The burden of infections and child health in Iraq

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Series of sanctions and prolonged wars have brought enormous calamities in Iraq. In a country where a major bulk of population are children, massive deterioration in physical, mental and social health has been witnessed during and after the conflicts in Iraq. With respect to the increasing burden of infections that have incapacitated children and their lives, the state of nutrition and unavailability of safe environment were thoroughly assessed in this article. Due to the recent deterioration of child health situation in Iraq recommendations are given to focus on the most crucial issues of child health.

Health of Iraqi children has been a matter of growing concern. Decade long sanctions imposed by the United Nations (UN) in 1991 and series of wars have brought the country to a devastating state. Classifying countries on the basis of various development indicators United Nations International Childrens Fund (UNICEF) report on the State of the World’s Children (2003) have ranked Iraq on the 33rd position for under 5 mortality rate. The infrastructure of Iraqi health system has become profoundly fragile. The reports further prove that Iraq's progress in child survival has been the worst among 201 countries in the world. It was worse than even Botswana and Zimbabwe, which have an adult prevalence of human immuno-deficiency virus close to 40%. The main cause of increase in infant, child and maternal mortality can be attributed to rising poverty the breakdown of basic services, of water and sewage disposal, poor sanitation and deterioration of health services, in terms of lack of drugs, equipment and quality. Children are dying of commonly preventable diseases such as diarrhea, cholera and from acute respiratory infections, malnutrition and other debilitating diseases. The article basically focuses on child health situation with respect to the previous and ongoing conflict in Iraq. With respect to 3 parameters like health development, burden of infections and nutrition an assessment is made that provides clues for emergency interventions.

Several aspects of child health in Iraq are still unknown, but various reports have shown a grim picture in the recent times. Bombing in the recent past has destroyed water and sanitation are at risk of sabotage of various factions across the country. Vital medicines have been robbed, and health facilities damaged after the fall of Saddam regimen. Health facilities cannot perform diagnostic tests as some reagents are missing. Shortages of vaccines and tuberculosis drugs are going to run short very soon. Public health and hygiene are constantly on the threat. The UN estimates that 5,000,000 Iraqis do not have access to safe water and sanitation. Combined with the high temperatures, the burden of diseases has been dramatically increased. The risk of infection is more than ever in the vulnerable communities. Knowing the fact that Tigris and Euphrates water serve main sources of drinking water, hundreds of thousands of tons of raw sewage are pumped into the these rivers. Supplies of water cleaning chemicals have been stolen or destroyed. Looting are piercing water pipes for commercial use; as a result, the quality of water being pumped into homes is extremely poor and proliferate fatal illnesses among children. Nearly 3 quarters of the children surveyed in Baghdad in the assessment had at least one bout of diarrhea over the previous month. Comparing the results with earlier findings it becomes clear that children who have generally grown over the past few years as of improved nutrition have suddenly and dramatically wasted. This coincides with war and the breakdown of social services. It might not be conclusive, but it does suggest that the shift of children into the acutely malnourished category is recent.

Reports show that one child in every 8 in Iraq dies before the age of 5 (Figure 1). As a result, The Infant Mortality Rate (IMR) increased 2.5 times during the last decade, under 5 mortality rate doubled and maternal mortality ratio, which is closely related to child survival, has tripled. The World Health Organization (WHO) is also extremely concerned regarding the psychological impact of conflict, fear, and the loss of family members or neighbors on Iraqi children. The physical and psychological damage of conflict could take years to heal, and is likely to leave many permanent scars.

The 3 biggest child killer diseases, for example acute respiratory infection, diarrheal diseases and measles are widely reported. Preventive measures
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Table 1 – Nutritional profile of Iraqi children (United Nations International Childrens Fund, 2003).

