Brief Communication

Determination of liver enzymes, serum ceruloplasmin and urine copper in parents of children with Wilson's disease

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Wilson's disease (WD) (hepatolenticular degeneration), first described by Kinnear Wilson in 1912, is an autosomal recessive defect in cellular copper transport, with a prevalence of approximately one case in 30,000 live births in most populations. Its clinical and pathological manifestations are the consequence of an excessive accumulation of copper in tissues, particularly in the liver, brain, cornea, and kidneys. An impairment in biliary excretion leads to the accumulation of copper in the liver. Over time the liver is progressively damaged, eventually becoming cirrhotic. The hepatic injury is believed to be caused by excess copper, which acts as a pro-oxidant, and promotes the generation of free radicals. Once cirrhosis occurs, copper leaks into the plasma, accumulates in, and damages other tissues, and causes the signs and symptoms of neurologic, hematologic, and renal damage. The incorporation of copper into ceruloplasmin is also impaired in WD. This accounts for the decreased serum ceruloplasmin concentration, which presents in most patients with WD. The clinical presentation of WD is variable, depending on the age of patients. Infants and children with WD have silent, however, ongoing hepatic deposition of copper, gradually producing clinical disease. Clinical manifestations arise as hepatic, and extrahepatic copper deposition progresses. The disease rarely presents clinically before 6 years of age, and almost always presents before the age of 30 years, although it was described in those as young as 3 years of age, and patients presenting in their 70's. The WD gene is localized on the long arm of chromosome 13, and was recently cloned by several research groups. The gene product ATP7B is a copper transporting type ATPase. The diagnosis of WD can be based on a combination of the presence of Kayser-Fleischer (KF) rings, a low serum ceruloplasmin level, and elevated 24-hours urinary copper excretion. Establishing the diagnosis of WD is usually straightforward if the major clinical and laboratory features are manifested as: typical hepatic or neurological symptoms and signs, presence of KF ring, low serum ceruloplasmin concentrations, and increased urinary copper excretion. Serum aminotransferases are usually mild to moderately elevated in patients with WD, including children and other patients diagnosed in a presymptomatic stage. The aspartate aminotransferase (AST) concentration is usually higher than the alanine aminotransferase (ALT). The degree of elevation correlates poorly with the extent of histologic changes. We know little regarding 24-hours urinary copper excretion of heterozygote carriers. The aim of this study was to evaluate the liver enzymes, serum ceruloplasmin, and urine copper excretion in parents of children with WD.

This prospective study was performed from February 2005 to May 2007 on parents of Wilsonian children in our center. This study was approved by the Medical Research Ethics Committee of the Shiraz University of Medical Sciences, Shiraz, Iran. The diagnosis of WD was made on the basis of hepatic or neurological clinical abnormalities, presence of KF rings, elevated 24-hours urinary copper (>100 µg/24 hr), and low ceruloplasmin level (reference range 20-40 mg/dL). All subjects with at least one Wilsonian child were included the study, and all patients with any type of liver diseases other than Wilson disease were excluded from the study. For parents, a questionnaire considering their age, gender, consanguinity, and history of previous diseases was completed. After receiving written consent, a complete physical examination was performed, and liver enzymes, serum ceruloplasmin, and 24-hours urine copper were checked. Serum ceruloplasmin was determined by radial immunodiffusion, and 24-hours urine copper level was determined by flame atomic absorption spectroscopy.

Thirty-one parents of Wilsonian children (16 female/15 male) with mean±SD age of 39.7±8.2 years (range, 26-64 year) were subjected to the study. Four families were first-degree relatives. All parents had normal physical examinations. Only one case (3.2%) had abnormal liver enzymes (high AST and ALT). Fourteen out of 31 subjects (45.2%) had high urinary copper excretion (higher than normal references, 75 µg/day) with a mean value of 110.3±35.8 µg/day (range, 79-179.5 µg/day). The mean serum ceruloplasmin level was 26.5±9.5 mg/dL. Six cases (19.4%) had low serum ceruloplasmin (reference range for adults 20-40 mg/dL). In this study, we observed elevated serum AST and ALT level only in one subject. The subject was obese, and the elevated liver enzymes was most probably due to fatty liver. Most patients with WD have low serum ceruloplasmin levels, however, most studies have demonstrated high, low, and normal values in adults and children, with and without WD. Approximately 10-20% of asymptomatic heterozygote carriers of WD have serum ceruloplasmin levels less than normal (20 mg/dL), as we found in our cases (19.4%). Urinary copper excretion is useful for the diagnosis of WD, and for monitoring of patient’s compliance during treatment. The disease is typically associated with 24-hours urinary copper excretion of 100 µg or higher, although lower values have been described in up to 25% of presymptomatic patients. High urinary copper
Copper status in parents of Wilsonian children

