Detection of antibodies to the extractable nuclear antigens by enzyme linked immunosorbent assay.

Khalil A. Aziz, PhD, Abdul A. Faizal, FRCP.

Anti-extractable nuclear antigen (ENA) antibodies are a group of autoantibodies that are directed against various components of the cell nucleus. Six different ENA have been well characterized and these include Sjogren’s syndrome-A antigen (SS-A also known as Ro), Sjogren’s syndrome-B antigen (SS-B also known as La), Smith (Sm) antigen, Scleroderma-70 (Scl-70) antigen, ribonuclear protein (RNP) and topoisomerase-1 (Jo-1). Antibodies to these antigens are closely associated with connective tissue diseases (CTD) with varying prevalence (Table 1). Connective tissue diseases are a group of systemic autoimmune inflammatory diseases comprising of systemic lupus erythematosus (SLE), Sjogren’s syndrome (SS), systemic sclerosis (Scl), polymyositis/dermatomyositis (PM/DM) and mixed connective tissue disease (MCTD).

Patients with CTD can present with clinical manifestations related to any organ-system of the body and often without the signs and symptoms that are classically associated with these diseases. Consequently, early diagnosis of these diseases, based on clinical examination, can prove very difficult and therefore, clinicians rely heavily on the use of anti-ENA antibody testing for the exclusion, or early diagnosis prognosis and monitoring of CTD (Table 1). Due to the importance of anti-ENA antibodies in the diagnosis and management of CTD, assays used for testing should, therefore, be sensitive, specific and have a quick turn around time. Testing for anti-ENA antibodies has traditionally been carried out using classical gel-assays including the simple immunodiffusion and the counter current immunoelectrophoresis (CCIE) assays. However, these methods are time consuming, require great skills and have rather low sensitivities for the detection of anti-ENA antibodies, particularly those directed against the SS-A and Scl-70 antigens. For these reasons, increasing number of clinical immunology laboratories are switching to testing for anti-ENA antibodies by enzyme linked immunosorbent assay (ELISA). The latter assays are more sensitive, require little skills, have a quick turn around time and are amenable to automation.

In the present study, we have investigated a number of different ELISA preparations with a view of changing our anti-ENA-antibody testing from the CCIE-method to an ELISA. The study was conducted at the regional department of Immunology, Birmingham Heartlands Hospital, during the period of 2003. We tested a number of ENA-positive and negative samples using 3

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Associated CTD</th>
<th>Prevalence %</th>
<th>Monitoring suggested</th>
<th>Prognostic indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-A/SS-B</td>
<td>SS</td>
<td>65/60</td>
<td>Yes</td>
<td>Associated with development of extraglandular manifestations (arthralgia, vasculitis, nephritis, lymphadenopathy and leucopenia).</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>35/15</td>
<td>Yes</td>
<td>Associated with subacute cutaneous lupus and neonatal lupus syndrome.</td>
</tr>
<tr>
<td>Sm</td>
<td>SLE</td>
<td>30-40</td>
<td>No</td>
<td>Renal disease and poor prognosis.</td>
</tr>
<tr>
<td>RNP</td>
<td>MCTD</td>
<td>90</td>
<td>No</td>
<td>Poor prognosis with cardiopulmonary disease and severe skin disease.</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>30-40</td>
<td>No</td>
<td>Predictor for the development of systemic sclerosis in patients with Raynaud’s phenomena.</td>
</tr>
<tr>
<td>Scl-70</td>
<td>Scl</td>
<td>20-40</td>
<td>No</td>
<td>Poor prognosis with cardiopulmonary disease and severe skin disease.</td>
</tr>
<tr>
<td>Jo-1</td>
<td>PM/DM</td>
<td>20-40</td>
<td>Yes</td>
<td>Predict an aggressive form of the disease with arthritis and interstitial lung disease (require close monitoring of pulmonary function for the early detection of lung involvement and aggressive treatment).</td>
</tr>
</tbody>
</table>

* Both Ro and La antibodies in pregnant patients can cross the placenta and cause fetal complete heart block and neonatal lupus.

different commercial ELISA preparations and then compared the results generated with that obtained by the traditional CCIE-assay. The results obtained are illustrated in Table 2. As can be seen from this table, the 3 ELISA preparations confirmed the positive results obtained by the CCI-assay for SS-A, SS-A/SS-B and Scl-70 antibodies. Moreover, all 3 ELISA preparations detected, additionally, SS-A and Jo-1 antibodies from samples previously shown to contain unidentified anti-ENA antibodies by the CCIE assay. The latter results can not be attributed to false positive results since, firstly, all 3 ELISA preparations produced the same strong positive results, and, secondly, such results correlated well with the clinical picture. In addition, 2 out of 3 ELISA detected 2 more additional antibodies to Sm and RNP. However, the significance of the latter results is questionable, since the results obtained were either equivocal or weak positive and they were not reproduced by the third ELISA preparation, or the CCIE-method. Therefore, these latter results would seem to be truly false positives and this would fit with the previous studies showing that ELISA have reduced specificity for some of the anti-ENA antibodies. In contrast to previous studies, the present study has revealed that some ELISA preparations can be more sensitive than, and as specific as, the CCIE method. There are a number of possible explanations for the generation of false positive results by some of the ELISA preparations observed in the present and previous studies. These include, firstly, the use of impure substrates to coat ELISA-plates, and, secondly, as a result of detection of low affinity antibodies by some of the ELISA preparations which would not be detected by the CCIE-assay. The possibility of contamination would be supported by the fact that, firstly, not all ELISA preparations produced false positive results (as shown in the present study) and, secondly, false positive results were only obtained by ELISA preparations coated with proteins purified from human materials (Sm and RNP), but not by recombinant proteins (Ro, La, Scl-70 and Jo-1). Refining the purification procedures for ENA, or the use of recombinant proteins should increase the specificity of ELISA for anti-ENA antibodies.