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<td>25</td>
<td>16</td>
<td>22</td>
<td>37</td>
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against the killer diseases in Iraq are abysmally poor. The burden of diseases during and after the war has gone beyond the limits of control efforts. In a WHO report 17 cases of acute watery diarrheal syndrome (8 May 2003) have been notified in 2 hospitals in Basra. Seven cases of clinically confirmed cholera were reported, mainly among very young children (between 13 months and 4 years old) and currently more than 30 admissions per day for diarrheal disease is causing a big concern. Out of 200 outpatients a day, 90% are for diarrhea; others are diagnosed with hepatitis, acute respiratory infections, malnutrition, shigella and typhoid. Surveillance and infection activities have been currently arrested. Any epidemic of serious impact cannot be ruled out if urgent actions are not taken. Several cholera outbreaks were reported in June through to August 2002. After the Gulf War outbreaks of diarrheal diseases and Cholera became endemic in all governorates of Iraq. In refugee and internally displaced persons’ camps during (and after) previous wars in Iraq, diarrheal diseases accounted for between 25% and 40% of deaths in the acute phase of the emergency. Eight percent of these deaths occurred in children under 2-years-old. Pertussis (whooping cough) incidence is on the March and more cases of diphtheria have been reported. In 1999 Iraq suffered a major outbreak of polio. Tuberculosis rates have risen significantly in the last decade. The number of new cases of tuberculosis nearly tripled from 46.1 per 100,000 people in 1989 to an estimated 131.6 per 100,000 people in 2000. A serious malaria outbreak (vivax strain) occurred with a peak of 100,000 cases per year in 1994 and 1995. The outbreak has been attributed to movement of people from endemic into malaria-free zones, delays in access to effective treatment and a lack of effective control measures.2

If robust actions are not taken to control the burden of infections, the consequences for child health will be enormous. The World Health Organization estimates that if 10,000 Iraqi people are unable to access health care for one month, at least 30 children with diarrhea will not be treated, 55 children with respiratory infections will go untreated and 5 children with pneumonia will not receive life-saving antibiotics. In the longer term, disruption of surveillance for monitoring disease in the general population, breakdown of public health programs, damage to health facilities, and malfunction of water and sanitation systems will lead to increased levels of illnesses and higher death rates. The incidence of community oriented, and vaccine-preventable infections will increase. New disease patterns and outbreaks of communicable diseases including measles, meningococcal meningitis, pertussis and diphtheria can be expected.2,4

An assessment undertaken by UN agencies in 2000 revealed a high prevalence of anemia in school children. Numerous cases of rickets (vitamin D deficiency) were also reported. Reports provided by the Iraqi Ministry of Health, 2001 documented 31,545 cases of kwashiorkor, 291,587 cases of marasmus (swelling of limbs and body) and 1,977,454 cases of other protein, calorie and vitamin malnutrition in children under 5 years.2-4 Approximately 22-25 million people live in Iraq of which 13,000,000 are children. Sixteen million Iraqi civilians are completely dependent on government-distributed food rations. One million or one-third of Iraqi children suffers from malnutrition.3 The child nutritional status in Iraq has also deteriorated rapidly from 1991-1996 and has shown some decline recently (Table 1).

United Nations International Childrens Fund have released troubling findings from a rapid nutrition assessment undertaken in Baghdad, which has found that acute malnutrition rates in children under 5 have nearly doubled in Baghdad since a previous survey in February 2002. Nevertheless, it shows that 7.7% of children under 5-years-old are suffering from acute malnutrition, compared with last year’s figure of 4%. Acute malnutrition signifies that a child is actually wasting away. The survey found that more than 1 in 10 children were in need of treatment for dehydration.1,4

The country suffers from frequent shortage of supplies including vaccines due to the procurement problems related to the Oil For Food Program. There is a lack of motivation among governorate staff at all levels, due to low salaries, and the very small public expenditure budgets of local authorities. This is compounded by a shortage of qualified staff, as many moved abroad or into the private sector. The distribution of infant milk formula in the food basket continues to jeopardize the promotion of exclusive breastfeeding and puts infants at higher risk of intestinal infections and contamination, which contribute directly to malnutrition. The recent improvement in the situation of children can be easily reversed as a large proportion of the Iraqi population is fully dependant on food rations and the public health
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Care system. The damage to the infrastructure and disruption in services will further contribute to the deterioration of the situation.

Health is a fundamental right of children. With half of the population children represent the future of the Iraq. Provision of health care and protection for the most vulnerable population has become an absolute priority. There is a need for concerted and prompt action by the international community to help safeguard children. Based on the population needs the goal should not only be an urgent, co-ordinated, flexible, effective intervention but should also reflect the interest and welfare of Iraqi children. Due to the recent outbreaks WHO has already set up a surveillance system which is conducting a survey of infectious diseases, and has established an outbreak committee that is implementing control interventions.¹² Still robust efforts in the provision of major health services and for improving of child health are widely lacking. In line with the local policies and strategies, best child health care practices need to be adopted across the country. The most pressing health-related actions will be ensuring of adequate, safe drinking water and access to sanitation, providing medical supplies and treatment for children affected by infections; trauma and other war related injuries. Building a safe environment, prevention of diseases outbreaks, and making sure that adequate stocks of essential drugs are available and functional health facilities are in hand to provide coverage to the population.

