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Case Report

Periodontal myiasis treated by open flap debridement: A case report

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Myiasis is an infestation of live vertebrate by dipterous larva, which feeds on living and dead host tissue, host products and byproducts. Here, we report a case of a female aged 35 years old, mentally retarded presented with severe pain, swelling, crawling worms in the upper teeth and lip region. She was a chronic mouth breather with very poor hygiene. On clinical examination, a larvae was seen in the periodontal pocket with relation to teeth #21 and 22, which was later by entomological examination identified as *Chrysomya bezziana*. The patient was managed by mechanical removal of the larvae from pocket using turpentine oil and then by open flap debridement with removal of all maggots. This case report emphasizes the importance of oral hygiene instruction, motivation and education in mentally challenged patients. Controlling fly vector population, maintaining good oral and personal hygiene contributes in checking this infestation.

Key words: Myiasis, mouth breather, oral hygiene, *Chrysomya bezziana*.

INTRODUCTION

The term myiasis was coined by Hope (1840) is derived from Latin word 'muia' which means fly and 'iasis' means disease. It is an infestation of live vertebrate by dipterous larvae which feeds on living or dead host tissue, liquid body substances, or undigested food (Gomez et al., 2003). It occurs frequently in countryside areas, infects live stocks, pets and in malnourished, medically compromised individuals in third world countries (Rossi-Scheider et al., 2007). Myiasis can be primary when larvae feed on living tissue and secondary when larvae feed on dead tissue (Shinohara et al., 2004). The most common anatomic sites for myiasis are the nose, eye, lung, ear, anus, vagina and rarely oral cavity (Abdo et al., 2006). Oral myiasis was first described by Laurence (1909). Persistent mouth opening as in chronic mouth breathers, poor oral hygiene, suppurative lesions, severe halitosis and facial trauma may predispose the patient to oral myiasis (Abdo et al., 2006). Here we report a case of

myiasis in which the maggot were found in the periodontal pocket in the upper anterior region of a chronic mouth breather and its subsequent management.

CASE REPORT

A 35 year old female reported with severe pain and swelling in the upper anterior teeth and upper lip was used as the case study. The patient complained of something crawling inside her upper anterior gums on the right side. She was mentally retarded and belonged to low-socioeconomic strata.

On clinical examination, her upper anterior teeth were protruded and there was spacing between them. She was a chronic mouth breather as it was evidenced by incompetent lips and high arched palate. Her oral hygiene was very poor and there was severe halitosis. There was generalized periodontal tissue loss with probing pocket depth ranging from 5 to 10 mm. The greatest probing pocket depth (≥ 10 mm) was seen between teeth 21 and 22. On exploration of periodontal pocket with a periodontal probe around teeth #21 and 22,

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Figure 1. Larvae coming out of the periodontal pocket between teeth #21 and 22.



Figure 2. Palatal incision given for open flap debridement to look out for remaining maggots.

a maggot was seen coming out of the mouth of the periodontal pocket (Figure 1) Thus a provisional diagnosis of periodontal myiasis was made.

The patient was transported to operating room and under intravenous conscious sedation and local anesthesia, cottons buds impregnated with turpentine oil was placed between teeth 21 and 22 for 10 min. Two more maggots appeared from there. Then, open flap

debridement, to look out for any missing maggots was planned. Buccal and palatal flap were elevated by giving crevicular incision using No. 15 blade followed by mucoperiosteal or fullthickness flap elevation using periosteal elevator (Figure 2). We found three more maggot plugged underneath the palatal flap. Then we thoroughly debrided the area with surgical curette and concomitantly irrigated the area with normal saline and



Figure 3. Open flap debridement did along with extraction of teeth #22.

5% povidine iodine solution and intensely searched for any remaining maggots. After ensuring that no more maggots were left, tooth number 22 was extracted as it was mobile (Figure 3) due to extensive damage caused by maggots. Then we replaced both flaps to their respective positions and sutured with 3-0 black silk and gave zinc oxide dressing.

The patient was prescribed Tablet Ivermectin drug for 2 days. Broad spectrum antibiotics amoxicillin 500 mg and metronidazole 400 mg TDS for 5 days were prescribed with analgesic Acetoaminophen 325 mg TDS for three days along with multivitamins and nutrition supplement. The patient was given tetanus prophylaxis and was instructed to practice oral hygiene measures using soft toothbrush along with Chlorhexidine mouthwash 0.2% (10 ml twice daily) for maintaining plaque control. The patient was recalled after a week for suture removal and re-evaluation. The healing was uneventful without any sign of any discomfort or swelling. There was no residual maggot infestation left and patient responded very well to the treatment.

The maggots isolated from the site were sent for entomological evaluation. The larvae were identified as *Chrysomya bezziana* by the entomologist. The maggots were 12 to 15 mm long, creamy white in color with transverse grooves and without body process. They also had open peritreme of the posterior spiracle and four to five lobes in the anterior spiracle suggesting larvae of *C. bezziana*.

DISCUSSION

Myiasis is caused by members of the Diptera fly family

that lay eggs on food, necrotic tissue, open wounds, and unbroken skin or mucosa. It can be obligatory, when larval flies develop in living tissue, or facultative, when maggots feed on decomposing matter or necrotic tissues.

C. bezziana also known as 'Old World Screwworm' is one of the causative organisms for obligatory myiasis. The species is widely distributed throughout South-East Asia, the Indian Subcontinent, China, tropical Africa, and Papua New Guinea. The species was first found in Hong Kong in July 2000, when animal cases were identified (Ng et al., 2003). The patient in the present case was of low socio-economic status having poor living conditions. Persistent mouth opening due to incompetent lips and high arched palate, protruded maxillary incisor with poor oral hygiene and advanced periodontal disease as seen in our case are the most commonly known predisposing factors for oral myiasis. The periodontal pocket act as an ideal habitat with very good nutrition supply, favourable environment and mechanical support for the larvae to grow. The mechanism of myiasis invasion involves the fly depositing their eggs in the periodontal pocket which penetrate deeper for larval development. The larva gets implanted and causes progressive destruction of periodontal supporting structure leading to cavitation and ultimately tooth loss (Shah et al., 1984). Feeble, old, paediatric or mentally handicapped patients who are unable to defend themselves, and who often present with a lack of labial closure as in this case, are attacked by flies and consequently by the disease (Gealh et al., 2009).

As the patient was in severe pain, hence there was an urgency to remove all the maggots from the periodontal pocket; the maggots which were visible were removed with a tweezer. Cotton impregnated with turpentine oil

were placed in the periodontal pocket to asphyxiate the remaining maggots, as turpentine oil acts as an irritant by blocking larvae's air sinuses and compels them to come out (Babu et al., 2010).

The current treatment protocol for myiasis includes maintenance of nutrition, antimicrobials for secondary infection and manual removal of larvae with or without topical asphyxiating drugs such as ether, chloroform, olive oil, turpentine oil that expels the larvae to come out. Ivermectin given orally in just one dose of 150 to 200 mg/kg body weight and repeated after 24 h has been reported to be effective in severe cases (Shinohara et al., 2004). It is activated by gamma amino butyric acid liberation, which leads to parasitic death and their spontaneous elimination by washing out larvae (Victoria et al., 1999). Failure of antihelminthic therapy has been reported by Gealh et al. (2009) with survival of larvae even after 5 days of therapy, warrants for surgical debridement.

The developmental cycle of *C. bezziana* from egg to adult fly is completed in 18 days under favourable conditions. The adult female fly lays eggs on live mammals and deposits eggs every two days at the site of the wound in body orifices. The eggs hatch after 12 to 18 h and the first-stage larvae, white in color and 15 mm in length emerges from the eggs and then burrow into wound or wet tissues. They feed not only on the host's dead tissues but also on the living tissues, and the wounds increase in sizes as they feed. In about four days, the larvae metamorphose into the second and third stages. After 5 to 7 days, the third-stage larvae leave the wound and fall to the ground to pupate and transform into adult fly in 7 days (Kwong et al., 2007).

Since the flies are the transmitters of the disease and are attracted to open putrefying wounds, poor oral hygiene, lack of manual dexterity, incompetent lips, open bite and rural locality are considered to be predisposing factors for larval infestation in this patient. The flies are attracted to the bad mouth odor due to neglected oral hygiene or fermenting food debris usually affecting the both upper and lower arches by direct infestation. In addition, the patient had incompetent lips with a class II overjet, which could be thought of as a contributing factor to his neglected oral hygiene. Therefore maintenance of oral hygiene is paramount importance for prevention of this disease.

Conclusion

The incidence of myiasis can be reduced by raising the standard of living and improving personal hygiene habits as they rarely occur in these patients. Controlling fly vector population and maintaining good oral and personal

hygiene contributes in checking this infestation. Special needs patients (mental and/or physical disability) have difficulties in maintaining good oral hygiene due to poor manual dexterity. Therefore a special needs patient should be exposed to the dental education, motivation, prevention and intervention as early as possible to promote co-operation and confidence and to prevent disease.

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Case Report

Uterine leiomyosarcoma presented as chronic inversion of uterus- A rare case report

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Sarcomatous change in uterine fibroid is termed leiomyosarcoma. The tumor originates from smooth muscle cells and is rare, accounting for 2 to 5% of all uterine malignancies. The said patient had the history of irregular bleeding, but not typical menorrhagia, which is the usual presentation in this tumor. A 40 year old, P₄₊₀ women of North Indian origin, was admitted with huge polypoidal fungating mass with bosselated surface and variegated mass protruding from introitus consistently. It was 12 ×10 cm in size with fundal cuping and cervical rim around the mass, which indicates inversion of the uterus. Total abdominal hysterectomy with bilateral salpingoophorectomy and bladder repair was done via abdomino-perineal route and the histopathology confirmed the diagnosis of leiomyosarcoma. Because of their rarity, uterine sarcomas are not recommended for routine screening. Surgery is the only treatment modality of leiomyosarcoma and prognosis depends upon the stage of the cancer.

Key words: Leiomyosarcoma, inversion, hysterectomy.

INTRODUCTION

Leiomyosarcomas are rare and aggressive form of uterine cancer (Melona et al., 2008). Compared to the more common endometrial carcinomas, uterine sarcomas behave more aggressively and are associated with a poorer prognosis. It arises from smooth muscle of the uterus and accounts for 2 to 5% of all uterine malignancies (Bergman et al., 2000). We report an original case of an unusual presentation of this rare tumor.

PRESENTATION OF THE CASE

A 40 years old Para₄₊₀ postmenopausal lady was presented to our OPD with the chief complaints of something coming out of vagina for the past 6 months with low grade fever and discharge per vagina. The patient was apparently asymptomatic 4 years back when she had undergone myomectomy for bleeding uterine

fibroid at a private hospital. Since previous records were not available, the site size and histopathology of previous fibroid was not known.

Examination

On examination, mild pallor was present and pulse rate was 100 beats/min and blood pressure was 120/70 mmHg. Local examination revealed a huge unhealthy looking polypoidal fungating mass of about 10 to 12 cm in size, smeared with thick foul smelling discharge coming out of introitus. Per vaginum examination revealed the same mass was present in the vagina, but actually originating from inside the cervical os, with the cervical rim felt all around it.

On bimanual examination fundal cupping was present. The mass firm to hard in consistency with irregular surface and it bled on touch. Exact uterine size could not be assessed due to huge size of the mass (Figure 1). A strong suspicion of chronic uterine inversion with probable recurrence of uterine fibroid was made.

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Figure 1. Inversion of uterus with fungating polypoidal growth protruding out of introitus.

Investigation

The patient had anemia with Hb% at 8 gm/dl. Serum chemistry showed liver and kidney function to be normal. Ultrasonography revealed empty uterine fossa with bilateral hydronephrosis, which was further confirmed by Intravenous Pyelography. Viral markers (HbsAg, HIV, and HCV) were negative. Urine culture was sterile and the blood (sugar fasting and postprandial) was within normal limits. The chest x-ray and EKG were unremarkable.