Existence of low affinity anti-ENA antibodies would be the other explanation for the generation of 'false positive' results by ELISA and this suggestion would be supported by previous investigations into other autoantibodies (dsDNA and Scl-70 antibodies). Anti-dsDNA antibodies measured by the Farr, or the Crithidia Luciliae assays were regarded as highly specific for SLE. However, when ELISAs were introduced, it became apparent that such antibodies were not restricted to SLE, but associated with many other conditions. Further work revealed that antibodies associated with SLE were of high affinity, whereas those associated with other conditions were of low affinity. Similarly, anti-Scl-70 antibodies obtained by the CCIE-assay were regarded as highly specific for scleroderma. However, when the latter method was replaced with ELISA, positive results for anti-Scl-70 antibodies were found associated with SLE in addition to Sclerodermal, and such results were initially labeled as false positives. However, these results were shown subsequently to be truly positive-results and corresponded to low affinity antibodies. These antibodies are now taken as a marker of a subgroup of SLE patients who are at an increasing risk of developing pulmonary hypertension and renal disease. It is important therefore, to audit anti-ENA antibody results,

<table>
<thead>
<tr>
<th>Methods</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
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<td>Wp-uENA</td>
<td>Ro</td>
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<td>Ro</td>
<td>Ro</td>
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<td>N</td>
<td>N</td>
<td>Jo-1</td>
<td>Ro</td>
<td>Ro</td>
<td>Ro</td>
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<td>Scl-70</td>
</tr>
<tr>
<td>ELISA-3</td>
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<td>Sm</td>
<td>Jo-1</td>
<td>Ro</td>
<td>RNP</td>
<td>Ro</td>
<td>Ro</td>
<td>Ro</td>
<td>Ro</td>
<td>Ro</td>
<td>Scl-70</td>
</tr>
</tbody>
</table>

Serum samples from 11 patients (P1-11) were assessed for antibodies to total and specific ENA using counter current immunoelectrophoresis (CCIE) and different preparations of enzyme linked immunosorbent assay (ELISA).

**Wp-uENA** - weak positive-ENA antibodies, **N** - negative, **ENA** - extractable nuclear antigen, **Sm** - Smith antigen, **Jo-1** - topoisomerase-1, **Ro** - Sjogren's syndrome-A antigens, **La** - Sjogren's syndrome-B antigens, **RNP** - ribonuclear protein, **Scl-70** - Scleroderma-70.
obtained by ELISAs, in order to determine their true significance.

In conclusion, the present study has revealed that some ELISA preparations can be more sensitive, and as specific as the CCIE method for the detection of anti-ENA antibodies. Laboratories that are still using the later method should consider switching to ELISA. However, it is important that laboratories evaluate a range of different ELISA preparations before selecting the most optimal one. In addition, it is recommended that laboratories then audit results in order to determine the true significance of such results. Finally, until the true significance of ELISA-generated results is known, positive ENA-results should be interpreted in conjunction with the clinical picture and this would require close liaison between the clinical Immunology Laboratory and clinicians.

References

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**Antiphospholipid syndrome among Bahraini patients**

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Antiphospholipid syndrome (APS) is defined as the presence of antiphospholipid (APL) antibodies, arterial or venous thrombosis, recurrent spontaneous abortions, and thrombocytopenia. However, not all patients develop such complications. The risk of thrombotic event in patients with APS is 0.5-30%. The syndrome can occur within the context of several diseases, mainly autoimmune, or it may be present without any recognizable disease, the so-called primary APS.

Systemic manifestations of APS are multisymptomatic and can affect most of the systems. The symptoms are secondary to thrombosis that can be located in the vessels of each caliber. Most commonly, APS is associated with systemic lupus erythematosus (SLE). Approximately 35% of SLE patients have elevated levels of APL antibodies. The diagnosis of APS is based on the presence of any clinical manifestation associated with the syndrome in addition to the presence of anticardiolipin (ACL) antibodies or lupus anticoagulant (LAC).