References


The oral hygiene habits of school students in Riyadh, Saudi Arabia

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People’s oral health behavior is important for the prevention and care of oral diseases. Their views of being able to cope with oral health behavior relate to actual tooth brushing, inter dental cleaning and dental visiting. The oral hygiene habits of a particular population depends upon its cultural background, religious norms, awareness of the problems that a lack of hygiene causes, knowledge of the existence of particular cleaning tools, education levels and socio-economic status. Today the toothbrush has become a necessity, and no conscientious person in the Western world and in some parts of the developing world can think of spending a day without the involvement of a toothbrush. In the developing world, various plants are used for oral hygiene purposes. In the Kingdom of Saudi Arabia (KSA), a study on school children revealed that 83% used a toothbrush while 16% used miswak.¹ Another study on secondary school students from Riyadh, KSA confirmed that 10% of non-smoker students never brushed their teeth.²

The aim of this study was to find out the prevalence and frequency of oral hygiene habits among intermediate and secondary school male and female students from Riyadh, KSA.

The study was carried out on intermediate and secondary school students (male, female) from Riyadh, KSA, over a period of 2 months, using stratified cluster sampling technique. A questionnaire was developed and used in Arabic language having 15 questions. The questionnaire was tested before embarking on the study. The questionnaire was distributed to 2000 students (1000 male and 1000 female).

The data was entered by a Fox Pro Program and analyzed by using statistical package for social sciences version 10. The data was analyzed for frequency distributions and Chi-square test for comparisons. The p value was set 0.05%. One thousand seven hundred questionnaires were returned. Fifteen hundred and ninety-six questionnaires were accepted for the analyses giving response rate of 80%. A total of 82% male (n=820) and 77.6% female (n=776) respondents were within the age range 12-20 years (mean age 15.39 and SD ± 2.08).

Among intermediate school students (age 12-15 years) 7% of male and 3% of female and in secondary schools 14% of male and 3.5% of female students never cleaned their teeth. The daily oral hygiene habit

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Better cleaning was given the most common reason of using both miswak and toothbrush by both male and female students.

A recent consensus statement on oral hygiene concluded that bacterial plaque plays an important role in the etiology of dental caries, gingivitis and periodontitis; that effective removal of dental plaque can result in the prevention or reduction of these diseases. The present study was conducted to look into 2 aspects of oral hygiene habits among school students, firstly to assess the prevalence of oral hygiene habits and secondly, the frequency of these hygiene habits and then comparing among male and female students from intermediate and secondary schools in Riyadh, KSA. Though questionnaire approach is acceptable but it has inherent limitations regarding over or under estimation of the investigation, in the absence of clinical examination of the subjects. In this study, the valid response rate of 80% shows keen interest of the students in their oral health matters. The oral hygiene habits of schools students in Riyadh, Saudi Arabia was prevalent among 19% male and 20% female students in intermediate and 25.4% male and 19% female students in secondary schools. A toothbrush was most commonly used by both male and female students at intermediate (28.9% male, 44.5% female) and at secondary school (28.8% male and 63.7% female). The use of miswak was less prevalent as compared to brush and was used by almost 24% of secondary school male students (Table 1).

Table 1 - Frequency of methods used for cleaning teeth.

<table>
<thead>
<tr>
<th>Methods used</th>
<th>Age 12-15 Male n (%)</th>
<th>Female n (%)</th>
<th>Age 16-20 Male n (%)</th>
<th>Female n (%)</th>
<th>p value (Sig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toothbrush</td>
<td>106 (28.9)</td>
<td>167 (44.5)</td>
<td>130 (28.8)</td>
<td>254 (63.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Miswak</td>
<td>35 (9.5)</td>
<td>14 (3.7)</td>
<td>108 (23.9)</td>
<td>11 (2.8)</td>
<td></td>
</tr>
<tr>
<td>Toothbrush and Miswak</td>
<td>219 (59.7)</td>
<td>183 (48.8)</td>
<td>199 (44.1)</td>
<td>130 (32.6)</td>
<td></td>
</tr>
<tr>
<td>Finger</td>
<td>4 (1.1)</td>
<td>6 (1.6)</td>
<td>10 (2.2)</td>
<td>2 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3 (0.8)</td>
<td>5 (1.3)</td>
<td>4 (0.9)</td>
<td>2 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>367 (100)</td>
<td>375 (100)</td>
<td>451 (100)</td>
<td>399 (100)</td>
<td></td>
</tr>
</tbody>
</table>