Management

The patient was planned for simultaneous retrograde vaginal and abdominal resection of mass. Intraoperatively, a vague mass was seen arising from lower portion of pelvis extending into vagina, which was densely adhered to the posterior surface of the bladder. In the process of removal of adhesion, small rent occurred in bladder, which was followed by bilateral ureteric catheterization and repair of bladder. Resection of the mass both by abdominal and vaginal route then followed. Ovaries and tubes could not be visualized clearly due to severe distortion of anatomy.

Pathology

Histopathology shows an infiltrative malignant smooth

muscle tumour disposed in intersecting fascicles with occasional whirling and palisading. The individual tumour cells are pleomorphic with high nucleo cytoplasmic ratio, oval to elongated blunt ended nuclei, fibrillary eosinophilic cytoplasm. Fair numbers of a typical mitoses are seen. Adjacent areas show extensive coagulative necrosis and haemorrhage, suggestive of leiomyosarcoma of uterus as shown in Figure 2.

Follow up

The patient was followed in the cancer clinic of institute monthly (for 3 months) by vaginal cytology and was negative for malignancy.

DISCUSSION

Uterine sarcomas are rare hence they are not included in routine screening. The incidence of sarcoma is 1 to 2% in postmenopausal women (Wickerham et al., 2002). Abnormal vaginal bleeding and pelvic or abdominal pain are the most frequent presenting symptoms. The pattern of bleeding ranges from spotting to menorrhagia and is often associated with foul-smell and vaginal discharge. Weight loss, weakness, lethargy, and fever are less common symptoms (Van Dinh and Woodruff, 1982; Barter et al., 1985; Schwartz et al., 1985). Our patient did not have any history of genital bleeding, which is the

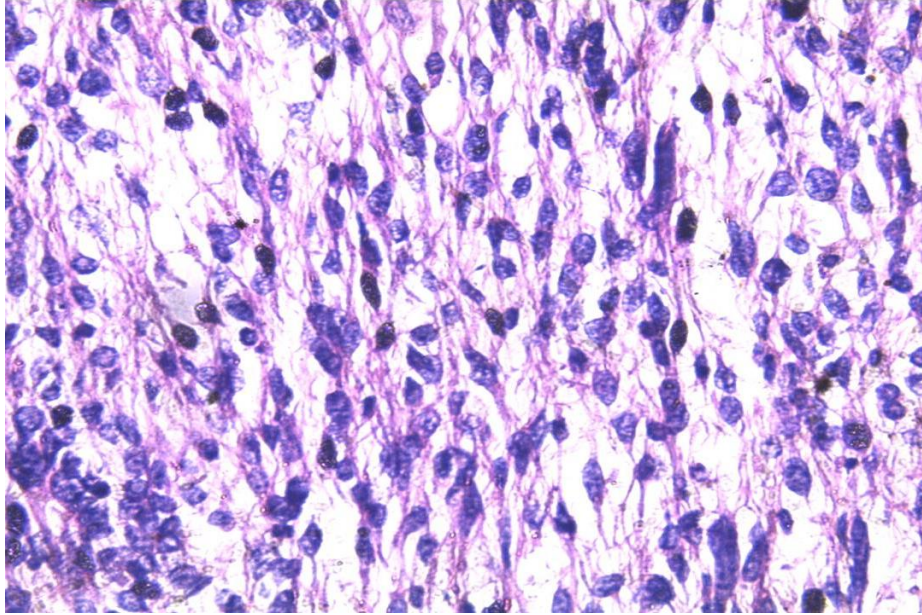


Figure 2. H and E: High power: showing an infiltrative smooth muscle tumor arranged in intersecting fascicles with oval to elongated blunt ended hyperchromatic nuclei with fibrillary eosinophilic cytoplasm (Leiomyosarcoma).

usual presentation, but she had low grade fever with foul smelling vaginal discharge. In our case, the lady had rapidly growing mass coming out of her introitus 6 months before she was presented.

A similar case was reported by Musa et al. (2005) in which patient had similar presentation but eventually turned out to be leiomyosarcoma of the uterus. Uterine leiomyosarcoma are considered neoplasm of high metastatic potential with 5 years overall survival rate varying between 0 and 73% (Bartsich et al., 1988; Hart and Billman, 1978). In this case, inspite of previous myomectomy, tumour had recurred, thus regular follow up may be suggested in perimenopausal women undergoing myomectomy. Women with tumor size more than 5 cm in maximum diameter have a poor prognosis (Evans et al., 1988).

CONCLUSION

Uterine leiomyosarcomas are rare, as such diagnosis is made only by histopathological examination, thus emphasizing the need for the same by an expert pathologist. Surgery is the mainstay of the treatment.

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Case Report

Klinefelter syndrome and specific learning disability

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47XXY aneuploidy is the most common disorder of gender chromosomes with a prevalence of 1: 700 males. However, only 1/3 cases are diagnosed due to phenotypic variability and insufficient professional awareness of the syndrome. School children with Klinefelter syndrome (KS) were presented with language delay, learning disabilities and behavioral/social problems. Although Klinefelter syndrome (KS) with language based learning disabilities is not uncommon, this case was unusual due to its coexistent condition of attention deficit hyperactivity disorder (ADHD) and specific higher cognitive deficits on psychoeducational profile.

Key words: Klinefelter syndrome, language delay, learning disabilities, behavioral problems, attention deficit hyperactivity disorder (ADHD).

INTRODUCTION

Klinefelter syndrome (KS) is a group of chromosomal disorders with an extra X chromosome added to a 46 XY male karyotype. 47XXY aneuploidy is the most common disorder of gender chromosomes with a prevalence of 1: 700 males. KS has a varied phenotype with characteristic features of tall height, gynaecomastia, sparse body hair, small testis, decreased muscle mass, feminine distribution of adipose tissue, azoospermia and infertility (Smyth and Bremner, 1998). Majority of KS children have a normal but a characteristic cognitive phenotype (Rovet et al., 1996). They have a deficit in verbal processing affecting comprehension and learning leading to significant under achievement and a generalized learning disability. Identification of this is of immense importance in planning early psychoeducational interventions.

CASE REPORT

An eleven year old boy was presented to the clinic with academic underachievement for the purpose of certification in order to make available the provisions laid down by the education authorities. He was multilingual from the upper middle social economic status (SES) in

the seventh grade, Central Board of Secondary Education (CBSE), and was presented with academic lags in the areas of reading comprehension in all languages, with poor written expression. He was frequently lost in thoughts and could not express them effectively both verbally as well as on paper. He had difficulty in both copying and writing from memory although oral expression was better than written expression. He made careless mistakes in computation but understood concepts well in both Mathematics and Science however reading comprehension was affected in languages. Occasional spelling errors particularly with unfamiliar words, lengthy words were present.

In mathematics, though analytic reasoning was preserved, simple computation errors occurred due to impulsivity. Additionally, the school reported that he was slow in copying, unable to express verbally, had attention deficits and had considerable emotional immaturity. Concerns of poor concentration and inattention to details were also noted by the parents and teachers alike.

He was reported to have difficulty in sustaining peer interactions and his behaviour was often considered socially inappropriate for his age. He had mild anger management issues and a poor self image and self esteem.

Developmental history was notable for mild motor delays walking (18 months) and language delay (2 years). On examination, he was tall (his height was measured at the 95th centile for his age), generalized mild

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Table 1. WISC IQ profile.

Verbal scale	IQ	Performance scale	IQ
Information	15	Picture completion	13
Comprehension	13	Picture arrangement	12
Arithmetic	05	Block design	16
Similarities	13	Object assembly	13
Vocabulary	14	Coding	02
Digit span	14	Mazes	16

Verbal IQ (VIQ) - 116 performance IQ (PIQ) – 115, full scale IQ (FSIQ) – 117.

Table 2. Woodcock Johnson III Psychoeducational Tests of Academic Achievement (Standard and Extended Battery).

WJ III achievement clusters	Actual achievement	Predicted achievement	Ability/Achievement SS difference	Discrepancy SD	Significant At -1.50 SD
Math calculation skills	107	110	03	0.25	NO
Math reasoning	96	113	17	1.70	YES
Broad reading	94	112	18	1.72	YES
Reading comprehension	79	112	33	3.29	YES
Written expression	88	109	21	1.68	YES

SS - Scaled scores, SD - standard deviation.

hypotonia, gynaecomastia, flat feet, and soft neurological signs like dysdydokinesia, grapesthesia were also present. He had mild incoordination issues with difficulty in hopping and inability to maintain a steady stance. Chromosomal evaluation done was significant for chromosomal aneuploidy [46 XXY] Klinefelter syndrome karyotype.

Based on the DSM IV-TR (Diagnostic Statistical Manual) clinical criteria, a diagnosis of the Attention based hyperactivity disorder, which predominantly consists of an inattentive subtype, was made. Psychological tests as the WISC-R (Weschler's Intelligence Scale for Children- Indian adaptation by Mahindrika Bhatt) provides a broad assessment of general cognitive skills and describes the typical functioning in specific areas of cognition broadly divided into the verbal intelligence quotient (VIQ), performance intelligence quotient (PIQ) and full scale intelligence quotient (FSIQ). The average above his cognitive profile done as per the WISC-R (Table 1) revealed a full scale IQ - 117 with a VIQ - 116 and PIQ – 115, that is, IQ was above average without significant differences in verbal conceptual and non verbal reasoning. But on further analysis of the WISC report, the scaled score of Arithmetic subset in the verbal scale was very low (05) suggesting that severe deficits in arithmetic reasoning were present. The coding subset in the performance scale had low scaled scores (02) implicating fine motor issues, poor processing speed and inattention. Psycho educational assessment for specific learning disability was done using Woodcock Johnson III (WJ III) test of

Academic Achievement (Standard and extended battery) (Table2). The WJ III measures broad reading, reading comprehension, written expression, mathematic reasoning and calculation skills among other clusters. Significant discrepancies in the scaled scores of predicted ability versus actual achievement were seen in areas of broad reading (94), written expression (88). High order cognitive skills such as reading comprehension were much lower (79) when compared to broad reading (94) skills, the mathematics reasoning cluster (96) was also low. The IQ scores and the educational assessment scores were compared to calculate discrepancy between potential and academic achievement for fulfilling the essential criteria in diagnosis of a learning disability. The significant discrepancies established in the areas of broad reading, reading comprehension, broad written expression suggested a language based learning disability. In spite of the child's innate intelligence skills of word decoding, spelling, fluencies were affected due to the poor processing speed.

DISCUSSION

Cytogenetic surveys of neonates have found that approximately one boy in 500 is born with an extra sex chromosome. Only about 10% children with KS are diagnosed prenatally, while another 25% are diagnosed during childhood or adulthood and alarmingly about two thirds of affected individuals tragically remain unidentified forever in their lifetime (Bogesen et al., 2003). Specific

learning disability especially in reading is seen in 50-75% of KS cases (Verri et al., 2010). Since children with KS have a predominantly language based learning disability, language difficulties are known to occur in almost 70-80% of them. Therefore, speech and language problems especially during early age of life, acts as a red flag sign in predicting and may actually predict the later academic achievement deficits (Boada et al., 2009). A recent study on the description of the cognitive phenotype of KS in a sample of 50 children concluded that specific language, academic, attention and motor abilities tend to be impaired and are a major cause of concern (Ross et al., 2008) Although the cognitive profile in KS is characterized typically by a low VIQ and high PIQ due to poor verbal skills versus the non verbal performance based skills in older children this discrepancy diminishes with advancing age (Verri et al., 2010). Many features in our assessment such as the language based learning disability, ADHD, social immaturity, poor motor coordination are seen to be in synch with that of the literature reviewed.