Vaccine-associated paralytic poliomyelitis in a pre-vaccinated infant

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Although poliomyelitis caused by wild-type poliovirus has been almost eradicated, especially in developed countries, vaccine-associated paralytic polio (VAPP) cases continue to occur in most of developed and developing countries. Live attenuated oral polio vaccine (OPV) has been successfully used to control wild-type polio over the past 30 years. It has several advantages such as low cost, ease of use, and high efficacy rate with herd immunity, yet OPV has a drawback of causing a rare but a serious complication of vaccine-associated paralysis. As per World Health Organization statistics, the overall risk for VAPP is approximately one in 2.4 million doses of OPV with a first dose risk of one in 750,000. A mutation of the polio vaccine virus known as a reversion causes previously attenuated poliovirus to revert to a more neurovirulent form leading to paralysis that is similar to wild virus and is usually permanent. Inactivated polio vaccine has no adverse effects and eliminates the risk of VAPP, yet OPV remains the vaccine of choice in countries where polio is endemic. Since implementation of the all-inactivated polio vaccine schedule in 2000 in the USA, no cases of VAPP have been reported. Vaccine-associated paralytic polio can occur in either recipients of the vaccine or in susceptible contacts. We hereby describe a case of VAPP in a pre-vaccinated infant caused by contact infection.

A 2-month-old male infant from the United Arab Emirates (UAE), was admitted to the pediatric ward with a 3-day history of fever, one day history of lethargy, irritability, and hoarseness of voice, followed by sudden onset of weakness of both upper and lower limbs. There was no history of convulsions or ingestion of honey. The patient had contact with a 4-year-old sibling who was vaccinated with OPV 22 days before the onset of the clinical picture. The patient received only BCG and single dose of Hepatitis B vaccines at birth. There is no family history of similar condition or immunodeficiency disorder. On examination, the infant was hypoactive with stable vital signs. Growth parameters (weight, length, and head circumference) were at the 75th percentile for age. Neurological examination revealed a flaccid and areflexic paralysis of the 4 limbs. Muscle power of right leg one over 5, left leg 2 over 5, right arm 2 over 5, left arm one over 5.

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References
Head drop sign was positive. There was no sensory loss. Kerning and Brudzinski signs were negative. Cranial nerves examination was normal.

**Laboratory data.** Complete blood count: white blood count 14.4/mm³, hemoglobin 12.9 g/dl, platelet 559,000 (150,000-400,000). Blood urea nitrogen 8 mg/dl, sodium 139, potassium 5.1, sugar 88, and creatinine 0.3 mg/dl. C-reactive protein negative. Blood and urine culture: no growth. Cerebrospinal fluid exam: clear with normal pressure, white blood cell 225 (0-22/mm³), 96% lymphocytes, and 4% polymorph. Red blood cell nil. Total protein 82 mg/dl (20-170 mg/dl), sugar 43 mg/dl. Serology for enteroviruses and herpes simplex virus were negative. Gram stain negative and culture no growth. Quantitative plasma immunoglobulin electrophoresis was normal.

Two stool specimens collected 24 hours apart were sent to the WHO accredited polio laboratory in Oman where Sabin Like vaccine strain poliovirus was isolated. Reports of electrophysiological studies conducted on this child included electromyography (EMG) and nerve conduction velocity (NCV) and were consistent with an asymmetric lesion of the anterior horn cells. The patient was discharged home 10 days after admission. She was re-assessed twice at one and 2 month intervals and was found to have persistent flaccid and areflexic paralysis of the left leg with muscle power of 1 over 5.

The World Health Organization defines acute flaccid paralysis (AFP) as "acute onset of flaccid paralysis in any child under the age of 15 years." In 1988, the WHO resolved to eradicate poliomyelitis worldwide. Through the effective use of the OPV of Albert Sabin, in 1994, the WHO declared that wild polio had been eradicated from the western hemisphere. However, wild polio virus is still detected by the Global Polio Laboratory Network from cases in polio endemic countries in Africa, Eastern Mediterranean, and South-East Asia. The OPV, which is composed of live attenuated poliovirus (Sabin Vaccine), is still the vaccine administered in the UAE. It is estimated that OPV can cause VAPP with frequency of one case per 2.5 million doses of OPV distributed. Approximately 4-8 cases of VAPP occur annually in the United States. Globally, the VAPP burden is 250-500 cases annually. In the UAE, the national immunization program for all children was initiated in 1980. The OPV coverage exceeded 90% in 2004. With the establishment and implementation of the national immunization program and improving socio-economic standards, the annual incidence of poliomyelitis in the UAE has decreased from 58 cases in 1982 to 9 cases in 1988. The last case of confirmed polio due to wild polio virus was reported in April 1992 from Ajman medical district. The AFP surveillance in UAE was established in 1998. In 2004, the AFP rate in the UAE was 1.07 per 100,000 population under the age of 15, equivalent to 11 cases, all of them were non-polio cases.