Horizontal technique among miswak user was common among male and vertical among female intermediate school students and at secondary school unspecific technique of miswak was common. Majority of the students used miswak more than 3 minutes per day. Horizontal technique among miswak user was common among male and vertical among female intermediate school students and at secondary school unspecific technique of miswak was common. Majority of the students 58.5-63% used old miswak. Most of the students preferred using toothbrush due to their perception of more cleaning effect by toothbrush, ranging from 46-58%. Sunnah (Prophet Muhammed’s (PBUH) way of Practice) was given the most common reason of using miswak by 58% male and 61.5% female intermediate school and 53% male and 47.2% female secondary school students. Better cleaning was given the most common reason of using both miswak and toothbrush by both male and female students.

A recent consensus statement on oral hygiene concluded that bacterial plaque plays an important role in the etiology of dental caries, gingivitis and periodontitis; that effective removal of dental plaque can result in the prevention or reduction of these diseases. The present study was conducted to look into 2 aspects of oral hygiene habits among school students, firstly to assess the prevalence of oral hygiene habits and secondly, the frequency of these hygiene habits and then comparing among male and female students from intermediate and secondary schools in Riyadh, KSA. Though questionnaire approach is acceptable but it has inherent limitations regarding over or under estimation of the investigation, in the absence of clinical examination of the subjects. In this study, the valid response rate of 80% shows keen interest of the students in their oral health matters. The prevalence of oral hygiene habits was interesting as 7% of male in intermediate and 14% in secondary schools never brushed their teeth while among female students this corresponded to 2.7-3.5%. That shows the increase in neglecting oral hygiene as higher age group is concerned. The frequency of daily brushing ranged from 19-25%. The trend shows that daily practice of oral hygiene method was decreased in secondary school female students as compared to male students. This trend is in agreement with a study of primary school children in Jubail. Toothbrush in combination with miswak was most commonly used in both male and female groups of students. Only toothbrush was used by 64% of the female students from secondary schools groups, while almost 24% of the male students from secondary schools, used miswak. Which shows more access to miswak and social and cultural norm of the society. Daily

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toothbrushing habit varied from 42.6% in male and 27.6% female in intermediate to 61.4% male and 26.8% female in secondary schools.

Miswak was used more than 3 times a day by 53% male and 24% female student at intermediate schools, while this increased to 56% in male and decreased to 17% in female at secondary schools. The over all time spent on brushing was less as compared to miswak in average more than 3 minute per use, and it is in agreement with previous study. This shows that more time is being spent on miswak compared to toothbrush but that does not reflect the meaningful cleaning of teeth by miswak as most of the male chew the miswak as a cultural norm. The majority of the students in both groups of schools expressed that toothbrush clean better than miswak, while majority of the miswak user, use it due to Sunnah with second feeling of better cleaning. The combined users of miswak and toothbrush ranged 53-68% in expressing the understanding of better cleaning. Recently, Darout et al. assessed and compared the periodontal status of adult Sudanese habitual miswak and toothbrush users. It was found that the periodontal status of the miswak users in the Sudanese population was better than that of toothbrush users and the efficacy of miswaks used for oral hygiene was comparable to or slightly better than that of the toothbrush.

All of the above-mentioned comparative studies are in favor of promoting traditional oral hygiene tool of miswak. This can be integrated for oral health promotion activities, and the target group of oral hygiene neglectors should be persuaded to start with miswak, which is socially and culturally accepted and religiously motivated as the finding of present study has highlighted. The other important finding in this study is that female students in secondary school groups have descending trend of oral hygiene practices. As we know that today's children or adolescent are tomorrow's parents, so the female students needed to be focused for effective orientation for toothbrushing, as before becoming mothers. Almost 10% of intermediate school students and 17% from secondary schools never cleaned their teeth. Female students have better oral hygiene practices as compared to male students at intermediate level but trend shifts towards the male students at secondary school level. Toothbrushing was most commonly used by female students at both intermediate and secondary schools. Both groups of students and both groups expressed the common use of toothbrush due to better cleaning perception. Miswak was used by 24% secondary school male students. Majority of the students used old miswak, and unspecific technique was most commonly used. Sunnah was expressed as common motivation of using miswak.

