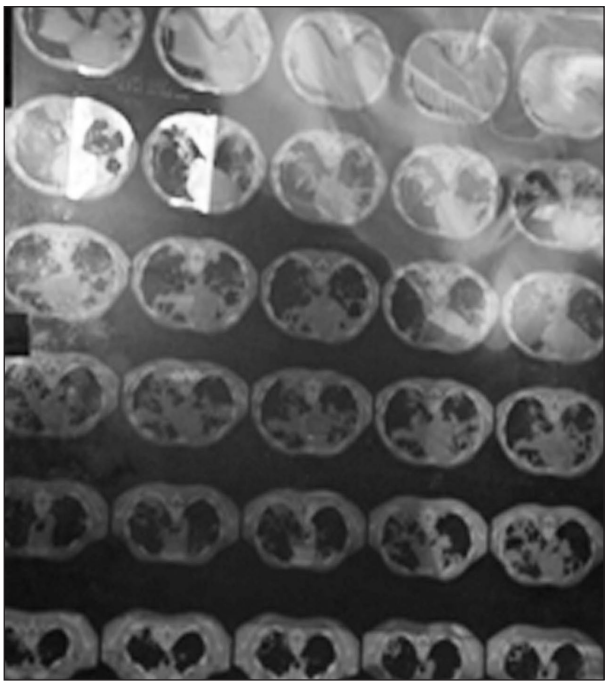
On admission routine blood tests revealed : white blood cells 53.9100/micL ,NEUT% =95.41% ; HGB =12.5g/dL ;HCT =35.5% (44-70)Low ,MCV =86.2fl(102-115)Low MCH=30.3pg (33-39)Low MCHC=35.2g/dl ,PLT=224.000/micL. ESR =70mm/hr .HBG dropped to 8.5 grams after 4 days .Blood biochemical tests showed total protein 8.1g/dL ; albumin , 3.5g/dL ; Globuline 4.6 g/dL ; serum aspartate aminotransferase 17IU/L ; alanine aminotranfserase 11 IU/L ; alkaline phoshpatase 201 IU/L ; C-reactive protein > 24mg/dL; total bilirubin 0.4 mg/dL ; direct bilirubin 0.2 mg/dL blood urea 104mg/dL ; creatinine 1.4 mg/dL ;serum sodium 140 mmol/L and serum potassium 3.9mmol/L .

Sputum for culture and sensitivity revealed Staphylococcus aureus ,sensitive to vancoycin and drugs were selected accordingly. Sputum was negative for ZN stain for 3 consecutive days and serology for HIV was negative .

Figure (1)

Chest x- ray showed cavitations mainly on right side and upper zones with remarkable patchy consolidation on right middle and left lower zones also pleural effusion was noted on right side **(Figure 1).**

Computerized tomography of chest showed multiple bilateral thick wall cavities with multiple pulmonary nodules , areas of consolidations with air bronchogram and minimal bilateral effusions



(Figure 2)

Impression was suppurative lung disease. Abdominal U/S as normal apart from an enlarged liver with no focal lesions.

In ER diagnosis of severe bronchopneumonia was considered and patient was started on Ceftriaxone , Azithromycin and Metronidazole intravenously . Salbutamol and ipratrpium bromide nebulizations were deliverd to the child .

After C/S results ,the patient was started on intravenous Ceftriaxone and vancomycin according to the consultation of the pediatric pulmonologist in Soba Hospital . Intravenous fluids in the form of (D5/0.45 NS)were restricted to 0.6% of the maintenance . Nebulized salbutamol and ipratropium bromide continued as needed .The patient received 2 units of packed RBCs .Antibiotics continued for 3 weeks and the patient showed dramatic improvement in terms of general condition , better appetite , . Follow up chest x–ray clearly showed improved aeration and less cavitations.

Firgure(3)

Patient and family were counseled for the issue of smoking and the patient was followed for 3 month in the outpatient clinic .

Discussion

The presentation of this adolescent boy mainly favors an aggressive form of bronchopneumonia characterized by severe disease , hypoxia and persistent systemic manifestations that cannot be explained in a well thriving ,previously healthy child .

Clinical findings supported the diagnosis of severe lung injury . Cavitations and necrotic changes were quite evident on both chest –X-ray and CT chest .Moreover , the significant anemia and hypoalbuminemia are characteristic features of necrotizing pneumonia .

Sheisha smokers are at the same risk of diseases cigarette smokers face including lung and stomach cancer ,reduced lung function

Staphylococcus aureus is estimated to cause 1-10% of community- acquired pneumonias (CAP)and 20-50 % of nosocomial pneumonias with high morbidity and mortality . Underlying risk factors and co- morbidities were evident in nearly half of the patients [6,7,8].Panton –Valentine Leucocidin is known to play an important role in the pathogenesis of necrotizing pneumonia .It forms pores in the cell and mitochondrial membrane of neutrophils and macrophages provoking cell lysis ,apoptosis and subsequent liberation of inflammatory mediators[9,10,11] .