The VAPP is a rare event where neurological damage is caused by a virus ingested from the live OPV. The paralysis that results, is identical to that caused by wild virus and is usually permanent. The diagnosis of VAPP is confirmed by isolating the vaccine-strain polio virus from stool samples collected within 14 days of onset of paralysis. The VAPP is the most serious disadvantage of the OPV. Inactivated polio vaccine (IPV) has no adverse effects and eliminates the risk of VAPP. Since implementation of the all-IPV schedule in 2000 in the USA, no cases of VAPP have been reported. The identification of VAPP consistently highlights the importance of maintaining vigilance for AFP, and the possible need to use IPV instead of OPV in the post-eradication period. Currently, the most urgent priority is to eliminate the remaining reservoirs of wild poliovirus endemcity, and this is best achieved by mass immunization campaigns of OPV. Universal introduction of IPV is not currently advocated as the WHO has not determined under what conditions the use of OPV can be safely ceased. The WHO has to develop a comprehensive strategy for the prompt cessation of OPV use as soon as possible after global eradication.

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**References**

A neglected cause of cervical lymphadenitis.  
Oropharyngeal tularemia

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There are 2 species known as *Francisella Tularensis* (*F. Tularensis*), and *Francisella philomiragia* (*F. philomiragia*) in the *Francisella* genus. Tularemia is caused by virulent, facultative intra-cellular bacteria, *F. Tularensis*. It is a zoonotic disorder. *Francisella tularensis* also has 4 subspecies. *Francisella Tularensis subsp. tularensis* is mainly found in North America, originates from rodents and is transmitted via vectors. It is also virulent. *Francisella Tularensis subsp. palaearctica* is found throughout the Northern Hemisphere. It is found in animals living near the water and is transmitted via food contaminated with water and via water itself. It is less virulent than *F. Tularensis subsp. tularensis*. Therefore, it is not fatal in humans and recovery may even occur without treatment. *Francisella Tularensis subsp. mediaasiatica* has only been isolated in Central Asia. It causes disease in both humans and animals, though less frequently. *Francisella Tularensis subsp. novicida* was recently described in North America. It causes an illness similar to tularemia, infrequently. The other species of the genus, *F. philomiragia* is an opportunistic pathogen causing illness in patients with immune deficiencies. The illness can develop in ulceroglandular, glandular, oropharyngeal, oculoglandular, pneumonic, and typhoidal forms. There are no pathognomonic clinical or laboratory signs for oropharyngeal tularemia. Therefore, the cases are usually diagnosed when there is an epidemic, and sporadic cases are probably overlooked. The oropharyngeal form of tularemia especially mimics pharyngitis, tonsillitis, or cervical lymphadenitis due to other infectious causes. Therefore, most of the cases are mis-diagnosed as tonsillopharyngitis or cervical lymphadenitis causing a delayed in diagnosis and treatment. In this report, we present 12 cases of the oropharyngeal form of tularemia with cervical lymphadenitis diagnosed between 2006-2007 in the Province of Kars in Turkey, and evaluate the reasons for late diagnosis and treatment.

Twelve patients, who were admitted directly to the Department of Infectious Diseases and the Pediatrics Department of Kaftkas University Medical Faculty or, to the Department of Otolaryngology of Kars State Hospital between November 2006 and April 2007, or who were examined in various departments due to neck swelling, referred later to the aforementioned departments and consequently diagnosed and followed-up in our department, were enrolled in the study. Tularemia was diagnosed by appropriate clinical presentation and positivity of the serological tests. Aspiration materials taken from the throats and suppurated lymph nodes of patients were cultured in standard blood agars. Direct preparations prepared from the same materials were stained with gram stain. A micro-agglutination test (MA) was used for serological diagnosis. The MA test was evaluated in the tularemia reference laboratory of Uludag University Medical Faculty. The cases investigated presented mainly in the winter and spring months, especially January and February. The approval was obtained for the local ethics committee prior to the commencement of the study and informed patient consent was received from all study participants.

The mean age of the patients was 30±18.9 (10-75) years, and 75% of the cases (9/12) were female. It was noted that the lymphadenitis mainly occurred after tonsillopharyngitis and that they lasted 3-5 months. The mean delay time for diagnosis was 30 days (1-45 days). All of the cases had cervical lymphadenopathy, 75% had fever, 33.3% had headache, and 25% complained of cold. All the cases had cervical lymphadenitis of differing radii and dimensions, which were of moderate firmness in the beginning, though they were later soft or fluctuating, sometimes with overlying hyperemia (Figure 1). All the cases were painful upon palpation. Three of the cases had cryptic tonsillitis upon the examination of the neck. Seventy-five percent of the cervical lymphadenitis were on the left, and 25% were on the right. No reproduction was observed on standard blood agars. No microorganisms were observed on direct preparations prepared with gram stain. The MA positivity varied between 1/80-1/640. The mean leucocyte count of the patients was 9.8±2.3 (5.6-12.8) K/µ, the mean sedimentation rate was 48±22.3 (10-80) mm/hours, and the mean C-reactive protein (CRP) was 49±26.5 (11.5-82.2) mg/dl (Table 1).