Oral health educational activities at schools should be integrated with oral health promotional approach. Female students at schools should be given more knowledge and incentives to improve and develop oral hygiene practices on regular basis. The parents and teachers should play a major role in promoting healthy oral habits among students. Further, research is needed to evaluate the effectiveness of oral hygiene habits and to compare the oral health status among the studied population.

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References


Comparison of PCR and disc diffusion methods in detecting methicillin resistance among Staphylococcus species from nosocomial infections

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Methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant coagulase negative Staphylococcus species (MRCON) have spread worldwide after the introduction of methicillin. Methicillin resistant Staphylococcus aureus (S. aureus) was first isolated in England in 1961 shortly after the introduction of methicillin. Resistance Staphylococcus species are an important cause of nosocomial and community infections. Nowadays, there is an increase in the prevalence of MRSA and MRCONS worldwide. This increase requires a rapid, accurate and sensitive method for isolation methicillin resistant isolates. Methicillin resistant Staphylococcus species are
resistant practically to all lactam antibiotics that are represented by penicillin and cephalosporin. It is difficult to detect all methicillin resistance by routine susceptibility methods as of the heterogeneous nature of methicillin resistance.\(^1\) Genetically, methicillin resistance determined by a chromosomal gene (meca), which has been cloned and sequenced from a Japanese MRSA mec A gene codes for a penicillin binding protein (PBP2a). Acquiring PBP2a\(^2\) by *Staphylococci species* convert them into resistant strains. Meca gene detected in methicillin resistant and absent in methicillin sensitive *Staphylococci*, so it is a useful molecular marker to differentiate between methicillin resistant and sensitive *Staphylococci species*. Evaluation of disc diffusion assay in detecting methicillin susceptibility *Staphylococcus* was controversial. Prasad et al\(^1\) considered disc diffusion the least reliable assay (87.7% sensitivity and 89.8% specificity) compared with microdilution and PCR assays for detection of methicillin resistance *Staphylococcus aureus*. Other reports\(^2\) showed that disc assay are reliable and has a positive predictive value 95% for coagulase negative *Staphylococcus* species and could detect over 94%of meca positive isolates. Many previous reports used PCR assay for the detection of methicillin resistance through amplification of meca gene and considered as a gold standard for detecting methicillin susceptibility.\(^2\) The aim of this study is to compare sensitivity, specificity and accuracy of disc diffusion with meca amplification PCR assay for detecting methicillin resistance *S.aureus* and coagulase negative *Staphylococcus species*.

**Bacterial strains.** One hundred and two *S.aureus* strains and 96 coagulase negative *Staphylococcus species* were collected from different clinical samples from Prince’s Basma Hospital in Irbid. *Staphylococcus aureus* (ATCC 25923) was used as a control. *Staphylococcus* isolates were classified into 4 groups: MRSA, MSSA, MRCON and methicillin sensitive coagulase negative *Staphylococcus species*. Upon receipt of clinical samples, they were subcultured on blood agar plates and incubated at 37\(^\circ\)C overnight. *Staphylococcus* isolates were identified by biochemical and physiological tests.

**Disc diffusion susceptibility test.** Disc diffusion test was carried out following the National Committee Clinical Laboratory Standard. The final inoculum was adjusted to 5 x 10\(^7\) to 9 x 10\(^7\) CFU/ml in Mueller-Hinton broth (Difco Laboratories). The inoculum was plated by sterile swab uniformly on Mueller-Hinton agar (Difco laboratories) and 5 \(\mu\)g methicillin discs (Sigma) were applied. Plates were incubated at 25\(^\circ\)C for 24 hours.

**Oxacillin microdilution.** Oxacillin broth susceptibility test was carried out as NCCLS recommendations in Muller-Hinton broth.

**Deoxyribonucleic acid extraction method.** Deoxyribonucleic acid was extracted form *Staphylococcus* strains using Wizard kit (Promega) with slight modification. Proteinase K enzyme (20mg/ml) was added with lysostaphin to increase the lysis of *Staphylococcus* cell wall.