Due to the oral and written language problems of boys between 5 to 12 years of age, anticipatory guidance is recommended for these boys to prevent secondary maladaptive behaviors (Graham et al., 1988). Boys with KS do poorly on word decoding, written language skills and these deficits result in language based learning disability, for example, reading and spelling (Graham et al., 1988). The poor reading comprehension severely compromises their capacity to derive information from print. Clinicians should be aware of the possibility of KS in a child with physical features and behavioral and academic problems. The inability of these children to convey their specific needs to the teacher leads to behavioral problems in them and possible psychiatric complications later. KS boys are shy, immature, sensitive, anxious and prone to psychiatric ailments later in life. Difficulties in fine motor and coordination issues results in limited participation of sports related activities which are essential in developing social relationship and peer approval during the schooling period. These may further impact peer relations thereby limiting the child's social milieu. Awareness of this particular psycho educational and neurological profile can help in planning multidisciplinary intervention goals such as remedial education, occupational therapy, speech therapy, social skills training, behavior modification and medications. Psychological and emotional support to parents while communicating the diagnosis is vital and of equal importance (Graham et al., 1988). Although hormonal treatment at puberty improves motor skills, testis development it has no known impact on improving the child's cognitive ability. A language based learning disability with features of KS should justify karyotyping in a child given the high prevalence of the syndrome especially if behavioral issues are also present. Physicians should have a low threshold for obtaining a

karyotype in children with ADHD and learning disabilities, particularly in boys who are tall. Early detection could improve the prognosis of the learning and behavioral problems through medications and other non pharmacological interventions. Hence the diagnosis of KS should be considered in tall boys with characteristic physical features, language, academic problems as this disorder has a significant impact on learning and academic success.

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Full Length Research Paper

Seroprevalence of *Toxoplasma gondii* in couples in Ramadi City using enzyme linked immunosorbent assay (ELISA)

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Toxoplasma gondii is an obligate intracellular protozoan parasite that represents an actual public health problem. This study aims to investigate the prevalence of *T. gondii* among 91 couples in Ramadi City who were examined for the presence of antibodies against *T. gondii* using Enzyme Linked Immunosorbent Assay (ELISA). The overall anti-*T. gondii* (IgM and IgG) in both couples were 38.4%, the seroprevalence in wives was only 30.7%, while 13.1% in husbands only. The study showed that abortive women and women with abnormal pregnancies had the highest percentage rates, 35.7 vs. 57.14%, of toxoplasmosis within the age range of 26 and 30 years old, while the lowest rate was found among those within the average age range of 36 and 40 years old. One miscarriage was 50% higher than the other groups. The group of 26 to 30 years old showed high rate of IgM antibodies of about 66.66%. The number of abortion in the first trimester was high in both patterns of antibodies, IgM only and IgM and IgG (62.5 and 29.16%), respectively. Analysis of variance revealed that there were no significant interactions between IgM and IgG seropositivity and the gestational age of the fetus.

Key words: *Toxoplasma gondii*, pregnancy, the couples, seroprevalence.

INTRODUCTION

Toxoplasmosis is an important zoonotic parasitic disease that affects millions of people and is caused by the protozoan *Toxoplasma gondii*. In immune competent individuals, *T. gondii* preferentially infects tissues of central nervous systems, which might be an adding factor of certain psychiatric disorders (Reischl et al., 2003; Xiao et al., 2010). It is a ubiquitous obligate intracellular protozoan parasite, widely prevalent in humans and other animals across continents (Dodds, 2006; Weiss and Kim, 2007).

In Iraq, Niazi et al. (1988) found that the prevalence of *Toxoplasma* antibodies among women in Baghdad was 39%, whereas Niazi et al. (1992) reported low rate (8.6%) of positivity from eight governorates in Iraq. Mohammed and Al-Nasiry (1996) reported a prevalence rate of

20.4% toxoplasmosis in Iraqi women. In a study carried out in Basrah by Yacoub et al. (2006) the prevalence of *Toxoplasmosis* had been shown to be 41.1 to 52.1%, whereas a previous study by Al-Hamdani and Mahdi (1997) showed low rate of 18.5% of *Toxoplasma* antibodies in Basrah population. In Duhok, North of Iraq, Razzak et al. (2005) found low *Toxoplasma* infections of about 0.97%. This result indicated that the contribution of toxoplasmosis to fetal loss is greatly overestimated. In Sulaimania, Karem (2007) found out that by using ELISA, the seropositivity was 32.6% in women. In Baghdad, Juma and Salman (2011) found that the infection of *T. gondii* in women was 19.17%. In Tikrit, Al-Doori (2010) showed the presence of infection of about 49 to 95% and higher rate of infection lies among those of 25 to 31 years old in the women and their husbands.

Seroprevalence of *T. gondii* infection in man rises with age and it does not vary greatly between sexes (Montoya and Remington, 2000). The prevalence of *Toxoplasmosis* significantly increases with age and the highest

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Table 1. Seropositivity of anti-toxoplasma IgM and IgG detected by ELISA in examined samples.

Month	Number of couples	Both		Wife infected only		Husband infected only		No anti- <i>Toxoplasma</i> Abs.	
		Couples infected		No.	%	No.	%	No.	%
November	10	4	40	2	20	0	0	4	40
December	11	4	63.6	5	45.4	2	18.1	0	0
January	16	5	31.2	6	37.5	3	18.7	2	12.5
February	18	5	27.7	6	33.3	3	16.6	4	22.2
March	11	8	72.7	3	27.2	0	0	0	0
April	13	8	61.5	1	7.1	3	23	1	7.1
May	12	1	8.3	5	41.6	1	8.3	5	41.6
Total	91	35	38.4	28	30.7	12	13.1	16	17.5

seropositivity rate, 35.4% was found among pregnant women in the age group of 35 to 44 years old in Slovakia (Studenicova et al., 2006).

The overall seroprevalence of Toxoplasmosis in South African was 29/160 (18.1%). Seroprevalence in males and females were 7/42 (16.7%) and 22/118 (18.6%), respectively and the difference was not statistically significant ($p > 0.05$). The age distribution was 0.63% (1/160) for individuals of 20 years old and below, 10.6% (17/160) for those between 21 and 35 years old and 6.9% (11/160) for individuals who were 36 years old and above (Bessong and Mathomu, 2010).

The serologic evidence of toxoplasmosis in Ethiopia was found in 60% (39/65) of them. A large number of the seropositives were females (64.1%), while in male was 53.8% (Negash et al., 2008).

The overall anti-*T.gondii* IgG prevalence in China was 12.3%, the seroprevalence was 10.5% in men versus 14.3% in women (Xiao et al., 2010).

The purpose of this research is to investigate infections as a result of *T. gondii* in couples using Enzyme Linked Immunosorbent Assay (ELISA).

MATERIALS AND METHODS

One of the few methods frequently used in the detection of *T. gondii* infection in humans and animals is Enzyme-linked Immunosorbent assay (ELISA). In the ELISA test, soluble antigen is coated to microtiter plates and sample serum is added to form an antigen-antibody complex (if specific antibodies are present). A secondary enzyme-linked antibody specific to the host species is added to detect antigen-antibody complex. This test requires an ELISA reader and also enzyme conjugation to the secondary antibodies. Numerous modifications of ELISA have been reported to enhance specificity and simplify the protocol of the conventional ELISA (Dubey and Beattie, 1988).

Serum samples were collected from 91 clinically and laboratory confirmed *Toxoplasma* infected patients. The samples were collected during the period from November 2010 to May 2011 from clinical laboratory in Ramadi Hospital. Collected samples were stored at -20°C until we started the ELISA test.

This assay was performed by using two kits. One for detection of IgG and another one for detection of IgM specific antibodies against

T. gondii antigens in the patient's serum (Biokit Diagnostics Company, Spain).

Detection of IgG and IgM titers in all samples were analyzed for *T. gondii* by the titer of IgG and IgM antibodies using ELISA kit as described by Biokit Diagnostics Company, Spain. The optical densities (OD) of the samples were measured at 450 nm, using the OD value of the blank well to correct all the OD reading from test wells (Biokit Diagnostics Company, Spain).

RESULTS

Seroprevalence data obtained are shown in Table 1. The overall percentage of positive reaction to *T.gondii* in both couples was 38.4% (35/91), wife infected was only 30.7% (28/91), while infected husband was only 13.1% (12/91). The most frequent age group for abortive women and abnormal pregnancy was among those of 26 to 30 years old and it represents 35.7 and 57.14% of the total number of each group, respectively. Moreover, It was not observed to have a significant difference in the prevalence of *Toxoplasmosis* between the age groups ($P < 0.05$) (Table 2).

In Table 3, the samples of wives were divided into three groups, abortive women, abnormal pregnancy and normal pregnant women, each of them was subdivided into four groups (0, 1, 2 and 3 miscarriages). The rate of one miscarriage in abortive women was 50% (35/70), while 40% (28/70) and 10% (7/70) are for the two and three miscarriages, respectively. The statistical analysis revealed a high significant difference ($P < 0.05$).

The prevalence of IgM only in age group 26 to 30 years shows a high percentage (66.66%) and in age group 36 to 40 years shows a high prevalence of IgG (60%), while in age group 31 to 35 years shows a high prevalence of 50% for both IgM and IgG. Its correlation to the different age groups was not statically significant (Table 4). The prevalence of IgM recorded the highest number with two miscarriages [58.83% (10/17)], then 23.52% (4/17) for IgG antibodies, while the percentage of both IgM and IgG recorded the highest number of one miscarriage [44.44% (8/18)] (Table 5). Statical analysis revealed a non

Table 2. Age group distribution in examined samples.

Age groups (year)	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
≤ 20-25	21	30	1	14.28	3	21.42
26 – 30	25	35.71	4	57.14	3	21.42
31 – 35	15	21.42	1	14.28	3	21.42
36 -40	9	12.85	1	14.28	5	35.71
Total	70	100	7	100	14	100

Statistical analysis cal. $\chi^2 = 5.817$; tab. $\chi^2 = 14.06$; $P \leq 0.05$.

Table 3. Ratio of previous miscarriages in examined samples.

Number of miscarriages	Abortive women		Abnormal pregnancy		Normal pregnant women	
	No.	%	No.	%	No.	%
0	0	0	5	100	2	12.5
1	35	50	0	0	7	43.7
2	28	40	0	0	6	37.5
≥3	7	10	0	0	1	6.2
Total	70	100	5	100	16	100

Statistical analysis cal. $\chi^2 = 67.16$; tab. $\chi^2 = 9.488$; $P \leq 0.05$.

Table 4. Seropositivity of anti-*Toxoplasma* IgG and IgM in relation to participants' age.

Age groups (year)	IgM +ve		IgG +ve		IgM +ve and IgG +ve		Total	
	No.	%	No.	%	No.	%	No.	%
≤ 20 to25	7	58.33	2	16.66	3	25	12	100
26 to 30	8	66.66	2	16.66	2	16.66	12	100
31 to 35	3	50	0	0	3	50	6	100
36 to 40	1	20	3	60	1	20	5	100

Statistical analysis cal. $\chi^2 = 8.59$; tab. $\chi^2 = 14.06$; $P \leq 0.05$.

Table 5. Ratio of anti- *Toxoplasma* antibodies according to the number of miscarriages in infected couples.

Number of miscarriages	Pattern of antibody							
	IgM +ve		IgG +ve		IgM +ve and IgG +ve		Total	
	No.	%	No.	%	No.	%	No.	%
0	0	0	0	0	0	0	0	0
1	7	38.88	3	16.66	8	44.44	18	100
2	10	58.83	4	23.52	3	17.64	17	100
≥3	0	0	0	0	0	0	0	0

Statistical analysis cal. $\chi^2 = 2.9$; tab. $\chi^2 = 3.841$; $P \leq 0.05$.

significant interaction between IgM and IgG seropositivity and the gestational age of the fetus. Comparable results of seropositivity of both IgM and IgG obtained in the first trimester gave a high percentage in IgM [62.5% (15/24)] and both IgM and IgG [29.16% (7/24)], while the number of women in their third trimester indicated no anti-

Toxoplasma antibodies (Table 6).