A retrospective study was performed on 22 patients with APS who where treated in Salmaniya Medical Complex (Ministry of Health), largest hospital in Bahrain (1000 bed), over 16 year period from 1988 - 2002. Anticardiolipin immunoglobulin G (IgG) and immunoglobulin (IgM) were tested by enzyme link immunoabsorbent assay (ELISA) technique.

Primary APS was 45.8% while the secondary 54.5%. History of thrombosis was present in 50% of primary APS, while 33.3% of the secondary APS patients. The female to male ratio was 10:1. Among our female patients, 90.5% were married and percentage of pregnancies was 71.4% and number of miscarriages was 57.1%. In the primary APS, history of miscarriage was 80% while 50% in secondary APS group. All secondary APS were SLE patients. Percentage of ACL IgG was higher in primary APS (80%) compared to secondary APS 66.7%. On the contrary ACL, IgM was higher in secondary (75%) compared to primary APS (60%). Venereal Disease Research Laboratory (VDRL) was reactive in 90% of the primary group while 50% in secondary group. Antinuclear antibodies were much common in secondary APS (100%) than in primary APS as expected (20%). Regarding anti double stranded DNA it was positive in 83.3% of the secondary APS while absent in the primary form. Prolonged partial thromboplastin time (PTT) was present in 90% of the primary APS versus 66.7% of the secondary form. Treatment was given to 95.2% after diagnosis: 33.3% received aspirin, 76.2% received steroid, 28.6% received heparin and 28.6% received warfarin.

The present study on Bahraini patients showed that Hughes syndrome is not a common problem among hospitalized patients in Bahrain. Neurological, ophthalmological and cardiac manifestations known to be among the manifestations of APS were uncommon in the present study.
All studied patients in the present study are Bahrainis, results of laboratory and clinical findings in primary versus secondary APS are summarized in Table 1, comparison with other ethnic groups namely Saudi and Argentini patients was carried out. The mechanism of action of APL antibodies is not known. It has been shown, that the antibodies bind to the anionic phospholipids of platelet membranes, endothelial cells and clotting components such as prothrombin, protein C and protein S. There is a discrepancy between prolongation of clotting time in vitro and thrombosis in vivo. No satisfactory explanation of this phenomenon has yet been established, but several theories are proposed. In vitro lupus anticoagulant is thought to prolong clotting times by binding with phospholipids and thereby, limiting the phospholipid surface necessary for binding of the prothrombinase complex. The mechanism of paradoxical thrombosis observed in vivo is not known. The simplest theory is that, there is an antibody directed against the patient’s own platelet and endothelial cell phospholipids that cause platelet aggregation and subsequent vascular occlusion. This also accounts for thrombocytopenia seen in these patients. However, the mechanism of action seems to be more complex and probably not all participating components are known yet.

Lupus anticoagulant and ACL antibodies can both cause a false-positive result of VDRL assay, although it is another phospholipid responsible for true-positive result of this assay in syphilis. Anticardiolipin antibodies are also directed against plasma proteins such as beta-2-glycoprotein I and prothrombin, cofactors, the absence of which can decrease the binding of antibodies to phospholipids. The recently proven cross reaction between beta-2-glycoprotein I and lipoproteins could explain the pathogenesis of both thrombosis and atherosclerosis in patients with primary APS. Familial occurrence of elevated levels of APL antibodies as well as an association with certain human leukocyte antigen, DR4, DR7, DQ7, and DR53 types were reported.

Approximately 4% of the normal population have elevated levels of ACL antibodies. Frequency in the United States of APL antibodies can be found in as many as 50% of individuals with SLE and in 1-5% of the healthy population. On the other hand, ACL antibody tends to occur more frequently in elderly individuals. It is noteworthy, that relatively decreased titers of APL antibodies were also observed in patients with an ongoing thrombotic event. This could be explained by the consumption of antibodies during the event.

Recent literature suggests that the occurrence rate of APS in patients with SLE is 34-42%. Study conducted on a large group of SLE patients in Canada concluded that the presence of ACL antibodies in patients with SLE is associated with prolonged activated PTT (aPTT), thrombocytopenia and positive Coombs' test result, but not with APS. Another study concluded that there is a significant correlation between presence of antibodies and both thrombotic events and recurrent spontaneous abortions in SLE patients and that occurrence of thrombotic complications is in direct correlation with the level of APL antibodies. In a study conducted on Saudi patients with a positive LAC there was a clear association between the presence of LAC and an abnormal aPTT, which was much less obvious with the PT. Regarding the association of isotypes of ACL antibodies and LAC with the clinical outcome, a study on that aspect concluded that in patients with primary APS, the presence of the 3 ACL isotypes plus LAC was associated with a higher number of recurrent spontaneous abortions compared to other possible combinations of ACL antibody isotypes. On the contrary, a study in Israel reported no correlation between APL antibody titers and manifestations of APS. In the present study, there was no correlation between clinical