**PCR assay.** The extracted *Staphylococcus* DNA samples were used to amplify *Staphylococcus* meca gene. Amplification of meca gene is a 997bp product. Amplification was performed with the following primers: 5’ -CAT TTT GAG TTC TGC ACT ACC 3’ and 5’ GCA ATA CAA TCG CAT TAA

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**Table 1 - Comparison of methicillin resistant *Staphylococcus aureus* and methicillin resistant coagulase negative *Staphylococcus species* by PCR with disc diffusion method.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Resistant strains</th>
<th>PCR Sensitive strains</th>
<th>Total</th>
<th>Reliability</th>
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<tbody>
<tr>
<td>MRSA</td>
<td>20</td>
<td>4</td>
<td>24</td>
<td>Sensitivity=76.9%</td>
</tr>
<tr>
<td>MSSA</td>
<td>6</td>
<td>72</td>
<td>78</td>
<td>Specificity=94.7%</td>
</tr>
<tr>
<td>MRCON</td>
<td>21</td>
<td>1</td>
<td>22</td>
<td>Sensitivity=87.5%</td>
</tr>
<tr>
<td>MCON</td>
<td>3</td>
<td>71</td>
<td>74</td>
<td>Specificity=98.6%</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>72</td>
<td>96</td>
<td>Accuracy=93.1%</td>
</tr>
</tbody>
</table>

MRSA - methicillin resistant *Staphylococcus aureus*, MSSA - methillin sensitive *Staphylococcus aureus*, MRCON - methicillin resistant coagulase negative *Staphylococcus species*, MCON - methicillin sensitive coagulase negative *Staphylococcus species*.
The presence of meca gene was tested for 102 Staphylococcus aureus and 96 coagulase negative Staphylococcus disc diffusion fails to detect 6/26 MRSA and 3/24 MRCON. This test also identifies 4/76 MSSA as MRSA and 1/72 MSCON as MRCON. The sensitivity, specificity and accuracy of disc diffusion was 76.9%, 94.7% and 85.8% for Staphylococcus aureus and 87.5%, 98.6% and 93.1% for coagulase negative Staphylococcus (Table 1). The discrepant MSSA (6/26) and MSCON (3/24) were retested by broth microdilution assay. The results showed that 4/6 MSSA and 2/3 MSCON had minimum inhibitory concentrations (MIC) of more than 8 μg/ml for methicillin and therefore had posses the methicillin resistance gene. The other 3 were mecA negative and had their MIC 0.5 μg/ml indicating sensitivity to methicillin. The other discrepant MRSA 4/76 and MRCON 1/72 strains were also retested by microdilution assay and their MIC to methicillin were 4 and 8 μg/ml (borderline resistant). They were also retested for amplified meca gene and showed PCR product (997bp) upon retesting.

Methicillin resistant Staphylococci are one of the most common causes of nosocomial infections. Standard bacterial identifications and susceptibility testing frequently require as long as 72 hours and there may be a difficulty in identifying methicillin resistance due to the heterogeneous nature of resistance to methicillin. Disc diffusion fails to detect 6 MRSA and 3 MRCON while their resistance was detected by PCR assay through meca gene amplification. Methicillin resistant Staphylococci using PCR.  J Clin Microbiol 2000; 38: 3407-3412.