The province of Kars is an area at an elevation of approximately 2000 m above sea level and has patchy forests. The epidemic was centered in the Selim district, which has a population of 4500 and is located 30 km west of Kars. The economics of the area depends mainly on agriculture and animal husbandry, thereby leading to a close contact between people and animals. The city's water system was suspected as the primary source of infection. All the patients resided in Selim city center, and the city's water source was located 30 km west in the Sarikamis forest. The water source was accessible to all types of small rodents and, to contamination by their waste products. The water was not chlorinated, and *E. Coli* colonization was well above the appropriate limits. Water samples were collected from 10 different areas of the city center and were sent to the Hifzisihha Public Health Center Laboratory in Ankara. However,
no agent of tularemia could be isolated from those samples. Three of the cervical lymphadenopathies (25%) in the tularemia cases suppurated and had to be surgically drained. Streptomycin (1 g/day, intramuscularly) + doxycycline (200 mg/day, orally) was given to patients for 14 days. The illness was nonfatal. The ulceroglandular form of tularemia, which is mainly transmitted via a vector causes 60-90% of cases in the USA, and in western, middle, and northern European countries, whereas the oropharyngeal form is reported to cause 1-7% of cases.\(^1\,^{1-3}\) The oropharyngeal form, which is mainly transmitted via contaminated water and foodstuffs, is reported to be the most common form of disease in Bulgaria and in Turkey. Its prevalence is reported to be 89.7% in Bulgaria, and 83% in Turkey. The ulceroglandular form is reportedly absent from Bulgaria, whereas it is reported to be around 1% in Turkey.\(^1\,^{1-4}\) The fact that our cases were mainly seen in winter and spring months, especially in January and February, and that they were from the same district center, of which the water source was unchlorinated with a high ratio of \textit{E. Coli} and contamination, may denote the possibility of waterborne infection.

The mean delay time for the diagnosis of tularemia was reported to be 2-3 months.\(^1\,^{2\,4}\) Therefore, the classification of causes of cervical lymphadenitis as acute bilateral (usually viral infections), acute unilateral, subacute, or chronic unilateral (usually mycobacterial infections) is important for etiology.\(^5\) Predominantly, \textit{Staphylococcus aureus}, and \textit{Streptococcus pyogenes} cause acute unilateral cervical lymphadenitis along with oropharyngeal tularemia.\(^5\) The main cause for the lag in diagnosis is the similarity between the oropharyngeal form of tularemia and bacterial pharyngitis and tonsillitis. Therefore, the patients were diagnosed as having bacterial pharyngitis or tonsillitis, either with or without exudates, and treatment with beta-lactam antibiotics (namely, penicillin, cephalosporin) were started. No microorganisms could be produced on throat or suppurate lymphadenitis cultures while this was happening. Since the patients also developed cervical lymphadenitis, the neck swelling enlarged further during the treatment. However, treatments for \textit{staphylococci} and \textit{streptococci}, which are the most frequent causes of cervical lymphadenitis, were usually continued, the disease was mis-diagnosed as tuberculous lymphadenitis due to its granulomatous reaction, and anti-tuberculous drugs were used needlessly. Eventually, the cervical lymphadenitis was drained either spontaneously or surgically. Thus, the lag in diagnosis was inevitable. The leucocyte count is usually either normal or slightly elevated in tularemia cases, whereas erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) are elevated at the baseline and decreasing thereafter.\(^2\) The leucocyte count in our cases was normal, whereas ESR and CRP were elevated. Although these findings were non-specific for the oropharyngeal form of tularemia, they suggested a bacterial infection.

In a study conducted in Bursa,\(^4\) it was reported that 70% of cervical lymphadenitis due to tularemia were unilateral, while 10% were bilateral. All our cases had unilateral lymphadenitis, and this was on the left

**Table 1** - Results by age, gender, and laboratory values of oro-pharyngeal tularemia cases.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Gender</th>
<th>Age</th>
<th>Anti-body titer</th>
<th>Leucocyte number (K/µl)</th>
<th>Sedimentation rate (mm/hr)</th>
<th>CRP (mg/dl)</th>
<th>Neck USG (Lymphadenopathy)</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>58</td>
<td>1/80</td>
<td>8.28</td>
<td>45</td>
<td>45</td>
<td>Left cervical, 31x26mm,</td>
<td>Histiocytes, mature lymphoid elements</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>47</td>
<td>1/80</td>
<td>12.8</td>
<td>65</td>
<td>82.2</td>
<td>Left cervical, 38x24mm</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>75</td>
<td>1/160</td>
<td>8.9</td>
<td>30</td>
<td>21.5</td>
<td>Left cervical, 40x30mm</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>30</td>
<td>1/160</td>
<td>9.5</td>
<td>50</td>
<td>73</td>
<td>Left cervical, 22x20mm</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>28</td>
<td>1/160</td>
<td>12.1</td>
<td>75</td>
<td>78.7</td>
<td>Right cervical, 25x20mm</td>
<td>Granuloma formations produced by epitheloid histiocytes</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>16</td>
<td>1/160</td>
<td>5.6</td>
<td>55</td>
<td>42</td>
<td>Left submandibular, 24x20mm</td>
<td>Mature lymphocytes and histiocytes</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>14</td>
<td>1/320</td>
<td>10.2</td>
<td>65</td>
<td>66.8</td>
<td>Left cervical, 38x26mm</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>21</td>
<td>1/320</td>
<td>10.2</td>
<td>10</td>
<td>11.5</td>
<td>Right cervical, 49x30mm</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>25</td>
<td>1/640</td>
<td>11.6</td>
<td>50</td>
<td>48</td>
<td>Left submandibular, 28x20mm</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>10</td>
<td>1/640</td>
<td>9.2</td>
<td>25</td>
<td>33.7</td>
<td>Left cervical, 30x25mm</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>31</td>
<td>1/640</td>
<td>7.6</td>
<td>80</td>
<td>75</td>
<td>Right cervical, 24x22mm</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>12</td>
<td>1/640</td>
<td>12.5</td>
<td>30</td>
<td>16.4</td>
<td>Left jugulodigastric 37x28mm</td>
<td></td>
</tr>
</tbody>
</table>