**Table 1 - Laboratory and clinical findings in Bahrainis versus other ethnic groups.**

<table>
<thead>
<tr>
<th>Findings</th>
<th>Bahraini population (%)</th>
<th>Saudi population (%)</th>
<th>Argentini population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total number</strong></td>
<td>N=22*</td>
<td>N=77†</td>
<td>N=92‡</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>(9.1)</td>
<td>(27)</td>
<td>(20.7)</td>
</tr>
<tr>
<td>Female</td>
<td>(90.9)</td>
<td>(73)</td>
<td>(79.3)</td>
</tr>
<tr>
<td><strong>Clinical findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>(50)</td>
<td>(49)</td>
<td>(43.1)</td>
</tr>
<tr>
<td>Abortion</td>
<td>(80)</td>
<td>(51)</td>
<td>(56.9)</td>
</tr>
<tr>
<td><strong>Secondary APL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>(54.5)</td>
<td>(48)</td>
<td>(21.7)</td>
</tr>
<tr>
<td>Abortion</td>
<td>(33.3)</td>
<td>(50)</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory findings</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACL antibodies</td>
<td>(100)</td>
<td>(87)</td>
<td>(100)</td>
</tr>
<tr>
<td>Primary APL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACL IgG</td>
<td>(80)</td>
<td>ND</td>
<td>(41)</td>
</tr>
<tr>
<td>ACL IgM</td>
<td>(60)</td>
<td>ND</td>
<td>(21)</td>
</tr>
<tr>
<td>ACL IgG and IgM</td>
<td>(40)</td>
<td>ND</td>
<td>(15)</td>
</tr>
<tr>
<td>LAC</td>
<td>ND</td>
<td>(100)</td>
<td>(51)</td>
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<tr>
<td>Secondary APL</td>
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<tr>
<td>ACL G</td>
<td>(66.7)</td>
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<td>ACL M</td>
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<tr>
<td>LAC</td>
<td>ND</td>
<td>(100)</td>
<td>(80)</td>
</tr>
</tbody>
</table>


*present study, †Owaidah et al, ‡Guglielmone et al.
presentation and ACL antibody isotype; yet we noticed that percentage of ACL IgG was higher in primary APS compared to secondary APS, on the contrary ACL IgM was higher in secondary compared to primary APS.

Patients with IgG ACL antibodies are at higher risk than those with IgM or IgA antibodies. The probability of thrombosis is higher if both ACL antibodies and lupus anticoagulant are present simultaneously. Significantly higher incidence of thrombosis was also described in patients who had elevated levels of IgG anti beta-2-glycoprotein I antibodies.

Anticoagulation and immunosuppression seem to be the most effective treatment. Long-term therapy with aspirin, warfarin or heparin was suggested, but duration of the treatment and the point at which it should be discontinued are not clear. Life-long anticoagulation is necessary in some patients. Laser photocoagulation is an additional treatment of non-perfused retinal areas.

Antiphospholipid syndrome is a life threatening and vision threatening multisymptomatic disorder. Laboratory tests are essential for the diagnosis and should be considered in patients with unexplained vascular occlusion. Long-term anticoagulation and immunosuppression seem to be the most effective treatment. The patients have to be monitored on regular basis.

The present study on Bahraini patients showed that Hughes syndrome is not a common problem among hospitalized patients in Bahrain.

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References


**Contribution study of visceral leishmaniasis in Syria**

Samar Al-Nahhas, PhD, Maha Shaaban, MSc, Lana Hammoud, BSc.

Leishmaniasis is a parasitic disease, endemic in many countries around the world in approximately 88 countries. An estimated 350 million people are at the risk of contracting the disease, with approximately 100000 new cases annually.1 Visceral leishmaniasis (VL) caused by *Leishmania infantum* and *Leishmania donovani* is prevalent in the Middle East. The major features of this disease are intermittent fever, enlargement of spleen and liver, anemia and weight loss. High mortality rate is expected if the disease is left untreated. Diagnosis of VL can be achieved either by demonstrating the parasite microscopically in Giemsa stained smears of spleen, lymph node and bone marrow aspirates, or *in vitro* cultivation. The last method was found to be more sensitive than microscopic examination and allows further characterization of the isolates by isoenzyme analysis.2 However, *in vitro* cultivation is time consuming, expensive and difficult.2 Due to limitations of the direct diagnostic methods mentioned above, a number of direct immunological methods have been applied, such as indirect immunofluorescent test, enzyme-linked immunosorbent assay test, with either whole parasite or purified antigen, the western blot analysis and the direct agglutination test with aqueous suspension and *Leishmania donovani* promastigotes.3 In recent years, the amplification of parasite kinetoplast DNA (kDNA) by polymerase chain reaction (PCR) has proved to be rapid, sensitive and a specific method for detection of *Leishmania* parasites in a number of different clinical materials: blood, bone marrow, lymph node and spleen.3,4 In this study, we reported the following: 1. the distribution of VL in Syria according to data collected between 1993 and 2003, due to little was known on the epidemiology and prevalence of VL in Syria; 2. the presence of VL was confirmed using PCR technique, as a new diagnostic test instead of the traditional methods.
Visceral leishmaniasis in Syria