Treatment is mainly by antibiotics based on cultures and sensitivity .Patients who fail to respond may need lung resection as an alternative treatment option .Disease outcome is governed by degree of disease progression and co-morbidities[12].

Morbidity and mortality of necrotizing pneumonia caused by Staph .aureus is based on series and case reports .Typically ,the patient will present with an influenza- like prodrome which progresses to septic shock and respiratory failure .In the context of multiloblar consolidation ,pleural effusion and airway hemorrhage ,pleural effusions are considered predictive of fatal outcome [3].Published mortality rates vary between 40% and 60%.

Sheishas are water pipes used to smoke specially made tobacco that is usually flavored .They are also called a number of different names including narjiles ,shisha goza & hooka . Smoking is typically practised in groups , with the same mouth piece passed from mouth to mouth which was thought to increase both the risk of infection and the virulence of the organism .Similar to cigarettes, sheisha smoking delivers the addictive drug nicotine and it is at least as toxic as cigarette smoking .While many sheisha smokers may consider this practice less harmful than smoking cigarettes, Sheisha smoking carries many of the health risks as cigarettes[12].

Though some people believe the myth that because sheisha employs a water bowl ,it makes it safer by

drawing the smoke through the water , a new study unveils some shocking facts about how harmful sheisha smoking is. It stated that one sheisha session delivers 125 times the smoke , 10 times the carbon monoxide ,25 times the tar and 2.5 times the nicotine of a single cigarette and reduced fertility [13]. Another study investigated 21 people using sheisha for less than 5 year .Investigators took samples from the airways of the study group using a fine brush through a lung tube to gather the cells. Changes were noted in the epithelium of lung tissue .The study also reported higher carbon monoxide levels in sheisha smokers and they concluded that sheisha pipe seemed to expose the user to 7-11 times more carbon monoxide compared to one cigarette [13,14].

Sheisha users reported more coughing ,bringing-up sputum and lower scores on lung function tests[14].

Smoking can induce acute oesinophilic pneumonia causing acute respiratory distress syndrome- like illness . The current diagnostic criteria are as follows : fever ,acute respiratory symptoms ,severe hypoxia and bilateral infiltrates on chest x –ray[15,16].

Recommendations

Sheisha **is** similar to cigarettes. Besides delivering the addictive drug nicotine, it carries the same health risks as cigarettes .In recent years ,there has been an increase in sheisha use around the world, most notably among youth and students with higher rates among boys than girls .Important policies to be adopted by health authorities include : education of health professionals , regulators and the public about the risks of sheisha smoking including high potential levels of second hand exposure among children .Also false claims of harm reduction and safety should be prohibited . Sheishas should be prohibited in places consistent with bans on cigarette and other forms of tobacco smoking .

Misleading labeling ,such as (contains 0 MG tar) , which may imply safety , should be prohibited.

References

1-Lina G, Peimont Y ,Godail-Gamot F, et al . Involvement of Panton –Valentine Leucocidin producing Staphylococcus aureus in Primary Skin Infections and Pneumonia .*Clin Inect Dis* 1999; 29 : 1128-1132.

2-Gillet Y ,Issartel B,Vanhems P, et al . Association between Staphylococcus aureus strains carrying gene for Panton –Valentine Leucocidin and highly lethal necrotizing pneumonia in young immunocompetent patients .*Lancet* 2002 ;359:753-759 .

3- Gillet Y , Vanhems P , Lina G , et al . Factors predicting mortality in necrotizing community-acquired Pneumonia caused by Staphylococcus aureus containing Panton –Valentine Leucocidin .*Clin Infect Dis* 2007 ;45:315 -321.

4-Tsai YF ,K uYH . Necrotizing Pneumonia : a rare complication of pneumonia requiring special considerations .*Curr Opin Pulm Med* 2012 ;18 :246-52 .

5-Don Rauf : Even light hookah use may cause airway problems , American Journal of Respiratory and Critical Care Medicine .Updated April 14 .2016 11:36 PM AST./U.S Centers for Disease Control and Prevention .

6 -Howards LS ,Sillis M ,Pasteur MC ,Kamath AV ,Harrison BD . Microbiological Profile of community acquired pneumonia in adults over the last 20 years .*J Infect* 2005 ; 107 :1013-1016 .

7 -Chichering HT ,Park JH, Jr: Staphylococcus aureus Pneumonia .*JAA* 1919; 72:617-626.