USG - ultrasonography, CRP - C-reactive protein, F - female, M - male
in 75%. Three of the cervical lymphadenitis (25%) suppurated and were drained surgically. Three of the patients had cryptic tonsillitis, and this was treated with streptomycin + doxycycline. The mean lag time for diagnosis was 30 days (10-45 days). Most of our patients used beta-lactam antibiotics. The findings that cervical lymphadenitis was usually unilateral and on the left may be due to the anatomical localization and function of the lymphatic system or to the lymphotropic features of tularemia. The thoracic duct receives all the lymph flow from the lower extremities and most of the abdomen. It opens to the junction of the left internal jugular vein and left subclavian vein. Lymphatic jugular trunks arising from the innermost cervical nodes carry the lymph from the head and neck to the right lymphatic duct on the right and to the thoracic duct on the left. The right lymphatic duct opens to the junction of right internal jugular vein and right subclavian vein. If it is considered that the oropharyngeal form of tularemia is transmitted via contaminated food and water, then it should cross the gastro-intestinal barrier. The microorganisms pass to the lymphatics of the intestine, probably due to their lymphotropic nature. They reproduce and reach a critical level before reaching the lymphatic nodules of the neck and thereby cause infection. All of the patients were treated with streptomycin (one g/day, intramuscularly) + doxycycline (200 mg/day, orally) for 14 days. Three of the patients who suppurated were surgically drained. A clinical response was seen after treatment and the lymphadenopathy resolved completely after a mean period of 3-5 months. The oropharyngeal form of tularemia should be borne in mind in cases that present with sore throat and fever of acute onset, followed by tonsillopharyngitis, or unilateral cervical lymphadenopathy (especially on the left), those with no reproduction on aspiration cultures from the throat and suppurated lymph nodes, and who are unresponsive to an antibiotic trial of 48-72 hours (especially beta-lactams).

References


Sputum eosinophil markers in monitoring asthmatic patients in United Arab Emirates

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Asthma is a common disease all over the world affecting approximately 6% of adults, and 12% of children with an increasing prevalence especially in the developed countries. In the United Arab Emirates (UAE), as in other Gulf regions, asthma constitutes one of the major public health problems affecting more than 13% of the adult population, and up to 40% of children making it the most common problem in pediatric practice. Airway inflammation plays a pivotal role in the pathogenesis of bronchial asthma. Yet, physicians evaluate the severity of asthma and select the line of therapy according to subjective indices reported by patients. These indices may not accurately reflect the extent of underlying inflammation due to differences in perception. Thus, it would be extremely useful for clinicians to have a reliable, objective method on which to base their clinical decisions in monitoring the response of asthmatic patients to therapy.

The asthmatic airway inflammation is initiated, in the genetically predisposed (atopic) patient, by exposure to certain environmental allergens which trigger the production of IgE antibodies that bind to specific receptors on mast cells scattered among cutaneous, respiratory, and intestinal tissues. Further exposure to the same allergen, may cause degranulation of the respiratory mast cells, leading to the release of certain mediators that attract inflammatory cells (mainly eosinophils) to the involved pulmonary site to produce the specific pathological changes of allergic asthma termed "remodeling". In non-atopic-asthmas, some other non-allergen -IgE mediated mechanism is involved leading to the attraction of other inflammatory cells (mostly neutrophils) with a similar remodeling outcome. The eosinophil has been considered to play a decisive role in the pathophysiology of asthma by releasing toxic granules and chemokines.
This role has been confirmed by bronchial biopsy or bronchoalveolar lavage from asthmatic patients, which reveals high eosinophilic infiltration. However, these measures are invasive, and can be substituted by the examination of induced sputum for its cellular and fluid constituents such as eosinophil cationic protein (ECP). Inhaled corticosteroids (ICS) are the first-line therapy in the management of asthma due to their potent anti-inflammatory effect. However, the relation between the dose of ICS and the degree of suppression of airway inflammation is not always linear and some asthmatics are found to be steroid resistant.  