We studied the cases admitted to 11 hospitals in major cities. Different diagnostic methods were applied on the bone marrow aspirates obtained from Darra's children (south of Syria) aged between 21 months to 6 years old. The following was conducted in combination: 1. The bone marrow samples were smeared onto a glass slide, air-dried, fixed in methanol, stained with Giemsa and directly examined with (x100) oil immersion objective. Each sample was examined twice before confirming the result; 2. Culture, material aspirate (approximately 0.5 ml) was mixed with 2 ml of culture medium (RPMI-1640, containing 10% fetal bovine serum heated for 30 minutes at 56°C). The mixture was incubated for 15 days at 25°C, and then examined by microscope. 3. The DNA isolation was performed as in Qiagen Kit. Briefly, bone marrow sample (approximately 1 ml) was mixed with 50 µl of Proteinase-K contained in 3 ml lyses buffer, and incubated for 10 minutes at 70°C. Absolute ethanol was added to the mixture, which was transferred to Qiagen column and centrifuged at 3000 rpm for 3 minute. The supernatant was removed; 0.5 ml of buffer 1 was added, centrifuged at 5000 rpm for 1 minute, then 0.5 ml of buffer 2, centrifuged at 5000 rpm for 15 minute. Finally, approximately 110 µl distilled water was added to rinse the column incubated at room temperature for 5 minute, then centrifuged at 5000 rpm for 5 minute in order to obtain the DNA sample. In addition to dNTP and Taq polymerase, 2 primers (1.7 µl for each) were used; RAV1: 5’- CTT-TTC-TGG-TCC-CGC-GGG-TAG-G-3’ and RAV2: 5’-CCA-CCT-GGC-CTA-TTT-TAC-ACC-A-3’. After initial denaturation at 94°C for 2 minutes, the samples were incubated for 45 cycles (Crocodile Thermocycler) as follows: denaturation at 94°C for 60 seconds, hybridization at 62°C for 90 seconds and extension at 70°C for 30 seconds. Amplification reactions were determined by 4% agarose gel electrophoresis using molecular weight marker. Samples were scored as positive when PCR product of 139 bp could be visualized.

A total of 350 VL human cases were reported from 11 hospitals in the major cities of the Syria (Table 1). One hundred and ninety five males (55.7%) and 155 females (44.3%) harbored the disease. By age, children under 5 years old constituted 86.6% of the reported cases. By provinces (Table 1), the highest number of reported cases were 127 from Idlep, 85 from Darra and 80 cases from Lattakia Governorates over a period of ten years (1993-2003). On the other hand, Damascus, and Hama Governorates showed the lowest number of clinically diagnosed cases (one case in each). Prolonged irregular fever (38-40°C) and hepatosplenomegaly, in addition to loss of weight, cough, diarrhea, and sometimes enlarged lymph nodes, were the main symptoms observed among VL patients. Regarding the diagnostic technique, the smears stained by Giemsa and examined microscopically on the same day for the presence of amastigotes, gave negative results, and the cultivation of bone marrow aspirates on RPMI-1640 medium gave identical results after 15 days of culture. Whereas, by applying the PCR technique, Leishmania parasites DNA was found in aspirates from 3 of 4 (75%) VL suspected children.

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especially useful for confirmation of VL, as an sensitive than the traditional diagnostic tests and sensitivity and specificity of the PCR technique. Results were both negative, which confirm the high microscopic examination and of bone marrow aspirates results were positive while with the results obtained within 24 hours.

1. The detection of low levels of Leishmania parasites, 8 multicopy target sequence combined with a copies of approximately 800 bp minicircles. 8 The kDNA was composed of up to 10000 samples. The kDNA was composed of up to 10000 copies of 4% gel and ethidium bromide staining. The 139 bp fragment corresponds to Leishmania amplified DNA and the 183 bp fragment corresponds to internal control amplified DNA. MW - molecular weight standards, S1, S2, S4 - positive samples of visceral leishmaniasis, S3 - negative sample Con - internal control.

(Figure 1), that all (4/4) were negative by microscopy and cultivation, probably due to the low number of Leishmania parasites in the aspirates. Despite the recent advancement and development of medical services in Syria within the last few years, VL is still endemic and considered one of the challenges confronting the medical profession. The presence of VL parasites in dogs as well as in Phlebotomus tobbi (vector), in areas around the humid and sub-humid coastal zone and the surrounding mountains in Syria was demonstrated. Indeed, Idlep and LattakiaGovernorates are located within this zone, and exhibited the highest prevalence. Dry arid regions such as Damascus and inland provinces maintained the lowest number of reported cases. Due to the high sensitivity and specificity of the PCR technique, it was used to diagnose suspected patients who presented the clinical symptoms of VL but its parasite could not be demonstrated by biological tests, which shows the parasites microscopically, in stained smears, in vitro cultivation and indirectly by serological means, whereas the PCR test depends upon detection of Leishmania DNA in VL suspected samples. The kDNA was composed of up to 10000 copies of 800 bp minicircles. The multicopy target sequence combined with a simplified sample preparation procedure, allowed the detection of low levels of Leishmania parasites, with the results obtained within 24 hours. The PCR of bone marrow aspirates results were positive while the microscopic examination and in vitro cultivation results were both negative, which confirm the high sensitivity and specificity of the PCR technique.