DISCUSSION

The main purpose of this study was to evaluate the

Table 6. Seropositivity of anti-*Toxoplasma* IgG and IgM in relation to gestational age.

Gestational age	Pattern of antibody							
	IgM +ve		IgG +ve		IgM +ve and IgG +ve		Total	
	No.	%	No.	%	No.	%	No.	%
First trimester	15	62.5	2	8.33	7	29.16	24	100
Second trimester	4	36.36	3	27.27	4	36.36	11	100
Third trimester	0	0	0	0	0	0	0	100

Statistical analysis cal. $\chi^2 = 2.97$; tab. $\chi^2 = 3.841$; $P \leq 0.05$.

seroprevalence of *T. gondii* antibodies between couples of Ramadi City. The overall seroprevalence of *Toxoplasma* in this study among both couples was 38.4% and when compared to the previous results of pregnant women, demonstrated a decreasing seroprevalence compared with the published data (Yacoub et al., 2006; Al-Rawi, 2009; Al-Doori, 2010; Juma and Salman, 2011), while other studies were in agreement with this results (Al-Khafajy, 2004; Al-Musauy, 2008).

These variable results may be due to the differences in the specimens used by each researcher and their variable condition and data of studies.

The study found that seroprevalence of Toxoplasmosis in wives was higher than in husbands. These results were similar to Negash et al. (2008), Bessong and Mathomu (2010), Xiao et al. (2010) and Sroka et al. (2010). One of the reasons for this high prevalence is related to women handling raw meat more frequently than men due to the fact that they spend more time cooking at home.

There are several causative factors responsible for both habitual and sporadic abortions. However, the prevalence of toxoplasmosis in women with bad obstetrics history is known to be significantly higher than in normal. The seroprevalence in pregnant women on worldwide scale varies from 7 to 51.3% and in women with abnormal pregnancies and abortions the seroprevalence varies from 17.5 to 53.3% (Kumar et al., 2004).

The seropositivity rate of abortive women in age group 26 to 30 years old was obviously higher (53.71%) than in other groups, which was similar to the results reported in Iraq (Shani, 2004; Kadhim, 2006; Al-Rawi, 2009; Juma and Salman, 2011). This is presumably due to the high presence of cats, climatic, hygienic and socioeconomic conditions in the regions. However, it is acknowledged that seroprevalence increases with age, as seen in studies conducted in various countries (Dodds, 2006).

Women who may get infection during pregnancy may show a variety of clinical signs and symptoms depending on many factors, such as the number of parasites, virulence of strain, and the time period the mother acquires infection (Tenter et al., 2000). If the mother is infected in the first trimester, the result is abortion, stillbirth or severe disease of fetus (Lin et al., 2000).

On the other hand, IgM antibodies titer to *T. gondii* was found to be more than IgG antibodies. Clearly, the overall prevalence of IgM antibodies was interpreted as a diagnosis of the acute form of the disease. In the present survey, it was shown that chronic form (which shows prevalence of IgG antibodies) was increased with age 16.66 to 60%. These results reflected the contact with cats or infected materials and vegetables in these age groups.

These results pointed out that most IgM and both IgM and IgG patterns of antibodies were increased in first trimester (62.5 and 29.16%, respectively), these patterns of antibodies were absent in third trimester. The severity of disease decreases if the infection occurs in the second or third trimester, but the risk for transmission from mother to fetus increase (Romand et al., 2001). In pregnant women, the primary infection of *T. gondii* may cause abortion, neonatal malformation, neonatal death, or severe congenital deficiency, such as mental retardation, retinochoroiditis, and blindness (Kravetz and Federman, 2005). In addition, Toxoplasmosis is one of the main causes of fetal abortion, stillbirth, and neonatal mortality in domestic animals, resulting in significant economic loss in the farming industry (McCallister, 2005).

Congenital Toxoplasmosis is most severe when the mother becomes infected in the first trimester, then approximately 10 to 20% of fetuses are infected. If the infection is acquired in the second trimester, 30 to 40% of fetuses are infected, but the disease is mild or asymptomatic at birth. These differences in transmission may be related to the placental blood flow, size of uterus, virulence of the parasite or to the immunocompetence of the mother (Singh, 2003).

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Full Length Research Paper

A new craniosynostosis syndrome

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We report on a patient with bilateral multiple craniosynostosis involving the coronal and lambdoid sutures, high myopia, obesity, vertebral anomalies, minor acral anomalies and normal intelligence. The clinical features are not typical of any known craniosynostosis syndrome. Search of POSSUM, London Dysmorphology Database (LDDb), online Mendelian Inheritance in Man (OMIM), and the medical literature failed to find any similar case. The constellation of manifestations in this patient suggests a previously unrecognized syndrome resembling Carpenter syndrome.

Key words: New syndrome, craniosynostosis, Carpenter syndrome.

INTRODUCTION

Craniosynostosis (premature suture fusion) can be either classified as simple or multiple. In simple craniosynostosis, only one suture is involved. In multiple craniosynostosis, two or more sutures are synostosed. Multiple synostosis occurs in approximately 5% of nonsyndromic cases of craniosynostosis (Cohen and MacLean, 2000). Two-suture synostosis accounts for about two-thirds of the cases, while more than two sutures are involved in one-third of the cases. The more sutures synostosed, the greater the risk for an individual to bear mental retardation. About 35% of cases with more than two sutures synostosed show lacunae (small cavities within the bone matrix containing osteocytes) in their skull radiographies (Cohen and MacLean, 2000).

A number of craniofacial syndromes with multiple suture synostosis have considerable overlap in their manifestations. Bilateral coronal synostosis is the most commonly observed, however bilateral coronal and lambdoid synostosis or a combination of bilateral coronal synostosis and sagittal synostosis have also been noted, amongst other combinations (Barkovich, 2000). When the sagittal suture is synostotic in conjunction with both coronal sutures (and, sometimes, both lambdoid sutures), the membranous bone of the calvaria expands between the sutures, resulting in a characteristic lobulated skull configuration known as cloverleaf skull [Kleeblattschadel] (Angle et al., 1967; Lodge et al., 1993; Cohen, 1993; Goh et al., 1997). Carpenter syndrome, although it was first described in 1901 and 1909 by George Carpenter (British physician), it was not recognized as a distinct nosologic

and genetic entity, and as a new syndrome (acrocephalopolysyndactyly type II) until Samia Temtamy's article (Egyptian physician) in 1966. Sakati et al. (1971) have suggested that this syndrome might more appropriately be called the Temtamy syndrome, as the sisters originally reported by Carpenter did have cranial sutures and had other differences from Temtamy's patient and others reported since. About 100 cases have been described in the worldwide medical literature, therefore, Carpenter syndrome is one of the rarest forms of the craniofacial disorders and has an estimated occurrence rate of approximately one in a million male and female live births. In addition to the multiple suture craniosynostosis, which may present as a cloverleaf skull, the most characteristic abnormality of the Carpenter syndrome is the presence of polydactyly of the fingers and or toes; brachydactyly and syndactyly of the hands (Saxena et al., 1970; Cohen, 1979; Goodman et al., 1979).

Other abnormalities include congenital heart defects, obesity, short stature and mental retardation (Robinson et al., 1985; Cohen and MacLean, 2000), and also bilateral sensorineural hearing loss (Tarhan et al., 2004). Mild to moderate mental deficiencies are common (about 75% of all cases) and IQ score have ranged from 52 to 104 (Frias et al., 1978; White et al., 1987; Jamil et al., 1992; Richieri-Costa et al., 1993).

Autosomal recessive inheritance is supported by the reports of families with consanguinity (Der Kaloustian et al., 1972; Richieri-Costa et al., 1993) and multiple

affected siblings (Carpenter, 1901; Frias et al., 1978; Cohen et al., 1987). Sporadic cases have also been reported by some authors (Sunderhaus and Wolter, 1968; Eaton et al., 1974; Cohen, 1975). Most of the affected patients have a surgical procedure between 3 and 9 months of age to open the cranial vault to make space for the brain to grow (McCarthy et al., 1978). Hidestrand et al. (2009) reported an adult patient with Carpenter syndrome who was unusual in that she has never had surgical intervention.

MATERIALS AND METHODS

We report on a patient with manifestations resembling Carpenter syndrome including multiple suture craniosynostosis, obesity, short stature and syndactyly of the feet. She was born in 1990, and was referred to our department for diagnosis and further investigations at birth, by her pediatrician. We followed up her health condition for 15 years until she left and immigrated to other country.

RESULTS

Clinical report

The proposita presented with craniosynostosis and multiple congenital anomalies was the second child born to a non-consanguineous Ashkenazi Jewish couple. The parents were both 33 years old at the time of birth and phenotypically normal. They had an older healthy daughter. The family history was negative for craniosynostosis.

Pregnancy history was unremarkable. The delivery was at 37 weeks of gestation by Cesarean section. Her birth weight was 2296 g (<3rd centile), OFC was 28.5 cm (<2nd centile) and length was 48.2 cm (20th centile). The abnormal head shape was noted at birth, along with a single umbilical artery. Physical exam at the age of 12 days showed an alert, small and active baby with an elongated head and large anterior fontanel (5x7 cm). She had an apparent hypertelorism, with short palpebral fissures, and shallow orbits. There was a flat nasal bridge with upturned nasal tip. She had a long philtrum. Her ears, oral cavity, chin and neck were unremarkable.

Head CT scan confirmed premature bilateral synostosis of the coronal and lambdoid sutures. The remaining sutures appeared to be patent. However, the base of the skull appeared quite short and there was some parietal bossing consistent with a Kleeblattschadel (cloverleaf skull). Bony dysplasia was present in the parietal occipital region. The calvarium did not appear to be enlarged. Hypertelorism was also noted on the skull X-ray, but did not show any lacunae. Renal ultrasound was normal.

The patient had several surgeries at the age of 2 weeks, 11 months and 2.5 years for suture release and cranial vault reshaping. In addition, she had tonsillectomy and adenoidectomy at 6 and 7 years of age. Her physical

and mental development has been normal and she was studied at the public school.

At the age of 10 years, examination showed her head circumference was 50.5 cm (20th centile), her height was 147.5 cm (95th centile) and her weight was 42.5 kg (95th centile). Her head appeared to be large with turricephalic shape with some bitemporal and biparietal narrowing and mild supra-auricular bossing. She had a round face with mild bilateral ptosis and slight antimongoloid slant of palpebral fissures. Her inner canthal distance was 3 cm and outer canthal distance was 9 cm. Her thumbs were relatively slender and there was mild fifth finger clinodactyly. Her feet were well formed.

At the age of 15 years, on examination, she was a very cooperative girl who had thick glasses for myopia and appeared obese. Her OFC was 51 cm (<2nd centile), height 151 cm (3rd centile) and weight 70.2 kg (95th centile). She had a high forehead with bitemporal narrowing and round face. She had shallow orbits with slightly prominent eyes, bilateral upper lid ptosis and recession of the lateral portion of the orbits. She had a narrow palate, retrognathia, and dental malocclusion (wears orthodontic brace). Her feet were small and had partial second and third toe syndactyly. Skin examination was normal with no evidence of acanthosis nigricans. Examination of cardiac, uro-genital and other organs was unremarkable.

Three-dimensional CT scan of the brain showed mild brachycephaly with turricephaly, but bilateral symmetry through the cranium, skull base and maxillo-facial skeleton, except for mild flattening of the right parieto-occipital region. Facial slope was flat in the mid-orbital and mid-maxillary regions, with bilateral mild to moderate proptosis.