Comparison of PCR and disc diffusion methods

References


Osmotic fragility and Na-K ATPase activity of erythrocytes of HIV/AIDS patients

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Symptomatology of human immuno-deficiency virus/acquired immuno-deficiency syndrome (HIV/AIDS) is very diverse. However, anemia is one of the most universal clinical symptoms of the disease. The etiology of anemia in HIV disease has been extensively researched primarily from the
physiological and pharmacological angles. Malnutrition in HIV/AIDS has also been widely reported. Nutrient deficiencies can result in disruption of supramolecular structures like biomembranes. We therefore decided to investigate the osmotic fragility of erythrocytes of HIV/AIDS patients. Since the sodium−potassium+ adenosine triphosphatase (Na−K+ ATPase) is the major transmembrane pump involved in regulating osmosis in the cell; we considered it pertinent to determine its activity in the erythrocytes of HIV/AIDS patients. This was a cross-sectional study involving 56 HIV seropositive subjects recruited and confirmed positive at the Nigerian Institute of Medical Research (NIMR) Human Virology Laboratory in Lagos, Nigeria. Confirmations were carried out with the Genie II HIV Confirmation kit. Participants who were on multivitamins, mineral supplements, or cardiac glycosides, or both, or prolonged non-AIDS related treatment were excluded from the study. Some minerals have been reported to reverse osmotic fragility in erythrocytes and cardiac glycosides are specific inhibitors of Na−K+ ATPase activity. The HIV positive group was subdivided into 2 groups: those who were on antiretroviral therapy (ARV) at the Institute and those who had not commenced any form of antiretroviral therapy (non-ARV). The HIV positive subjects were grouped either as ARV (35 persons) or non-ARV (21 persons) subjects. Ten HIV seronegative persons served as the control group. The age range was between 20 and 60 years. Blood samples were collected by venous puncture into potassium EDTA bottles. Four ml were aliquoted for CD4+ T-lymphocyte count within 6 hours of collection using the Dynabeads method; one ml was aliquoted for erythrocyte ghost membrane (EGM) preparation while the remaining one ml was used for osmotic fragility assay. Two ml of blood were taken from the control subjects since CD4+ counts were not conducted on them. All tests were conducted on the day of collection. The HIV disease-stage classification was according to the Centers for Disease Control revised 1993 classification for HIV infection among adolescents and adults. Osmotic fragility of the erythrocytes was determined by using saline solutions of 0.85%, 0.65%, 0.35%, and 0.1% NaCl to which, erythrocytes was determined by using saline solutions consisting of 50mM Tris-HCl (pH 7.4), 120mM NaCl, 20mM KCl, 4mM MgCl2, 240mM sucrose, 1mM EDTA and 3mM disodium ATP. Fifty µl of EGM suspension were aliquoted into 2 tubes labeled one and 2. One hundred µl of incubation medium were also added to each tube but 100µl of one mM ouabain solution was added to tube 2 only. The reaction mixtures were incubated at 37°C for 20 minutes. They were stopped by adding 100µl of one percent SDS. Inorganic phosphate produced from the hydrolysis of ATP by ATPase and protein concentration of the EGM was determined.

At 0.6% hypotonic saline concentration the red blood cells of the non-ARV and ARV groups were found to have higher percentages of hemolysis than the control group. (Table 1) The non-ARV group had a greater degree of red blood cell fragility than the ARV group. Both groups showed statistically significant results. (p<0.05) The Na−K+ ATPase activities for the non-ARV and ARV groups were higher than the activity of the control group. (Table 1) The differences were however, not statistically significant. (p<0.05) On detailed investigation the Na−K+ ATPase of non-ARV and ARV subjects with CD4+ T-lymphocyte count of less than 200 cells per microliter of blood showed higher activity than subjects with CD4+ counts of between 200-499 cells per microliter.

Osmotic fragility has been associated with lower concentration of protein sulphhydrals in erythrocyte ghost membranes. Xia et al14 suggested in their report that an important function of zinc is to protect cysteine residues in critical plasma membrane proteins from auto-oxidation. Auto-oxidation of the cysteine residues will ultimately lead to a significant conformational change in these proteins, which may in turn cause structural fragility of the plasma membrane. Micronutrient deficiency has been reported in AIDS patients. The deficient micronutrients include zinc, vitamin A, iron, iodine, and trace elements. These nutrients are essential as cofactors for the proper functioning and structural integrity of various biomolecules – especially proteins. Some act as antioxidants. A deficiency in a critical micronutrient can completely upset the homeostatic functioning of a cell or the entire organism. It could be attributable to reduced intake, increased utilization or urinary excretion.

### Table 1: Degree of hemolysis of red blood cells and Na- K+ ATPase activities of HIV negative (control), non-ARV and ARV HIV/AIDS in 0.65% saline solution.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Percent Hemolysis</th>
<th>Na+ -K+ ATPase activity (nmol Pi/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.83 ± 0.36</td>
<td>2.22 ± 0.81</td>
</tr>
<tr>
<td>Non-ARV</td>
<td>3.19 ± 1.11</td>
<td>3.01 ± 0.52</td>
</tr>
<tr>
<td>ARV</td>
<td>2.56 ± 0.81</td>
<td>3.69 ± 0.58</td>
</tr>
</tbody>
</table>

* values represent mean ± standard error of mean

Non-ARV - non antiretroviral

ARV - antiretroviral

The report that reduced micronutrient intake may lead to nutritional deficiency lends credence to osmotic fragility of red blood cells observed in the HIV positive subjects. Anorexia, nausea, vomiting, and diarrhea are conditions that can result in reduced nutrient intake, which leads to malnutrition. These secondary symptoms have been observed and reported in acute and late stage HIV disease.