In order to determine the relationship between sputum eosinophils markers and asthma severity and prognosis in atopic and non-atopic patients in UAE, we investigated 98 asthmatic patients attending the Allergy Clinic of Al-Jazeira & Mafra Hospitals, during the period from February 2005 to February 2007. They were classified as having mild persistent or moderate persistent asthma types according to The National Asthma Education Program (NAEPP) GINA classification. Those with intermittent asthma type, those who received corticosteroids during the 3 months period preceding the study, and those with concomitant pregnancy or chronic illnesses were excluded. Fifteen of the 98 patients were also excluded due to inability to obtain satisfactory sputum samples. The remaining 83 (38 males, 45 females) aged from 9-53 years together with 30 healthy non-smoker adults (with no symptoms suggesting past or current asthma) completed the whole course. All subjects underwent complete medical history and physical examination. They were clinically categorized into 4 groups as mild (58 patients) or moderate asthmatics (25 patients) according to asthma guidelines and as atopic (65) and non atopic (18) according to the results of skin prick tests. Most of the studied patients (51) were UAE nationals, the remaining were from different nationalities resident in Abu Dhabi, including South Asian (11), Arabs (9), Iranian (6), and Europeans (6). Informed consent was obtained from each patient or his caretaker and the study was approved by the local Ethics Committee of Al-Jazeera hospital (Ref. 109-04).

During the first visit, patients were given inhaled bronchodilator (2 puffs of salbutamol 200 micrograms/dose) and sent for sputum collection. Those who produced satisfactory sputum were then skin prick tested for the common inhalant allergens and their sera were collected for ECP estimation. Patients were then given inhaled steroid therapy (Budesonide Pulmicort Turbuhaler) in a divided dose of 400 mcg daily for children, 600 mcg daily for adults with mild persistent asthma, and double that doses for the moderate cases for a period of 4 weeks. Full instructions about the proper use of Pulmicort Turbuhaler were demonstrated for each patient. Patients were also supplied with bronchodilator inhalers (salbutamol 200 micrograms/dose) to be used on need. They were instructed to record fully their symptoms and the frequency of bronchodilators use and to return to the hospital for a 2nd visit four weeks later. On the second visit, patients were clinically assessed and second sputum and serum samples were collected. Healthy controls were investigated twice within a 4-week interval, but did not receive any intervention. Sputum was induced by the method described by Pizzichini, using hypertonic saline with monitoring of lung function. The subject is pretreated with 400 ug of salbutamol and asked to inhale aerosol of increasing

### Table 1 - Mean values of % sputum eosinophilia; Sputum ECP and serum ECP levels for healthy and asthmatics at baseline and 4 weeks later.

<table>
<thead>
<tr>
<th>Clinical groups</th>
<th>Baseline results of SPT</th>
<th>Mean % sputum eosinophilia</th>
<th>Mean sputum ECP ug/L</th>
<th>Mean serum ECP ug/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline 4 weeks later</td>
<td>Baseline 4 weeks later</td>
<td>Baseline 4 weeks later</td>
</tr>
<tr>
<td>Healthy group (n=30)</td>
<td>Negative</td>
<td>&gt;80% of predicted</td>
<td>1.5</td>
<td>24.1</td>
</tr>
<tr>
<td>Non atopic mild asthmatic (N= 11)</td>
<td>Negative</td>
<td>&lt;80% of predicted</td>
<td>3.3</td>
<td>146.6</td>
</tr>
<tr>
<td>Atopic mild asthmatic (N= 47)</td>
<td>&lt;80% of predicted</td>
<td>10.7</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Non atopic mod asthmatic (N= 7)</td>
<td>Negative</td>
<td>60-80% of predicted</td>
<td>5.1</td>
<td>217.2</td>
</tr>
<tr>
<td>Atopic mod asthmatic (N=18)</td>
<td>Positive</td>
<td>60-80% of predicted</td>
<td>24.2</td>
<td>341.0</td>
</tr>
</tbody>
</table>

NB: SPT - skin prick test, N - number, mod - moderate, ECP - eosinophil cationic protein, FEV₁ - forced expiratory, ECP - normal range for serum ECP was taken as 2.8 - 9.0 µg/L and for sputum ECP as 7.1-50.0 µg/L.
concentrations of hypertonic saline (3%, 4%, and 5%) each for 5 minutes using De Vilbiss ultrasonic nebulizer (DeVilbiss Health Care Inc., Somerset). A nose clip was applied, and the patient was encouraged to expectorate sputum into a sterile container whenever he feels the need or every 5-minutes. Lung function was monitored every 5 minutes using (Microlab 3300 Spirometer, Micro Direct). Sputum induction was ceased once an adequate sample of sputum was obtained, or the maximum duration of mobilization was reached. Supplemental B2-agonist was administered if lung function dropped by more than 10%. Attempts to obtain sputum spontaneously after inhalation of nebulized salbutamol were always tried. The sputum sample was considered adequate when there was at least 0.5 ml of sputum containing 3 or more opaque, mucocellular clumps. Sputum was separated from saliva and treated with 4 volumes of 0.1% dithiothreitol followed by 4 volumes of Dulbecco’s phosphate buffered solution. The resulting suspension was filtered and centrifuged. The supernatant was aspirated and stored in Eppendorf tubes at -70°C for later ECP assay. The cell pellet was re-suspended in Dulbecco’s phosphate buffered solution, cytopsins were made and the yield was stained by the Wright method. A differential cell count was carried out on 400 nucleated non-squamous cells. The ECP levels in serum and in sputum (separated supernatant portion as above) were estimated by fluoro enzymatic immunoassay, using the UNICAP 100 machine (Pharmacia Diagnostics AB, Uppsala, Sweden). Sputum portions were diluted 1:20, while serum samples were used undiluted. According to the fluorescence of each immunoCAP the result of ECP level in the patient’s sample was determined automatically by the machine and expressed in µg/L. Skin tests were performed according to the European Academy of Allergy and Clinical Immunology recommendations, by the prick method as described earlier. The standardized allergen preparations, obtained from (Stallergen France), include the 5 most common inhalant allergens in the UAE, namely, house dust mites, molds mix, grass pollens mix, tree pollens mix, and cat dander).