In conclusion, the PCR technique is more sensitive than the traditional diagnostic tests and especially useful for confirmation of VL, as an endemic disease in Syria, which should receive more attention from the health authorities and the health professionals in the country.

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References

Vitiligo and human herpesvirus 6. Is there a relationship?

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Vitiligo is the acquired loss of melanocytes leading to areas of depigmentation. It affects approximately 1% of the population. Among
Vitiligo and human herpesvirus 6

Vitiligo etiology theories, autoimmune theory remains the most popular one. Latent viral infections have been postulated to be the triggering factors in the development of autoimmune diseases. Human herpesvirus 6 (HHV-6) attacks the cell nucleus, it is double-stranded and often remains dormant for many years and are large enough to scatter ultraviolet light. Human herpesvirus 6 demonstrates predominantly CD4+ cell trophism. Similar to HHV-6, at the onset of vitiligo the main lymphocytes are CD4 T-lymphocytes and vitiligo causes alterations in T-lymphocyte subsets, an aberrant natural killer cell activity and antibody dependent cell-mediated cytotoxicity.1 We aimed to study the role of HHV-6 in the etiology of vitiligo. To our knowledge there is no study in determining the relation between vitiligo and HHV-6 so far.

Eighty vitiligo patients with 80 age and gender matched controls who have minor dermatological problems were included in the study from those admitted to the Dermatology Department of the University Hospital. Patients’ age, gender, duration of illness, age at onset of the disease, course of vitiligo as active or stable, type of vitiligo and percentage of involvement and treatment received were recorded. All subjects provided signed informed consent after approval of the experimental protocol by the Medical School Ethics Committee. Serum anti-HHV-6 immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies were measured by enzyme-linked immunosorbent assay (ELISA) method (Panbio Inc., Maryland, United Stated of America) and quantitative serum C-reactive protein (CRP) levels were measured by Behring nephelometry system using particle enhanced immunonephelometry method (N High Sensitivity CRP, Behring, Germany). Statistical analyses were performed using Statistical Package for Social Sciences for Windows (version 10). Categorical data of sera results were analyzed by chi-square test. Fisher’s exact test was used for smaller groups. Independent samples T-test were used for comparison of quantitative sera results.

Of the 80 patients recruited 37 (46.3%) were male and 43 (53.7%) were female. The mean age of patients was 39.6 ± 16.7 (10 - 84 years), the mean age at onset of the disease was 30.3 ± 16.7 (4 - 78 years), the mean duration of the illness was 9.3 ± 10.6 (1 - 40 years) years. Thirty-three of patients (41.3%) had active vitiligo and 47 (58.7%) had stable vitiligo. Sixty-four patients (80%) had less than 25% involvement, 11 patients (13.7%) had between 25-50%, 4 patients (5%) had between 50-75% and one patient (1.3%) had more than 75% involvement. Thirty-seven patients (46.2%) were diagnosed as localized, 9 (11.3%) were segmental, 21 (26.3%) were acral or acrofacial and 13 (16.2%) were generalized types of vitiligo. Twenty-eight (35%) patients received systemic psoralen plus ultraviolet radiation (PUVA), 2 (2.5%) patients received local PUVA, and 18 (22.5%) patients were treated with local corticosteroid treatment. Thirty-two (40%) patients did not receive any treatment at all. Anti-HHV-6 IgG seropositivities were detected in 66 (82.5%) of 80 patients and 49 (61.3%) of 80 controls and the difference was statistically significant (p=0.003, x²= 8.93). Anti HHV-6 IgG positivity ratio was significantly higher in segmental vitiligo patients (13/13; 100%) compared with acral and acrofacial (12/19; 63.2%) involvement (p=0.0252, odd’s ratio=16.2) (Fisher’s exact test). Anti-HHV-6 IgM was positive in 9 (11.3%) of patients and 5 (6.3%) of controls, which was not enough for a significant relation. Six of 33 (18.2%) active vitiligo patients were CRP positive whereas only 4 of 47 (8.5%) of stable vitiligo patients were CRP positive but the difference was not statistically significant (p=0.198, x²=1.658).