Skeletal survey showed changes in the skull consistent with prior craniosynostosis repair, calcification of the anterior longitudinal ligament in the cervical spine with minor changes of bone formation, and elongated vertebral body height, most evident in the lumbar spine. Asymmetric spinal dysraphism in lower lumbar/upper sacral segments, and mild bilateral hallux valgus were also noticed. Orthopedic examination and the X-ray of the flexion/extension views of the neck showed that there were some congenital abnormalities of the vertebral bodies with some anterior osteophytes, but she seemed to have a good range of motion of her neck and was stable.

Ophthalmological examination showed severe myopia of -14 Diopters. Her chromosomal study was normal.

Mutation analysis for *FGFR1* (P252R), *FGFR2* (exons IIIa and IIIc), *FGFR3* (P250R), and *TWIST* (entire coding region) genes revealed no mutations (O'Rourke et al., 2002; Morriss-Kay and Wilkie, 2005).

Differentiation of clinical features of Carpenter syndrome and our patient are shown, in most details, in Table 1. Unfortunately, our patient and her parents were not consented to take photographs of her.

Table 1. Differentiating characteristics of Carpenter syndrome and presented patient.

Manifestation	Carpenter syndrome	Presented patient
Craniosynostosis sutural involvement	Sagittal, bilateral coronal and lambdoid	Bilateral coronal and lambdoid
Acrocephaly/turricephaly	+	+
Brachycephaly	+	+
Clover leaf skull	+	+
Short neck	+	-
Sloping/high forehead	+	+
Flat nasal bridge	+	+
Upturned nasal tip/anteverted nostrils	+	+
Hypertelorism	+	+
Shallow orbits	+	+
Bilateral ptosis	+	+
Epicanthic folds	+	+
Sclerocornea/microcornea/optic atrophy	+	-
High myopia	-	+
Midfacial hypoplasia (flat facial profile)	+	-
Micrognathia/retrognathia	+	+
High-arched/narrow palate	+	+
Dysplastic ears	+	-
Low-set ears	+	-
Single flexion crease of the hands	+	-
Polydactyly of the hands	+	-
Partial syndactyly and camptodactyly of the hands	+	-
Brachydactyly of the hands	+	-
Clinodactyly of the hands	+	+
Preaxial polydactyly of the feet	+	-
Partial syndactyly of the feet	+	+
Hypoplasia/aplasia of the middle phalanges of fingers and toes	+	-
Subluxation at distal interphalangeal joints	+	-
Angulation deformities at knees	+	-
Umbilical hernia	+	-
Omphalocele	+	-
Postnatal growth less than 25 th centile	+	+
Short stature	+	+
Acral anomalies	+	+
Obesity	+	+
Genital abnormalities/hypogenitalism	+	-
Mental deficiency/developmental delay	Common	-
Congenital heart defects (ASD, VSD, PDA, PS, TF, TGV)	Common	-
Genu valgum	Common	-
Lateral displacement of patellae	Common	-
Vertebral anomalies	Occasional	+
Proptosis	Occasional	+
Lacunae (in skull x-ray)	Occasional	-
Marked cranial asymmetry	Occasional	-
Slight downslanting palpebral fissures	Occasional	+
Dental problems (partial anodontia/malocclusion)	Occasional	+
Hearing loss (conductive and neurosensory)	Occasional	-
Duplication of second phalanx of thumbs	Occasional	-
Hallux valgus	Occasional	+
Coxa valga	Occasional	-

Table 1. Contd.

Metatarsus varus	Occasional	-
Flare to pelvis	Occasional	-
Flat acetabulum	Occasional	-
Absent coccyx	Occasional	-
Spina bifida occulta	Occasional	-
Scoliosis	Occasional	-
Pilonidal dimple	Occasional	-
Hydronephrosis with/without hydroureter	Occasional	-
Accessory spleen	Occasional	-
Precocious puberty	Occasional	-
Inheritance	AR	AR

AR, Autosomal recessive. ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TF, tetralogy of Fallot; TGV, transposition of the great vessels.

DISCUSSION

Multiple craniosynostosis including coronal and lambdoid sutures is present in a vast range of syndromes (Cohen and MacLean, 2000).

The constellation of anomalies in our patient is dissimilar to those previously reported. However our patient does resemble Carpenter syndrome, particularly with regard to the craniosynostosis, which appeared to cause a Kleeblattschadel anomaly, syndactyly of the feet, short stature and obesity. The differences, however allow nosologic splitting.

A few authors observed Carpenter syndrome among siblings (Gershoni-Baruch, 1990; Islek et al., 1998). Perlyn and Marsh (2008) reported a retrospective review on three siblings, all affected with Carpenter syndrome. They concluded that the diverse anatomical variation seen in these three siblings supports the notion of marked phenotypic variability within this syndrome. Therefore, our patient may represent an extension of the Carpenter syndrome phenotypic or more probable a new craniofacial syndrome. Further research will help resolve this and similar case reports, in the medical literature.

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Full Length Research Paper

Prevalence of β -lactamase producing and non-producing *Staphylococcus aureus* associated with patients in intensive care unit

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A total of one hundred and twenty five samples were collected from the intensive care unit of two main hospitals in Basrah: 74 clinical samples; Skin, blood, eye, nose, wounds and urine and 51 inanimate samples; bed, wall, instruments and addresses. A total of 334 isolates of bacterial types isolated from various sources and their percentages are shown as follows: *Staphylococcus aureus* 45 (13.47%), *Staphylococcus epidermidis* 31 (9.28%), *Staphylococcus saprophyticus* 18 (5.38%), *Staphylococcus xylosus* 11 (3.29%), *Staphylococcus capitis* 7 (2.09%), *Streptococcus pyogenes* 28 (8.38%), *Viridance streptococci* 35 (10.47%), *Streptococcus pneumonia* 12 (3.59%), *Pseudomonas aeruginosa* 41 (12.27%), *Escherichia coli* 19 (5.68%), *Klebsiella* spp. 20 (5.98%), *Proteus* spp. 10 (2.99%), *Enterobacter* 9 (2.69%), *Propionibacterium acnes* 24 (7.18%), *Acinetobacter* spp. 9 (2.69%) and *Corynebacterium* spp. 15 (4.49%). 31 isolates of *S. aureus* in percentage 68.89 were isolated as β -lactamase producers, while 14 isolates (31.11%) were β -lactamase non-producers. The prevalence of multiple drugs resistance *S. aureus* against eight antibiotics were carried in the present study, the resistance against three antibiotics has a biggest percentage (25.8) for β -lactamase producing *S. aureus* with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β -lactamase non-producing *S. aureus* in percentage 35.71% with no resistance against more than four antibiotics. The study found that vancomycin, cefotaxime and gentamicin were the most effective antibiotics against β -lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of intensive care unit in percentages of resistance (42.22, 44.44 and 44.44%), respectively and tetracycline was the antibiotics that has the highest percentage of resistance (82.22%) against the above strain. While for β -lactamase non-producing *S. aureus* strains, vancomycin and cefotaxime were also the most effective antibiotics but have the lowest percentages of resistance in comparison to the first above group which recorded (13.33 and 20.0%) of resistance respectively. And tetracycline still the weakest antibiotic has great resistance of 53.82% of isolates. The plasmid profiles in β -lactamase producing and non-producing MDRSA was also determined in this study. The ranges of band molecular weight were between 300 to 600 base pairs with clear main band in 550-570 bp for β -lactamase producing *S. aureus*. While the ranges of band molecular weight were between 200 to 700 base pairs with clear main band in 450-470 bp and 690-700 bp for β -lactamase producing *S. aureus*.

Key words: *Staphylococcus aureus*, antibiotics, β -lactamase, intensive care units.

INTRODUCTION

The hospital environment is uniquely suited to the spread of infections as it houses both susceptible patients and patients with difficult-to-treat infections. There is a great risk that some patients may contract hospital-associated infections other than those they were admitted for

because of nosocomial pathogens around them (Chambers, 2001). The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes (Anupurba et al., 2003).

Staphylococcus aureus, a spherical aerobic Gram positive, catalase positive, oxidase positive, non-motile, spore-forming coccus, is an opportunistic pathogen in human and animal, and is one of the most frequent sources of hospital- and community-acquired infections. Generally, *S. aureus* is responsible for superficial infections and toxic epidermal necrolysis, systemic infections, such as endocarditis inflammation of bone or bone marrow, pneumonia and toxinoses, such as food poisoning or toxic shock syndrome. However, among gram-positive cocci, only β -lactamase of major clinical significance is Staphylococcal β -lactamase, which rapidly hydrolyses benzylpenicillin, ampicillin, cephalosporins, and related antimicrobials (Foster, 1996; Boyce, 1994). Methicillin-resistant *S. aureus* (MRSA) is a special strain of *S. aureus* that is resistant to the antibacterial activity of methicillin and other related antibiotics of the penicillin class. Although, MRSA has traditionally been seen as a hospital-associated infection, community-acquired MRSA strains have appeared in recent years, notably in the USA and Australia (Shakibaie et al., 2002). Several new strains of MRSA have been found showing antibiotic resistance even to vancomycin and teicoplanin; these new evolutions of the MRSA bacteria are called Vancomycin Intermediate-resistant *Staphylococcus aureus* (VISA) (Tuo et al., 1995; Sampathkumar, 2007). Community-acquired MDRSA (multi drugs resistant *S. aureus*) infections in the absence of identified risk factors have been reported. Many outbreaks of infections due to MDRSA have occurred and it has now become endemic in several centers in the world (Schito, 2006; Mansouri and Khaleghi, 1997). The emergence of community acquired MDRSA that is capable of causing infections in otherwise healthy people has also been reported (Sieradzki and Tomasz, 1997). Staphylococcal antibiotic resistance has been associated with resistant plasmids that have the ability to mediate the production of drug inactivating enzymes such as β -lactamases (Daini and Akano, 2009) and other functions (Diep et al., 2008). MDRSA also differ in their resistance to antibacterial agents and in the genetic location of these resistance determinants. Studies have shown that the genetic determinants for antibiotic resistance reside on plasmids, chromosomal DNA, or on transposable elements (Diep et al., 2006; Maltezou and Giamarellou, 2006). In Bangladesh, as reported previously, the frequency of MDRSA was alarming due to indiscriminate and incomplete uses of antibiotics (Rahman et al., 2002). In 2002, 47.2% MDRSA was reported in an investigation on clinical *S. aureus* isolates (Diep et al., 2006). Both of these prevalence rates of MRSA were higher than the rate in some developed countries like Austria 21.6%, Belgium 25.1%, Spain 30.3%, and France 33.6% (Gradie et al., 2001). Therefore, the current situation of the susceptibility patterns of local strains is essential for the judicious use of antibacterial agents as well as to become aware of the MDRSA in hospitals and community arenas.

Staphylococcus aureus is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (Anupurba et al., 2003; Mansouri and Khaleghi, 1997). *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis and mycosis (Herwaldt and Wenzel, 1996). They live as commensals in anterior nares of over half the population of humans (Hotu et al., 2007). The cocci are spread from these sites into the environment by the hands, handkerchief, clothing and dust.

S. aureus is an opportunistic pathogen in the sense that it causes infection most commonly in tissues and sites with lowered host resistance such as in individuals with diabetes, old malnourished persons and other chronic cases (Foster, 1996; Maltezou and Giamarellou, 2006). *S. aureus* causes folliculitis, boil, furunculosis, scalded skin syndrome, conjunctivitis, paronychia, mastitis, and toxic shock syndrome for menstruating women who use tampons. Staphylococcal pneumonia can occur if staphylococcal infection spreads to the lungs (Foster, 1996). Hospital acquired Staphylococcal infections are common in newborn babies, surgical patients and hospital staff. Patients develop sepsis in operation wounds, which take place in the theatre during operation, and others post-operations in the ward (Hsueh et al., 2005). Attempts to control these diseases by chemotherapy through the use of antimicrobial agents particularly antibiotics have resulted in increased prevalence of resistance to these agents (King et al., 2006). Several investigations have been conducted to study the antimicrobial resistance pattern of *S. aureus* and it has been shown that the organism is resistant to β -lactam antibiotics, amino glycoside and macrolides (Francis et al., 1997; Marples and Reith, 1996). *S. aureus* strains carries a wide variety of multi-drug resistant genes on plasmids, which can be exchanged and spread among different species of Staphylococci (Mehta et al., 1998). The multi-resistance determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Iraq where antimicrobial drugs are readily available to consumers across the counter with or without prescription from a medical practitioner. Such a practice has led to misuse of antimicrobial drugs with the associated high prevalence of drug resistance among the Staphylococci (Foster, 1996; O'Brien et al., 1999; Okuma et al., 2002).