Data were entered electronically into a pre-programmed MS Excel database. Descriptive data are presented in terms of means ± SD or percentage. Comparisons among the groups were performed using Analysis of Variance test. A p-value of <0.05 was considered statistically significant.

Forty-seven (81%) out of the 58 mild asthmatics, and 18 (72%) out of the 25 moderate asthmatics showed positive skin prick tests to one or more of the common aero allergens tested, and were regarded as atotics, the rest who showed negative skin prick tests were regarded as non-atopic patients. Relative eosinophil count in induced sputum sample was expressed as percentage of the total non-squamous cells in the examined specimen. The results of estimation of % sputum eosinophilia, sputum ECP level in µg/L and serum ECP level in µg/L for the healthy controls and for the asthmatic patients at baseline and after 4 weeks of therapy are tabulated as the mean value ± SD in Table 1. The normal range for serum ECP was taken as 2.8-9.0 µg/L, and for sputum ECP as 7.1-50 µg/L. Although a wide individual variation was seen among asthmatics, and to a lesser degree among healthy controls, yet the mean values of sputum eosinophilia and sputum ECP at baseline for all studied asthmatic groups were higher than those for the healthy controls. The values were found to be higher in the moderate asthmatics than in the mild cases and higher in the atopic asthmatics than the non-atopic ones. Healthy subjects show no statistical difference in the mean values of sputum and serum markers at baseline and 4 weeks later, however, the level of sputum eosinophilia and sputum ECP of all asthmatics showed a marked reduction after 4 weeks of ICS therapy, while serum ECP does not show such a dramatic change after therapy.

This study has confirmed that the mean relative sputum eosinophil count and mean sputum ECP levels are higher in asthmatics than in healthy controls, emphasizing the role of eosinophils in the pathogenesis of asthma. However, we found that some patients with mild asthma and fewer with moderate severity have normal sputum eosinophil counts. Moreover, most of the non atopic (intrinsic) asthmatics have mildly elevated levels of sputum eosinophils markers, as compared with the high level of the atopic asthmatics regardless the degree of severity, indicating that in non atopic asthmatics, other cells or factors are more important than eosinophils in producing the pathology. Considering atopic patients as one group and non atopic patients as a different group, we found that the moderately severe atopic asthmatics showed a higher value of sputum eosinophilic parameters than the mild forms, emphasizing that eosinophil number and activity are directly proportional to the clinical state. We also found a good correlation between sputum eosinophilia and sputum ECP level in all groups of asthmatics, thus, sputum ECP level can be used to assess the severity of asthma. Our most interesting finding is the observed marked reduction of these parameters in response to (ICS) therapy. After 4 weeks treatment with (ICS), all patients showed a sharp decline in sputum eosinophil counts and sputum ECP levels, albeit at a higher level than in healthy controls. This finding may be attributed to the fact that steroid therapy reduces, but not eliminates, airway inflammation. The reduction of airway inflammation by steroids may be indirectly assessed by
measuring sputum eosinophilic activity. The relatively elevated post therapy levels of sputum eosinophilia indicate that eosinophilic inflammation persists to a certain degree even when the clinical features of asthma are suppressed by high-dose (ICS) therapy. Moreover, those with very high sputum eosinophilia showed the best response to ICS therapy while those with very low or normal baseline level were not good responders. This finding is very important, since in clinical practice there is no recognized surrogate inflammatory marker to predict benefit from anti-inflammatory treatment in these patients. We found that serum ECP level was higher in asthmatics than in normal subjects, however, in contrast to sputum ECP, did not show a marked decline after ICS therapy. This indicates that serum ECP cannot be used as a reliable marker for asthma severity or monitoring.

In conclusion, asthmatic patients in UAE, especially with atopic background, possess higher levels of sputum eosinophils and sputum ECP than healthy controls. These levels drop dramatically in response to ICS therapy. Analysis of induced sputum for its cellular and fluid contents can, therefore, provide a unique opportunity to follow eosinophil activity in asthmatic patients, and can be used as a surrogate for monitoring the severity of the disease and its response to inhaled steroid therapy.