Human herpesvirus 6 is the major cause of roseola infantum. Supposed roles for HHV-6 in several diseases, which are linked to viral infections have been reported; multiple sclerosis, infectious mononucleosis-like illness, lymphadenopathy, lymphoma, leukemia, pityriasis rosea, oral and cervical cancer, gloves and socks syndrome, Gianotti-Crosti syndrome and severe drug eruptions.2 The relation between HHV-6 and vitiligo has not been studied up to now. In our study, HHV-6 IgG seropositivity was found to be higher in vitiligo group compared with the control group. All HHV-6 IgM positive patients were also HHV-6 IgG positive. The prevalence of HHV-6 IgG level in the healthy group (61.3%) was similar to previous studies. The prevalence of HHV-6 in different populations ranged from 63.5% to 88.1%.3,4 To our knowledge, there has been no epidemiological study detecting HHV-6 seropositivity in healthy population in our country. In our study, the mean value of quantitative CRP levels of patients and controls did not differ significantly and the mean serum CRP levels did not differ between HHV-6 IgG or IgM seropositives and seronegatives in patient group. C-reactive protein is synthesized from hepatocytes after acute inflammation or tissue destruction and it is not diagnostic for specific diseases and increases with any kind of inflammation. C-reactive protein was also found to increase in vasculitis and in atherosclerotic coronary artery diseases5 in which there is chronic infection. These studies imply that acute or chronic infections increase CRP levels. Although chronic infections have been suggested to initiate vitiligo, there may not be a substantial amount of inflammation enough to increase serum CRP levels in our study. The relation of vitiligo and microorganisms other than herpesviruses has also been studied. Grimes et al6 studied herpes simplex virus (HSV), varicella zoster, cytomegalovirus

LMA for rhinoplasty

(CMV), Epstein-Barr virus, HIV and human T-cell lymphotrophic virus DNA in skin biopsy specimens in 29 vitiligo patients and 22 control subjects and only CMV-DNA was identified in 38% of the patients, whereas all control subjects were negative. Intercellular adhesion molecule-1 (ICAM-1) on the surface of epidermal keratinocytes and melanocytes is likely to greatly influence cytotoxic damage of these cells in diseases like photosensitive lupus erythematosus, lichen planus, erythema multiforme, and vitiligo. It is proposed that disease-specific induction of ICAM-1 by factors such as ultraviolet radiation and herpesvirus infection, is an important determinant in triggering these skin diseases and in determining the pattern of disease.7 Our results also comply with the immunologic proposal of vitiligo as herpesviruses may trigger ICAM-1 expression on melanocytes which may activate autoimmune destruction of these cells.

In conclusion, HHV-6 IgG seropositivity showing past HHV-6 infection is related to vitiligo. C-reactive protein, which is elevated in acute or chronic inflammation and tissue damage, is related neither to vitiligo nor to HHV-6. Human herpesvirus 6 infection in a genetically susceptible host could potentially mediate the destruction of melanocytes by induction of aberrant humoral and cell-mediated immunological responses eventually causing vitiligo. Nevertheless, advanced immunologic and pathologic proofs of viral infection in skin is needed to confirm vitiligo-virus relationship.

References


Use of laryngeal mask airway for the care of rhinoplasty

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Some operations on the face, such as rhinoplasty, require care to preserve the delicate surgical work. In anesthetized patient after extubation, firm application of face mask (such as Rush™) is required for adequate spontaneous or manual ventilation. Pressure exerted on the nose by the face mask may change the shape of newly reconstructed nose. Subcutaneous emphysema has been reported after rhinoplasty where air was pumped through the lateral osteotomy incision.1,2 The risk of emphysema would probably increase if patient receives positive pressure ventilation via face mask. Awake extubation is often associated with straining or bucking increasing the nasal bleeding, which is undesirable in this situation. To avoid both tracheal extubation response and face mask ventilation, the trachea can be extubated during deep anesthesia and ventilation can be maintained with laryngeal mask airway (LMA). As compared to Guedal airway, LMA provides easier airway maintenance.3 Use of LMA after extubation during emergence from anesthesia compared with awake extubation or extubation in anesthetized without LMA, has been associated with less respiratory complications during recovery period.4 Laryngeal tube has been used during emergence from anesthesia in a patient with an unstable neck.5

We present results on 15 cases of rhinoplasties where at the end of the operation patients were extubated during deep anesthesia and ventilation was maintained through LMA until patients regained consciousness. Mean age of patients were 32 years (standard deviation (SD) of 5) and mean body weight is 68 kg (SD = 8.7), and they were either American Society of Anesthesiologist class I or II. All our patients received oral pre-medication of diazepam and metoclopramide 10 mg each orally 2 hours before the operation. After establishing IV line and standard monitoring, general anesthesia was induced with fentanyl (2 µg/kg), thiopentone (3-5 mg/kg) and cisatracurium (0.5 mg/kg). Patients were ventilated with face mask until all 4 twitches of train of 4 disappeared, and were then intubated. Anesthesia was maintained with sevoflurane, nitrous oxide and oxygen. Additional narcotic (morphine 1.5 mg/kg, intramuscularly), nonnarcotic
analgesics (diclofenac 100 mg; rectally) and dexamethasone 8 mg were given before the start of the operation. Average duration of operation was 125 minutes (SD = 17). After the operation, throat was cleared and when the fourth twitch of train of 4 was detected, residual effect of neuromuscular blocking drug was reversed with neostigmine (2.5 mg) and glycopyrrolate (0.4 mg). Anesthesia was maintained with sevoflurane with 100% oxygen and carbon dioxide was allowed to rise (end-tidal CO₂ between 40-60 Kpas). Patients were extubated when bleeding from the naso-pharynx appeared to have stopped and LMA was inserted along with a roll of gauze between the teeth. Sevoflurane was then switched off and patients were either breathing spontaneously or ventilated manually receiving 100% oxygen. Adequate placement of LMA was judged with: a) the ability to hand ventilate easily and b) after disconnecting the breathing circuit, clear breath sounds heard at the tubal end of LMA.