Hospital strains of *S. aureus* are usually resistant to a variety of different antibiotics. Few strains are resistant to all clinically useful antibiotics except vancomycin. Some workers have reported however the presence of vancomycin resistant strains (Daini and Akano, 2009; Olukoya et al., 1995).

This work was undertaken to determine the prevalence of β -lactamase-producing and non-producing *S. aureus* associated with patients and inanimate sources in intensive care of hospital populations in Basrah hospitals

of Iraq.

MATERIALS AND METHODS

Sample collection

Various clinical and inanimate swabs were collected from hospitalized patients in intensive care unit of two main hospitals in Basrah city (Al-Sadder teaching and general Basrah hospitals) from June to November, 2011, using sterile swab saturated with Brain – Heart Infusion. All specimens were transported immediately to the laboratory and cultured within 3 to 4 h of collection.

Isolation and characterization of bacteria

The swab specimens were inoculated on various ordinary media; blood agar base, nutrient agar, MacConkey agar (Hi Media, India) to obtain discrete colonies. The plates were incubated at 37°C for 24 h under aerobic conditions. After 24 h of incubation, the culture plates were examined recording the appearance, size, color, and morphology of the colonies. Gram stain reaction, catalase test and coagulase test growth on differential and selective media such as mannitol salt agar, triple sugar iron agar, eosin methylene blue agar (Hi Media, India) and other biochemical tests were carried out according to standard techniques (Forbes et al., 2002; Cowan and Steel, 2004).

Isolates that were gram-positive, that is, cocci, catalase positive, coagulase positive, and form yellow colonies on mannitol salt agar were considered *S. aureus* in this study.

Susceptibility of Isolates to various antibiotics

Antibiotic sensitivity test was carried out on all isolates using paper disc diffusion technique. A total of eight antibiotics were tested. A 0.1 ml of 18 h brain heart infusion culture of the test organism was used to inoculate on a dry sterile Mueller-Hinton agar plate by using a sterile glass L-shaped spreader and allowed to dry for about 15 to 30 min. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antibiotic discs were then incubated at 37°C for 24 h to observe the zones of growth inhibition produced by the antibiotics. The antimicrobial disks were sourced from the HiMedia Laboratories Ltd., Mumbai, India as follow:

Tetracycline (TET), Gentamicin (GEN), Amoxicillin (AMOX), Ciprofloxacin (CIP), cefotaxime (CEF), Amoxycillin/Clavulanic Acid (AMOX/CLA), Vancomycin (VAN), Methicillin (METH).

The zone diameters measured around each disk were interpreted on the basis of Bauer et al. (1966), according to guidelines by the NCCLS (2002).

β -Lactamase test (Adegoke and Komolafe, 2009; Ekramul et al., 2011)

β -Lactamase production was assayed by the acid-formation method. A piece of Whatman No.1 filter paper (5x6) was briefly placed in a sterile Petri dish. The bluish penicillin solution was added drop wise to saturate the paper. Thick masses of bacterial colonies of the test organism were transferred with a bacteriological loop from the test culture to the filter paper and spread over an area of 5 mm diameter. The paper was then incubated at 37°C for 30

min with the Petri dish covered. The paper was examined and yellow zones formed by β -lactamase producing strains were noted.

Plasmid profile and molecular studies on MDRSA strains (Maniatis et al., 1989; Adeleke and Odelola, 1997; Hamid et al., 2011)

Plasmid profiles of 14 MDR *S. aureus* strains were determined in Laboratory of Biotechnology, College of Veterinary Medicine and Oklahoma Laboratory of Biotechnology, College of Science, University of Basrah by the Mini Prep method followed by band separation on horizontal gel electrophoresis in 1.5% agarose in 1X Tris-Borate-EDTA (TBE) buffer at room temperature (Lech and Brent, 1987; Kraft et al., 1988). Briefly, single purified bacterial colonies were seeded each into 10 ml Mueller-Hinton broth (HiMedia) in screw cap tubes and incubated overnight at 37°C. After centrifugation of 1.5 ml of the overnight culture for 1 min, the pelleted cells was dissolved in 300 μ l of TENS solution (Tris 25 mM, EDTA 10 mM, NaOH 0.1 N and SDS 0.5%), the tube inverted a few times for thorough mixing and iced for 5 min. An addition of 150 μ l of 3.0 M sodium acetate (pH 5.2) was made and the tube vortexed until it was completely mixed. The solution was micro-centrifuged for 5 min at 13,000 rpm to pellet cell debris and chromosomal DNA.

Supernatant (400 μ l) was decanted into fresh Eppendorf tube, mixed with 800 μ l ice-cold absolute ethanol, and centrifuged for 10 min to pellet the plasmid DNA. The supernatant was discarded; pellet rinsed twice in 1 ml of 70% ice-cold ethanol and dried at 45°C for 15 min. The dried pellet was re-suspended in 40 μ l TE buffer and stored at 4°C till further analysis. For the separation of plasmid DNA, a horizontal tank loaded with 5 mm agarose gel stained with 20 μ l of 1 mg/ml ethidium bromide was connected to a power supply at 80 V for 4 h. The loading dye used was bromocresol purple. For each well, 15 μ l of plasmid DNA solution was mixed with 2 μ l loading dye, carefully loaded onto the gel and allowed to run for 2 h. DNA bands were visualized and photographed using digital camera (Sony, 7.2 megapixel). The molecular weight of unknown plasmid DNA was extrapolated using the band mobilities in the gel.

Statistical analysis

The results were statistically analyzed by using ANOVA and T-test in the SPSS (Statistical Package for the Social Sciences) package (Version 17).

RESULTS

A total of one hundred and twenty five samples were collected from the intensive care unit of two main hospitals in Basrah (74 clinical samples and 51 inanimate samples) (Table 1), with no statistical differences at $P \geq 0.05$:

1. Clinical samples: Skin 16, blood 10, eye 11, nose 11, wounds 14, and urine 12 samples.
2. Inanimate samples: bed 13, wall 12, instruments 15 and addresses 11 samples.

Table 2 show the total of 334 isolates of bacterial types isolated from various clinical and inanimate sources of

Table 1. Numbers of samples collected from clinical and inanimate sites.

Samples type		Numbers of samples*	%
Clinical samples	Skin	16 [#]	12.80
	Blood	10	8.00
	Eye	11	8.80
	Nose	11	8.80
	Wounds	14	11.20
	Urine	12	9.60
Inanimate samples	Bed	13	10.4
	Wall	12	9.60
	Instruments	15	12.00
	Dresses	11	8.80
Total		125	100

*: One sample was taken from each types at time once. #: there are no statistical differences between sites of postoperative wounds ($P \geq 0.05$).

Table 2. Bacterial types isolated from different clinical and inanimate sources of intensive care unit.

Bacterial types	Total number of isolates (%)	Numbers of isolate (%)									
		Skin	Blood	Eye	Nose	Wounds	Urine	Bed	Wall	Instruments	Dresses
<i>Staphylococcus aureus</i>	45(13.47)**	5	3	2	4	7	5	5	3	9	2
<i>Staphylococcus epidermidis</i>	31(9.28)	7	0	5	0	2	0	4	4	6	3
<i>Staphylococcus saprophyticus</i>	18(5.38)	2	0	0	2	4	4	1	0	3	2
<i>Staphylococcus xylosus</i>	11(3.29)	2	0	1	1	2	2	0	0	2	1
<i>Staphylococcus capitis</i>	7(2.09)	2	0	0	0	1	0	1	0	2	1
<i>Streptococcus pyogenes</i>	28(8.38)	4	3	2	1	6	0	3	2	5	2
Viridance streptococci	35(10.47)	7	3	4	3	3	0	3	4	5	3
<i>Streptococcus pneumoniae</i>	12(3.59)	2	2	2	0	2	0	1	0	2	1
<i>Pseudomonas aeruginosa</i>	41(12.27)	4	0	2	2	6	4	4	6	10	3
<i>Escherichia coli</i>	19(5.68)	3	0	0	2	4	3	1	0	4	2
<i>Klebsiella spp</i>	20(5.98)	2	0	2	0	2	3	2	2	5	2
<i>Proteus spp</i>	10(2.99)	0	0	0	1	3	2	0	0	2	2
<i>Enterobacter</i>	9(2.69)	1	0	0	1	2	2	1	0	2	1
<i>Propionibacterium acnes</i>	24(7.18)	7	0	2	2	3	0	2	2	4	2
<i>Acinetobacter spp</i>	9(2.69)	2	0	0	1	2	1	1	0	2	0
<i>Corynebacterium spp</i>	15(4.49)	3	2	3	0	2	0	0	0	3	2
Total	334	53 (15.86)	13 (3.89)	25 (7.48)	20 (5.98)	51 (15.26)	26 (7.78)	28 (8.38)	23 (6.88)	66 (19.76)	29 (8.68)

** : There are a highly statistically differences between numbers of isolates and various clinical and inanimate sites $P < 0.01$.

Table 3. Categorization of β -lactamase producing and non-producing *S. aureus* isolates.

Type of isolates	Total no of strains (%)	Numbers of isolates (%)									
		Skin	Blood	Eye	Nose	Wounds	Urine	Bed	Wall	Instruments	Dresses
β -lactamase producing <i>S. aureus</i>	31** (68.89)	3 (9.67)	3 (9.67)	1 (3.22)	2 (6.45)	7 (22.58)	3 (9.67)	3 (9.67)	2 (6.45)	6 (19.35)	1 (3.22)
β -lactamase non-producing <i>S. aureus</i>	14 (31.11)	2 (14.28)	0	1	2 (14.28)	0	2 (14.28)	2 (14.28)	1 (7.14)	3 (21.43)	1 (7.14)
Total	45 (100.00)	5 (11.11)	3 (6.66)	2 (4.44)	4 (8.89)	7 (15.55)	5 (11.11)	5 (11.11)	3 (6.66)	9 (20.0)	2 (4.44)

** : There are a highly statistically differences between numbers of β -lactamase producing and non-producing *S. aureus* isolates in various clinical and inanimate sites $P<0.01$.

Table 4. Prevalence of Multiple drugs resistance isolates of β -lactamase producing and non- producing *Staph. aureus*.

Type of isolates	Total no of tested strains (%)	Numbers of resistant isolates (%)									
		0	1	2	3	4	5	6	7	8	
β -lactamase producing <i>Staph. aureus</i>	31** (68.89)	3 (9.67)	3 (9.67)	5 (16.13)	8 (25.80)	5 (16.13)	3 (9.67)	2 (6.45)	1 (3.22)	1 (3.22)	
β -lactamase non-producing <i>Staph. aureus</i>	14 (31.11)	3 (21.43)	2 (14.28)	5 (35.71)	3 (21.43)	1 (7.14)	0	0	0	0	
Total	45 (100.00)	6 (13.33)	5 (11.11)	10 (22.22)	11(24.44)	6 (13.33)	3 (6.66)	2 (4.44)	1 (2.22)	1 (2.22)	

** : There are a highly statistically differences between numbers of resistant isolates of β -lactamase producing and non- producing *S. aureus* $P<0.01$.

the intensive care unit. The number of their isolates and percentages are shown as follows: *Staphylococcus aureus* 45 (13.47%), *Staphylococcus epidermidis* 31 (9.28%), *Staphylococcus saprophyticus* 18 (5.38%), *Staphylococcus xylosus* 11 (3.29%), *Staphylococcus capitis* 7 (2.09%), *Streptococcus pyogenes* 28(8.38%), Viridance streptococci 35 (10.47%), *Streptococcus pneumonia* 12 (3.59%), *Pseudomonas aeruginosa* 41 (12.27%), *Escherichia coli* 19 (5.68%), *Klebsiella spp* 20(5.98%), *Proteus spp* 10 (2.99%), *Enterobacter* 9 (2.69%), *Propionibacterium acnes* 24 (7.18%), *Acinetobacter spp* 9 (2.69%) and *Corynebacterium spp* 15 (4.49%).