It may seem surprising that patients who are undergoing antiretroviral therapy, and are showing significant improvement in health, should also be significantly susceptible to osmotic stress. These patients may have osmotically fragile cells also as a result of micronutrient deficiency. Stephensen et al and Jordao et al reported that deficiency might occur in HIV/AIDS as a result of increased urinary excretion. Antiretroviral therapy involves a cocktail of drugs taken under a strict regimen. In an attempt to detoxify or metabolize, or both, these drugs the liver increases their water solubility. Ultimately, there is an increase in urine production and a depletory loss of vital water-soluble nutrients like the metallic ions may occur. The patients may therefore suffer from conditions like anemia. Zidovudine (AZT) therapy has been reported to be the most frequent cause of anemia in HIV-infected persons. This supports our findings of a possibility of anemia in patients on antiretroviral therapy. Whereas previous works have attributed this condition to marrow erythroid hypoplasia, aplasia, and megaloblastic maturation, we believe that from the present data and cited literature, osmotic fragility resulting from a micronutrient deficiency is critical to the development of anemia in antiretroviral therapy patients. Sodium-potassium adenosine triphosphatase activities of the erythrocytes were found to be increased in non-ARV and ARV HIV positive patients compared to HIV negative patients, though they were not statistically significant in the present study. The increased activities may be a consequence of the osmotic fragility of the plasma membrane of the erythrocytes discussed above. The Na+-K+ ATPase pump is the primary mechanism by which the cell prevents lysis from osmotic stress. The activity of the pump increases when the cell is threatened with plasmolysis. The pump performs a continual surveillance role in maintaining normal cell volume. The Na+-K+ ATPase activity of the erythrocytes is only marginally increased in an attempt by the cells to reverse the deleterious effects of osmotic fragility in HIV/AIDS disease.

References


Tonsillectomy blood splash

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Hepatitis B virus and human immunodeficiency virus (HIV) can be transmitted via certain routes including conjunctiva.1 Tracheostomy, air drilling, local anesthetic infiltration and beside other numerous surgical procedures, tonsillectomy puts otolaryngologist
Tonsillectomy blood splash

A telephonic survey was carried out of major medical centers in the Kingdom of Saudi Arabia (KSA) to inquire if otolaryngologist use protective eye cover during tonsillectomy; none of the surgeons took precaution to prevent blood splash.

A prospective study was carried out at the North West Armed Forces Hospital in Tabuk, from February through to September 2002 of 100 patients who under went tonsillectomy with or without adenoidectomy. Five Surgeons, grade Senior Registrar/Consultant, were involved. The method of surgery was the classical dissection and electrocautery. All involved surgeons wore protective eye cover or spectacles, which were examined before and after surgery for blood contamination.

A total of 100 patients were included in the study, 63 males and 37 females, of these 74 were pediatric and 26 were adults. Only one pediatric case that had adenotonsillectomy was noted to have caused blood splash. There were 3 blood spots on the left lens of the spectacle ranging in size from 0.2-0.5mm. The surgeon was aware of the splash. Method of surgery, patient’s age and type of surgery did not affect the outcome. The danger of transmission of blood-borne viral infection, hepatitis B, C and HIV, during surgery is on the rise, with the increase of infected cases. In the KSA, according to an official report, there are 1,238 HIV carriers. Sexual contact, blood transfusion, skin prick and transconjunctival are known routes of transmission of these infections; nevertheless, the latter is underestimated. It is worth noting here, medics and paramedics in KSA, who may get infected in line of duty, are compensated, as stated in the civil service regulations in vague terms and there are no rehabilitation programs.

It is assumed that senior surgeons may be exempted from using protective eye cover during tonsillectomy. Their experience may well prevent splattering of blood as of their tidy surgery, in contrast to junior doctors. However, experience of the surgeon may decrease the risk, but does not eliminate it, as seen here in our study (1%), which was the least in comparison to other studies 46% and 23.7%. In either case, the center for disease control (CDC) recommends goggle use during any procedure that may result in eye splash by bodily fluids. Fluid shield mask that covers nearly all of the face was used by one surgeon, but proved to be cumbersome, especially when using a headlight. The second eye cover used, could sit comfortably with the headlight, was a plastic goggle. Two surgeons used spectacles as eye cover, which provided partial protection.

They were the most comfortable. Age of the patient as previously reported did not affect the result and the method of surgery had no impact on the outcome. It would be interesting to compare these and other results with laser surgery, as the set up is completely different where the use of eye cover is mandatory and bleeding is minimal.

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