8- Kallen AJ ,Bruncard J ,Moore Z et al . Staphylococcus aureus community acquired Pneumonia during the 2006-2007 influenza season . *JA nnEmergMmed.2008* ; 365:101-116.

9-Miles G , Movileanu L ,Bayley H : Subunit composition of a bicomponent toxin : Staphylococcal Leucocidin form an octameric

transembrane pore Protein . *Sci* 2002 ; 11:894-902.

10 –Bubeck w J , Palazzolo –Ballance AM, Otto m, Sckneewind O ,DeLeo FR , : Panton –Valentine Leucocidin is not a virulence determinant in murine models of community associated methicillin resistant staphylococcus -aureus disease .*J Infect Dis*2008 ; 198:1166-1170.

11 – Olsen RJ , Kobayashi SD , Ayeras AA et al : Lack of a major role of staphylococcus –aureus Panton –Valentine Leucocidin in lower respiratory tract infection in nonhuman primates .*Am J Path* 2010 ; 176:1346-1353.

12 -Lukas K , Emmanuel C ,Phillips E :Community –acquired necrotizing pneumonia due to methicillin sensitive staphylococcus aureus secreting Panton –Valentine Leucocidin : a review of case reports .*Annu Intens car* 2011 ;1:52.

13- Centers for Disease Control and Prevention(CDC): Dangers of Hookah Smoking ,2012 (http:// www.cdc.gov/pcd/ issues/2012/120082htm) : Page last reviewed : November 9,2015.Page last updated November 9 ,2015.

14- Marie Ellis : One hookah session delivers 125 times the smoke of one cigarette. Published : 2016 .http:// www.medicalnewstoday.co/ articles/305137.php.

15- Ji -Seok Youn, Ji-won Kwon,Byoung –Ju Kwimand Soo –Jong Hong : Smoke- Induced Acute Oesinophilic Pneumonia in a 15 year old girl : A case report .*Allergy Asthma Immunol Res* 2010 ; 2:144-148.

16-McEachem EK, Huang JH ,Sladewski KM .DP: Analysis o the Effect of Cigarette smoke on staphylococcal virulence phenotypes .*Infect Immun*2015 ; 83: 2443-52.

Instructions to Authors

Authors are advised to read these instructions carefully. Adhering to the format of the journal guidelines will facilitate and limit the time needed for the processing of the paper.

Types of papers

Please specify the type of paper submitted for publication. The journal accepts the following categories: original articles, short communications, case reports, review articles, letter to the editor, medical news and quiz cases relevant to medical education.

Covering letters

1. Should specify the type of paper according to the first paragraph of this document.
2. If the authors wish, they can include in the covering letter information on related publications.
3. All authors should sign the covering letter.
4. Address all correspondence to:-

   The Editor, Khartoum Medical Journal, P.O.Box 102, Khartoum, Sudan.

   E.mail:kmj @meduofk.net

   E-mail:khartoummedicalj@gmail.com

Copyright

Submission of original articles for publication is an undertaking by the author/s that:-

1. The manuscript is not under consideration for publication elsewhere.
2. The manuscript is original, truthful and free of fabrication, fraud or plagiarism.
3. All authors have read the manuscript, agree to its contents and share in the responsibility of its publication.
4. All authors have made a substantial contribution to the work submitted e.g. conception and design, experimental work or clinical studies, analysis and interpretation of data, drafting and critical editing. Contributions such as obtaining material or other support does not justify authorship.
5. All funding and support for the work should be acknowledged.
6. Any part of the manuscript not owned by the authors requires that permission should be obtained by the authors from the owner of the copy right.
7. All papers published by the journal will be KMJ copyright.
8. Please also supply information or related papers in press or submitted for publication elsewhere.

The manuscript

1. Use of English Language according to Oxford English Dictionary style.
2. Formatting the manuscript: should be typed, double spaced, with margins not less than 3 cm.
3. The title should not be more than 100 characters and spaces.
4. The abstract should not be more than 250 words presented as follows: objectives or background – about 50 words, methods about 60 words, results about 60 words and conclusion about 60 words.
5. System of international units should be used. Equivalents may be given in parenthesis. Symbol and abbreviations: A Guide for Biological and Medical Editors and Authors, 5th Edition, 'London, Royal Society of Medicine Press 1999'.
6. Tables should be on separate pages.
7. Legends for tables and figures should be submitted separately.
8. Please supply two hard copies of manuscript, tables and figures as well as a digital copy which may be sent through the e-mail.