There is a highly statistically differences between numbers of isolates and various clinical

and inanimate sites ($P<0.01$).

Staphylococcus aureus was the main predominant bacterial pathogens isolated from both types of samples, so we used these bacteria to demonstrate its ability to produce β -lactamase and determine the antibiotics profile. The Categorization of β -lactamase producing and non-producing *S. aureus* isolates from various sources are illustrated in Table 3. 31 (68.89%) isolates of *S. aureus* were isolate of β -lactamase producers, while 14 isolates (31.11%) were β -lactamase non-producers. There was a highly statistically differences between numbers of β -lactamase producing and non-producing *S. aureus* isolates in various clinical and inanimate sites $P<0.01$.

Table 4 illustrated the prevalence of multiple drugs resistance of *S. aureus* against eight

antibiotics. We found that resistance against three antibiotics has the highest percentage (25.8) for β -lactamase producing *S. aureus* with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β -lactamase non-producing *S. aureus* (35.71%) with no resistance against more than four antibiotics.

There is a highly statistically differences between numbers of resistant isolates of β -lactamase producing and non- producing *S. aureus* ($P<0.01$).

Tables 5 and 6 illustrates profile of antibiotic resistance of β -lactamase producing and non-producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care unit. The study found that vancomycin, cefotaxime and gentamicin were the most effective antibiotics

Table 5. Profile of antibiotic resistance of β -lactamase producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care unit.

Samples type	Total no of tested strains	Number of resistant isolates								
		TET	GEN	AMOX	CIP	CEF	AMOX/CLA	VAN	METH	
Clinical samples	Skin	5	5	3	4	3	2	3	2	5
	Blood	3	2	0	2	2	1	2	0	3
	Eye	2	2	1	2	2	0	2	1	2
	Nose	4	3	2	3	3	2	3	1	2
	Wounds	7	5	5	4	6	4	5	3	5
	Urine	5	3	3	4	5	3	3	2	3
Inanimate samples	Bed	5	4	2	3	4	3	0	3	2
	Wall	3	3	0	3	2	2	1	2	2
	Instruments	9	8	4	7	7	3	5	4	7
	Dresses	2	2	0	1	2	0	0	1	2
Total	45 (100.00)	37 (82.22)	20 (20.00)	33 (73.33)	36 (80.00)	20 (44.44)	24 (53.33)	19 (42.22)	33 (73.33)	

Tetracycline (TET), gentamicin (GEN), amoxicillin (AMOX), ciprofloxacin (CIP), cefotaxime (CEF), amoxicillin/clavulanic acid (AMOX/CLA), vancomycin (VAN), methicillin (METH).

Table 6. Profile of antibiotic resistance of β -lactamase non-producing *S. aureus* strains isolated from clinical and inanimate samples of intensive care unit.

Samples type	Total no of tested strains	Number of resistant isolates								
		TET	GEN	AMOX	CIP	CEF	AMOX/CLA	VAN	METH	
Clinical samples	Skin	5	5	1	2	1	0	2	1	3
	Blood	3	1	0	0	0	0	0	0	0
	Eye	2	2	1	1	1	0	0	0	0
	Nose	4	2	1	2	1	2	1	0	1
	Wounds	7	4	3	4	3	2	3	2	3
	Urine	5	2	1	2	2	0	2	0	2
Inanimate samples	Bed	5	2	1	2	2	1	0	1	1
	Wall	3	2	0	2	2	2	1	0	0
	Instruments	9	3	2	5	3	2	3	2	4
	Dresses	2	1	0	1	1	0	0	0	2
Total	45 (100.00)	24 (53.82)	10 (22.22)	21 (46.66)	16 (35.55)	9 (20.00)	12 (26.66)	6 (13.33)	16 (35.55)	

Tetracycline (TET), gentamicin (GEN), amoxicillin (AMOX), ciprofloxacin (CIP), cefotaxime (CEF), amoxicillin/clavulanic acid (AMOX/CLA), vancomycin (VAN), methicillin (METH).



Figure 1. Gel electrophoresis result of multidrug resistant β -lactamase producing *Staphylococcus aureus* strains.

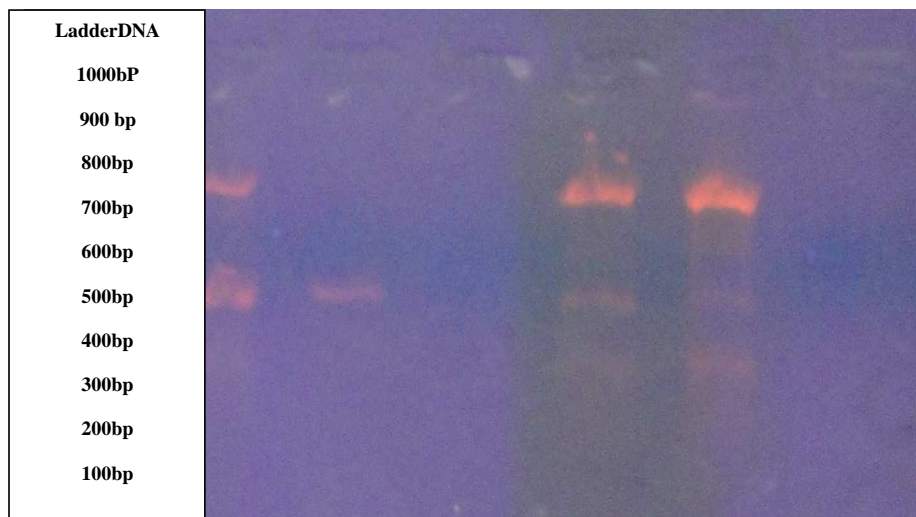


Figure 2. Gel electrophoresis result of multidrug resistant β -lactamase non-producing *Staphylococcus aureus* strains.

against β -lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of intensive care unit in percentages of resistance (42.22, 44.44 and 44.44%) respectively. And tetracycline as antibiotics has the highest percentage resistance (82.22%) against the aforementioned strain. While for β -lactamase non-producing *S. aureus* strains, also vancomycin and cefotaxime were the most effective antibiotics, but have the lowest percentage resistance compared to the first aforementioned group, which recorded 13.33 and 20.0% of resistance, respectively, and tetracycline as the weakest antibiotic has great resistance of 53.82% of isolates.

To look into the plasmid profiles in β -lactamase producing and non-producing MDRSA, we selected 14

multi-drug resistant strains, isolated the plasmid DNA by alkaline lysis miniprep method and analyzed using agarose gel electrophoresis (Figures 1 and 2). The ranges of band molecular weight were between 300 to 600 base pairs with clear main band in 550-570 bp for β -lactamase producing *S. aureus*, while the ranges of band molecular weight were between 200 to 700 base pairs with clear main band in 450-470 and 690-700 bp for β -lactamase producing *S. aureus*.

DISCUSSION

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to

escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world (Udo et al., 1993). The present study isolated a total of 334 isolates of bacterial types from various clinical and inanimate sources of intensive care unit (ICU); with the numbers of isolate and their percentages given as follows: *Staphylococcus aureus* 45 (13.47%), *Staphylococcus epidermidis* 31 (9.28%), *Staphylococcus saprophyticus* 18 (5.38%), *Staphylococcus xylosus* 11 (3.29%), *Staphylococcus capitis* 7 (2.09%), *Streptococcus pyogenes* 28 (8.38%), Viridance streptococci 35 (10.47%), *Streptococcus pneumoniae* 12 (3.59%), *Pseudomonas aeruginosa* 41 (12.27%), *Escherichia coli* 19 (5.68%), *Klebsiella* spp. 20 (5.98%), *Proteus* spp 10 (2.99%), *Enterobacter* 9 (2.69%), *Propionibacterium acnes* 24 (7.18%), *Acinetobacter* spp. 9 (2.69%) and *Corynebacterium* spp. 15 (4.49%). All these isolates have been shown to cause different nosocomial infections, especially intensive care unit infections. The result of our study were approved by results of recent studies (Maltezou and Giamarellou, 2006; Rahman et al., 2002; Vidhani et al., 2001; Karlowky et al., 2003; Laupland et al., 2007; Levy, 1998). Test for β -lactamase production revealed that 68.89% isolates produced β -lactamase. The highest numbers of isolates were from wounds and instruments.

The data obtained in this study showed that resistance against three antibiotics has the highest percentage (25.8) for β -lactamase producing *S. aureus* with resistance of eight antibiotics, while resistance of two antibiotics was the predominant mode of β -lactamase non-producing *S. aureus* (35.71%) with no resistance against more than four antibiotics. Vancomycin, cefotaxime and gentamicin were the most effective antibiotics against β -lactamase producing *S. aureus* strains isolated from both clinical and inanimate samples of intensive care unit with percentage resistance of 42.22, 44.44 and 44.44%, respectively and tetracycline as an antibiotic has the highest percentage resistance of 82.22% against the aforementioned strain. While for β -lactamase non-producing *S. aureus* strains, vancomycin and cefotaxime, were the most effective antibiotics, but have the lowest percentage resistance in comparison to the first aforementioned group, which recorded 13.33 and 20.0% resistance, respectively. And tetracycline as the weakest antibiotic has great resistance of 53.82%.

The selection of an antimicrobial agent is determined by the most likely pathogen and its expected susceptibility pattern. Monitoring antibiotic susceptibility patterns of bacterial pathogens at a local level will yield important information regarding the emerging problems of antibiotic resistance and provide assistance in managing empirical therapy (Francis et al., 1997; O'Brien et al., 1999). The widespread use of antibiotics has been responsible for the development of numerous problems, including the emergence of multi drug resistance

bacteria, increased number of nosocomial acquired infections and increased health care costs (Maple et al., 1989). Rising to the challenge posed by hospital acquired infections, which are emerging as a global health concern, over 1.4 million people worldwide are suffering from hospital acquired infections. In this study, few isolates have been found susceptible to tetracycline. On the other hand, all the isolates were susceptible to vancomycin. These findings are similar to the findings of Rahman et al. (2002) and Cunha (2006). But they observed less percentage of MDRSA, which is much lower than the present study. Treatment of this infection is a major community indication of antibiotic usage (Felmingham, 2002).

In this study, *S. aureus* isolates were resistant to tetracycline. The indiscriminate use of antibiotics may be a cause for this multidrug resistance (Hotu et al., 2007). Among the eight drugs used in the present study, vancomycin and cefotaxime are the best choice for the treatment of *S. aureus* caused ICU infections. *S. aureus* is capable of causing a variety of human infections, including fatal invasive and toxic conditions and also possesses a differential ability to spread and cause hospital associated outbreaks of infections (Foster, 1996; King et al., 2006).

Reports from the International Infection Control Consortium (INICC) surveillance study showed that nosocomial infection is markedly higher in the ICUs of the INICC hospitals (Adegoke and Komolafe, 2009). The emergence of multidrug resistant *S. aureus* (MDRSA) strains, has posed a challenge in the treatment of infection (Ekramul et al., 2011).