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References


Continuous positive airway pressure compliance in Saudi men and women with sleep apnea

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Among the various modalities available for the treatment of obstructive sleep apnea (OSA), continuous positive airway pressure (CPAP) remains the most effective. Nevertheless, there are many factors that limit its usage including the cost, practicality, need for training and acceptance. Even those patients who accept to use CPAP initially may not comply with it later on. Possible reasons include difficulty tolerating high air flow and pressure especially during exhalation, intolerance of the interface, claustrophobia, and nasal irritation.1 No study has assessed the acceptance and hence compliance to CPAP therapy in Saudi patients with OSA. Additionally, limited data are available in the literature comparing compliance to CPAP therapy in men and women. Therefore, we conducted this study to assess the initial acceptance of CPAP titration in the sleep disorders center (SDC) as well as later compliance to treatment in Saudi patients with OSA, and to explore possible gender differences.

We included 148 Saudi women, and 169 Saudi men with OSA who underwent split night study, during which the final portion of the polysomnography (PSG) was used to titrate CPAP using C-FLEX (REM Star auto-CPAP Respironics, Inc., Murrysville, PA, USA), at the SDC of King Khalid University Hospital, Riyadh, Saudi Arabia, between January 2006 and December 2007. Pressure titration was considered according to a described protocol.2 Obstructive sleep apnea was diagnosed according to the International Classification of Sleep Disorders (ICSD 2005). The study was approved by the ethics committee. Continuous positive airway pressure was considered initially accepted if the patient completed the titration trial in the SDC under PSG recording and described his/her sleep on CPAP as being good and was willing to use it at home. Those who refused the titration trial or continued but were still not satisfied with the machine were considered as
not accepting it. Afterward, those who initially accepted to use the machine during split night study and bought it were followed up in the sleep clinic 3 months later. The number of patients who reported using CPAP at home for at least 4 hours/night for 5 or more nights per week was recorded. All CPAP machines were provided with heated humidifier.

The mean age of the study group was 54.5±11.8 years for women and 43.5±12.5 years for men (p<0.001). Their body mass index was 42.1±9.7 for women and 37.3±9.8 for men (P<0.001). Epworth sleepiness scale (ESS) was 10.5 in both groups. The apnea hypopnea index (AHI) was 56.5±41.7/hour in women and 61.6 ± 34.9/hour in men (p=0.005). There was no statistically significant difference in arousal index, desaturation index, lowest recorded saturation or time spent during sleep with saturation <90% between men and women (Table 1). Continuous positive airway pressure titration was initially accepted by 266 patients (83.9%) of the total study group. More men accepted the CPAP titration trial in the SDC than women (p=0.008) (Table 1). At the end of follow up (3 months), 124 patients (39% of all patients who underwent split night study) reported using CPAP at least 4 hours/night for 5 or more nights per week with higher compliance among men compared to women, but the difference did not reach statistical significance (43.7% of men versus 33.7% of women, p=0.09).

In the present report, the overall percentage of Saudi subjects who reported using CPAP at 3 months (39%) was lower than that reported by patients in other countries; 79% in Europeans, and 72% in Chinese. Additionally, the present study demonstrates that initial CPAP acceptance during titration was significantly lower in women compared to men. Similarly, there was a trend of lower usage at 3 months among women compared to men. Female gender has been reported previously to be a predictor of poor CPAP compliance. Females were also more likely to refuse the initiation of CPAP therapy. The lower compliance in women in our study could be related to their older age and their significantly lower AHI. However, social and cultural factors could play an important role as well. Furthermore, larger local studies are needed to confirm the findings of this report and to explore the causes of lower usage and compliance to CPAP in Saudis in general and in Saudi women in particular.

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### Table 1 - Polysomnographic variables and compliance with CPAP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Female n=148</th>
<th>Male n=169</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI</td>
<td>56.5 ± 41.7</td>
<td>61.6 ± 34.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Desaturation index</td>
<td>36.8 ± 33</td>
<td>39.8 ± 30.9</td>
<td>0.20</td>
</tr>
<tr>
<td>Time (min) saturation &lt;90%</td>
<td>30.7 ± 38.8</td>
<td>19.9 ± 29.67</td>
<td>0.13</td>
</tr>
<tr>
<td>Lowest oxygen saturation</td>
<td>76.9 ± 15.18</td>
<td>80.24 ± 11.38</td>
<td>0.09</td>
</tr>
<tr>
<td>Arousal index</td>
<td>59 ± 36.6</td>
<td>60.6 ± 34.8</td>
<td>0.57</td>
</tr>
<tr>
<td>Accept CPAP (%)</td>
<td>115 (77.7)</td>
<td>151 (89.3)</td>
<td>0.008</td>
</tr>
<tr>
<td>Used CPAP (%)</td>
<td>50 (33.7)</td>
<td>74 (43.7)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

CPAP - continuous positive airway pressure, AHI - apnea hypopnea index

References