Illustrations

1. Illustrations should be kept to the minimum. Illustrations in color are acceptable; however, an extra charge may be required to be paid by authors.
2. Care should be taken that illustrative material may have to be reduced in size to fit pages or columns. It is recommended that the size of figures to be about 12.5x20 cm.
3. All illustrations should be numbered on the reverse side and the tope of the figure indicated.

4. Graphics should be clear, camera-ready and all symbols explanations included on the figure or in the legend.
5. Permission to reproduce illustrations or tables should be obtained by the authors and submitted with the manuscript.

Statistical analysis

1. Statistical methods used should be clearly identified and if necessary described.
2. Means and standard errors of the mean and P values should be given to two decimal places.

References

1. Please use the Vancouver Style as shown below.
2. References should be listed numerically by order of their appearance in the text.

The Vancouver Style of Reference Formatting

With the growth of medical knowledge and research, it had become necessary that the formatting of reference citation both within the text of scientific writing and in reference lists should be widely agreed. The first steps to establish a uniform system for formatting manuscripts and references were begun by the Conference of Biological Editors in 1960. The International Committee of Medical Journal Editors (CMJE) held a meeting in Canada in 1979 to launch a uniform style of reference formatting for medical journals and proposed the Vancouver Style. Since then the major medical journals have adopted the 'Uniform requirements for manuscripts submitted to biomedical journals'[(1)], a common style for presentation of papers for publication.

- The justification of an internationally accepted style of reference citation can be summarized as follows:-
- Correct and complete referencing of scientific and medical publications is an essential component of the 'scientific method' when recording the outcome of research.
- To facilitate formatting scientific papers for more efficient peer reviews and publications.
- An unambiguous system of referencing allows other researchers and reviewers of manuscripts to access the cited literature to validate claims and arguments.
- To successfully secure research funding, the research proposal including the existing literature on which it is based should be convincing and easily accessed by reviewers.
- Uniform and complete citation formats facilitates quotation and reference compilation for researchers and postgraduate students.

The following is a summary to supplement the Instructions to Authors for referencing of manuscripts submitted to KMJ. It is based on the Vancouver Style and is the preferred referencing format for writing of dissertations, theses and other referenced writing in the Faculty of Medicine, University of Khartoum:-

1. References should be numbered consecutively throughout the text in the order in which they appear.
2. No references should be included in the abstract.
3. Identify references in the text, tables and legends by numerals in parenthesis e.g. (1), (2,3) or (3-6).
4. When citing authors in the text, acknowledge only the first author where there are three or more authors, e.g. Smith et al (1998) stated that ….(1).
5. Where there are two authors cite both, e.g. Adam and Ehsan (2003) reported that ….(2). Note that numerals in parenthesis at the end of a sentence are written before the full stop.
6. The list of references should begin on a new page and given the numbers which indicate order of citation.
7. All authors should appear in the list of references i.e. all references are listed in full.
8. Where more than 6 authors are registered, write the first 3 authors followed by et al.
9. The order of author/s initials, punctuation, title of article, year, journal title – in accepted abbreviated form, volume and page numbers, constitute a full reference citation. The following are examples

of commonly used reference sources:

Reference in journals

General format including punctuation,

Author/s, title of article, title of journal (in italics with no full stops), year; volume number: page numbers.

e.g. Rose ME, Huerbin MB, Melick J, JK et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res 2002; 935: 40-6.

References in books

Author(s) of a book

General format including punctuation.

Author(s) Title: sub-title. Edition. Place of publication: Publisher; Year

e.g. Guyton AC, Hall JE. Textbook of Medical Physiology. 10th Ed. Philadelphia: Saunders; 1990.

Author(s) of a chapter in a book

General format including punctuation

Author(s) of the chapter. Title: sub-title of chapter. In: Author(s) (or editors) of the book. Title: sub-title of book. Place of publication: Publisher; Year; page numbers.

Elmunshid HA. Special senses. In: Sukkar MY, Elmunshid HA, Ardawi MS, editors. Concise Human Physiology 2nd Edn. Oxford: Blackwell Science; 2000.p.401-23.

Reference on-line

Example (from The Michener Institute for Applied Health Sciences, Learning Resource Centre: Irc@michenere.ca).

Book on the Internet

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/o309074029/html/.

Internet homepage/website

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org.

For a fuller range of examples of citation from other sources of references, there are innumerable sites on the internet. Please also consult the publications cited in KMJ instructions to authors and the references cited below:-

1. Uniform requirements for manuscripts submitted to biomedical journals: writing and editing for biomedical publication [home-page on the Internet]. Philadelphia, PA: International Committee of Medical Journal Editors; [updated 2003 Nov; cited 2004 Oct 9]. Available from: http://www.icmje.org/.
2. Style manual for authors, editors and printers. 6th Ed. Milton, Qld: John Wiley & Sons; 2002.