In India, ICU infection rate is over 25% and is responsible for more mortality than any other form of accidental death. The prospective observational study describes that isolates of *Acinetobacter*, *Pseudomonas*, *Klebsiella* and *Escherichia coli* are resistant to the third generation cephalosporins, and it also states that the increased duration of the time spent in intensive care units and days of intervention are associated with the incident (Vidhani et al., 2001). *S. aureus* is a pathogen of greater concern because of its virulence, its ability to cause a diverse array of life threatening infections, and its capacity to adapt different environment conditions (Aubry-Damon et al., 1998). Recent report about the rate of MDRSA in nosocomial infections in Isfahan Iran showed that 67.2% of isolates were MRSA geographical, and health system capability in running infection control program has role in variability of prevalence MDRSA (Fatholahzadeh et al., 2008). Most isolates of MDRSA were observed in wound specimens (43%). Instead of many similar and independent studies that is not showing any relationship between sex, age, site of infection and rate of MDRSA (Maltezou and Giamarellou, 2006; King et al., 2006).

In this study, the highest level of resistance is observed in tetracycline, which is in agreement with the other

reports. So, in the present investigation, the variation that occurs in the antibiotic sensitivity pattern of *S. aureus* confirms the emergence of antibiotic resistance. The resistance in bacterial pathogens to antibiotics increases the chance of severe infections in human beings (Foster, 1996; Herwaldt and Wenzel, 1996).

However, the data indicated that among eight antibiotics used in the present study, vancomycin and cefotaxime should be the drug of choice to treat *S. aureus* infection. For proper treatment, the physician should perform the antibiotic sensitivity test before antibiotic treatment. It is as an important factor while prescribing antibiotics also observed that resistance of most of the antibiotics tested shows increased resistance with increasing age. These results suggest that clinicians should consider age and sites of infections.

In this study, investigation was carried out to know the prevalence of multiple-drug resistant (MDR) gene carrying plasmids in the MDRSAs but no vivid result was found. However, multi-drug resistant isolates showed more plasmid bands and all the isolates which did not show any plasmid were sensitive to almost all the antimicrobials. Our studies showed a 24.44% prevalence of MDRSA in the tested clinical samples which was almost similar to that reported by Adeleke and Odelola (1997). Such high rates of MDRSA have also been reported in India: 20 and 32.8% MDRSA in some regions of India (Vidhani et al., 2001).

In this present study, most of the isolates which showed plasmids were found to be resistant to three antibiotics. On the other hand, no correlation was observed between tetracycline resistance and plasmid profiles. However, no inter-relation was found between the 2nd and 3rd generation cephalosporin-resistance used in this investigation and plasmid profiles. Although, in the present study, it was observed that there is a tendency that multi-drug resistant isolates contain plasmids but no solid evidence could be provided. In order to clarify this issue, further studies are to be initiated. Abuse and irrational use of antibiotics will lead to development of drug resistance. In developing country like Iraq, there is lack of guidelines in the practice of antibiotic prescriptions. However, our studies might provide a platform for physicians to choose and prescribe rational antibiotics in the treatment of MRSA in hospital and community infections (Khorvash et al., 2008; Kohner et al., 1999; Zhang et al., 2005).

In conclusion, this study indicates that some antibiotics commonly used in treatment of ICU infections are still effective. They may be of immense value for use to determine drugs of choice in the treatment of ICU infections prior to the outcome of laboratory investigations while vancomycin, cefotaxime and gentamicin could be considered for first-line therapy for ICU infections, in agreement with previous reports. Although there are some others "old antibiotics" such as tetracycline with a role that may be underestimated for

ICU infections (Honderlick et al., 2006), prudent and rationale use of antibiotics must encourage prescribing vancomycin and other indicated antibiotics parsimoniously for uncomplicated UTIs.

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Short Communication

Study of oxidative stress in relation with antioxidant status in chronic bronchitis

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Lipid peroxide plays an important role in inflammatory lung diseases. Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion of the Total Antioxidant Capacity. Oxidative stress has been recognized as a central feature of smoke induced chronic bronchitis. Imbalance between oxidants and Total Antioxidant Capacity is also an established fact in these patients. 60 patients with chronic bronchitis were included in the study. Their base line clinical examination, malondialdehyde (MDA), nitric oxide, alpha tocopherol and Total Antioxidant Capacity were measured. 100 healthy non-smokers' served as controls. The mean malondialdehyde levels and nitric oxide in the patients at base line were higher than controls ($p < 0.001$). Plasma alpha-tocopherol and total antioxidant capacity were lower ($p < 0.001$) in the patients compared to controls. The present study shows that initially the plasma lipid peroxide (MDA) levels were high and antioxidants (alpha-tocopherol, total antioxidant capacity) were low in patients with chronic bronchitis. Our results suggest the presence of oxidative stress and decrease in total antioxidant capacity in chronic bronchitis.

Key words: Malondialdehyde, alpha-tocopherol, total antioxidant capacity, chronic bronchitis.

INTRODUCTION

Lung is the organ which is constantly exposed to many atmospheric pollutants such as cigarette smoke, ozone and nitrogen dioxide and is also at risk from oxidant injury by inhalation (Irfan and William, 1999). Inhaled ozone induces toxic processes that impair lung function. Lipid peroxide plays an important role in inflammatory lung diseases. Increased epithelial permeability produced by cigarette smoke is likely to be mediated through depletion Total Antioxidant Capacity (Rahman and Adcock, 2006). Oxidant-antioxidant balance is essential for the normal lung function. Both an increased oxidants and or decreased antioxidants may reverse the physiologic oxidant-antioxidants balance in favors of oxidants leading to lung injury.

Chronic bronchitis is defined clinically that it is present in any patient who has persistent cough with sputum

production for at least two consecutive years in the absence of any other identifiable cause. In simple chronic bronchitis, patients have a productive cough but no physiologic evidence of airflow obstruction. Some individual may demonstrate hyperactive airways with intermittent bronchospasm and wheezing. This condition is called chronic asthmatic bronchitis while some patients, especially heavy smokers, develop chronic airflow obstruction usually with evidence of associated emphysema and are classified as obstructive chronic bronchitis.

The earliest feature of chronic bronchitis is hyper secretion of mucus in the large airways associated with hypertrophy of the sub-mucosal glands in the trachea and bronchi. As chronic bronchitis persist there is also marked increase in goblet cells of small airways – small bronchi and bronchioles – leading to excessive mucus production that contributes to airway obstruction (Thurbleck, 1991).

Now a day's attempts towards oxidative stress status are continuing. The study of antioxidant capacity in lung

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Table 1. Illustrate the the levels of MDA, NO•, Vitamin E and TAC in the healthy controls and chronic bronchitis patients.

Test	Healthy controls (n=100)	Chronic bronchitis patients (n=60)
Sr. MDA ($\mu\text{mol/L}$)	1.66 \pm 0.289	4.61 \pm 2.7*
Sr. NO•($\mu\text{mol/L}$)	33.15 \pm 6.13	33.58 \pm 12*
Sr. Vit E (mg/dl)	0.927 \pm 0.12	0.32 \pm 0.09*
Sr.TAC ($\mu\text{mol/L}$)	1253.12 \pm 170.22	354.43 \pm 88.*

n = Number of cases; all values are expressed in mean \pm SD; * = significant when compared with control group.

disease patients opens a promising field in prevention of oxidative stress related complications in these patients.

Aims and objectives

1. To explore the existence of possible peroxidative damage in lung disease patients by estimating the level of serum malondialdehyde as an index of lipid peroxide.
2. To estimate nitric oxide as a marker of oxidative stress.
3. To study possible alteration in Total antioxidant Status in lung disease patients by estimating the total antioxidant capacity.
4. To study non enzymatic antioxidant vitamin E.

MATERIALS AND METHODS

The present study was conducted in the department of Biochemistry Dr. Vikhe Patil Medical College and Hospital Ahmednagar. This study included 60 clinically stable COPD Patients in the age group of 35 to 60 years, 100 healthy controls who were diagnosed by physicians on the basis of detailed clinical history, clinical examination and relevant biochemical examinations.

Hypertensions, malignancy, overt cardiac failure recent surgery, severe endocrine, hepatic or renal diseases and lung disorders other than COPD were excluded from the present study.

Informed consent was obtained from each patient in the study. The study was cleared by institutional ethics committee. 10 ml blood was collected from each patient. Serum was separated by centrifugation at 3000 rpm for 10 min at room temperature. Following parameters were carried out on the samples on the same day of collection.

1. The level of serum total lipid peroxide in terms of Malondiadehyde (MDA) was determined by Kei Satoh method (1998).
2. Serum Nitric oxide (NO•) as nitrite was estimated by Najwa Cortas and Nabil Wakid method (1990).
3. Serum Vitamin 'E' (α – Tocopherol) was estimated by the method of Baker and Frank (1968).
4. Total antioxidant capacity in plasma (TAC) was assayed by FRAP analysis (Iris and Strain, 1996).

RESULTS AND OBSERVATIONS

Statistical analysis

Analysis was carried out using students unpaired' test.

Probability values < 0.05 were considered as significant. Also data were expressed in mean \pm SD form.

DISCUSSION

Table 1 shows significant high levels ($p < 0.001$) of oxidants serum lipid peroxide (MDA) and serum nitric oxide (NO•) were observed as compared to healthy controls. The mean plasma levels of vitamin E ($P < 0.001$) and total antioxidant capacity (TAC) was lower than controls.

Lung cells, in particular alveolar epithelial type II cells, are susceptible to the injurious effects of oxidants. Lungs are continuously exposed to oxidants, either generated endogenously by metabolic reactions or exogenously, such as air pollutants or cigarette smoke and since cigarette smoking, another environmental hazard, also delivers oxidants and free radicals to the lungs. Cigarette smoke contains many oxidants and free radicals, both in the gas and the tar phase and causes sequestration of neutrophils into the pulmonary microcirculation and accumulation of macrophages in respiratory bronchioles. Once recruited, these cells become activated and generate ROS. ROS, which may also be released by lung epithelial cells, may also stimulate inflammatory cells directly, thereby amplifying lung inflammatory and oxidant events. There by increases the MDA significantly in chronic bronchitis patients (Paul and Irfan, 2006).

In the respiratory tract, NO• is generated enzymatically by all three distinct isoforms of NO• synthase that is, NOS-1, NOS-2 and NOS-3. Of these three forms, NOS-2 activity is primarily regulated transcriptionally and is commonly induced by bacterial products and pro-inflammatory cytokines. Inflammatory diseases of the respiratory tract such as chronic bronchitis is commonly characterized by an increased expression of NOS-2 within respiratory epithelial and inflammatory immune cells, and markedly elevated local production of NO• in the patients with lung diseases (Chambers and Tunnccliffe, 1998).

Vitamin E is the most important lipophilic antioxidant in humans in this study we observed the reduced vitamin E level in lung disease patients could be due partly to its overconsumption as an antioxidant subsequent to

increased production of free radicals by cigarette smoke and inflammatory reaction.

The total anti-oxidative potential of the plasma reflects the ability of an individual to resist the oxidative stress. Ferric reducing ability of plasma (FRAP) evaluates plasma total antioxidant capacity due to known and unknown antioxidants in the plasma (Aysen et al., 2004). Therefore in the present study significant reduction observed in total ferric reducing ability of plasma may be due to increased free radical activity either because of inflammation or complications that results in imbalance between antioxidant capacity and peroxidant affecting lung function. Extensively amplified oxidant burden and declined individual antioxidant levels might be responsible for the observed significant fall in total antioxidant capacity of patients with lung disease.

Conclusion

Thus evaluating oxidative stress in lung disease patients by measuring lipid peroxidation and antioxidant status can lead to better understanding of free radical mediated damage in chronic bronchitis patients. An inequity between oxidative stress and antioxidative capacity has been proposed to play an important role in the development and progression of chronic bronchitis and it is related to the severity of disease.

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