To stimulate spontaneous ventilation, small doses of doxapram (20 - 60 mg) were used in 60% of the patients, and in addition 2 out of 15 patients required naloxone (80 µg). With adequate spontaneous breathing through LMA, patients were transferred to post anesthesia care unit where oxygen was given through LMA. When patients regained consciousness, LMA was removed, throat was cleared and oxygen was given through Hudson mask. Good perioperative analgesia also helped in smooth recovery of all except one patient.

We concluded that after rhinoplasty, LMA after extubation avoids the needs of application of face mask, provides adequate ventilation and smooth recovery of patients. This technique may also be useful in other conditions, which require the avoidance of firm application of the face mask (after deliberate facial surgery) or endo-tracheal extubation response (such as hypertension, straining, bucking and coughing).

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References

Myasthenia gravis with respiratory failure in an elderly patient

Myasthenia gravis is an autoimmune disorder due to antibody mediated immune attack directed against acetylcholine receptors at neuromuscular junction. Clinically, it presents with muscular weakness and fatigability. In majority of patients initial presentation is due to the involvement of extraocular muscles. With the progression of the disease facial, bulbar, proximal limb muscles, neck extensors and diaphragm get involved.

An 80-year-old lady was admitted twice with history of progressively increasing breathlessness and respiratory failure. On first admission the diagnosis was elusive. During the second admission when she developed ‘head drop’, only then we realized the exact nature of her illness. Previously, she was in good health and did not experience any muscular weakness or fatigability, suggestive of myasthenia gravis. First admission was with 4 weeks history of progressively increasing breathlessness on exertion. There were no other associated symptoms and she was a nonsmoker. She suffered from mild hypertension which was well controlled with quinapril. Physical examination did not demonstrate any abnormality. Oxygen saturation was 91% on room air. Full blood count, urea, creatinine, electrolytes, electrocardiogram (ECG) and echocardiogram were all normal. Chest x-ray was normal except for small, ill defined shadow at left costophrenic angle. We were unable to point the cause of her breathlessness. She improved symptomatically and was discharged home for follow up in the out patient clinic after pulmonary function testing and high resolution computed tomography (CT) of the chest. Four weeks later, she was readmitted due to worsening of breathlessness. She was now in atrial fibrillation with a ventricular rate of approximately 120 per minute, blood pressure 120/80, respiratory rate 30 per minute,

Breathlessness and respiratory failure in myasthenia gravis patient

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Brian F. Tregaskis, MBBS, FRCP(Edin), Shahid Barlas, FRCP(Edin).
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oxygen saturation was 84% on room air and bibasal crepitations, more at right base posteriorly. Electrocardiogram confirmed atrial fibrillation without ischemic changes. Chest x-ray differed little from previous films aside from some consolidation in the right lower zone. High resolution chest CT showed atelectasis at both bases and some pneumonic consolidation of posterior segment of right lower lobe. There was no evidence of interstitial lung disease or pulmonary embolism. Her arterial blood gas was consistent with type II respiratory failure (pH 7.37, PO\textsubscript{2}: 6.9, PCO\textsubscript{2}: 9.1, HCO\textsubscript{3}: 38.6). We considered the possibility of left sided heart failure and pneumonia but were unsure on the cause of her respiratory failure. Despite treatment with digoxin, frusemide and antibiotics there was no improvement. Over the next few days, she had developed a ‘head drop’ being unable to hold her head upright. At this point it became clear to us that we were dealing with a case of neuromuscular junction disorder. She denied any previous history of diplopia, dysphagia or muscular weakness and fatigability. Tension test was positive with dramatic improvement in the power of her neck extensor muscles and oxygen saturation rising from 88-93% on room air. We were unable to check arterial blood gas as she had high international normalized ratio being on warfarin for atrial fibrillation. Auto antibodies screening was negative. Muscle enzymes, serum calcium, magnesium, electrolytes and thyroid function tests were all normal. Anti acetylcholine receptor antibodies assay titer was positive at 222 x 10\textsuperscript{10}M (normal range <5 x 10\textsuperscript{10} M). She responded very well to prednisolone and pyridostigmine and was discharged home. She lived a normal life until unfortunately succumbed to death.

Myasthenia gravis classically affects young patients in their fourth and fifth decades. Incidence of myasthenia gravis is increasing and this may be explained by the aging population, improvement in prognosis and higher detection rates of patients with milder symptoms.\textsuperscript{1} Due to late onset occurrences, myasthenia gravis is still substantially under diagnosed in older people.\textsuperscript{2} The majority of patients initially present with ptosis and diplopia and later on develops weakness of proximal limb muscles and respiratory muscles. Approximately 15% of patients have weakness, remained confined to extraocular muscles.\textsuperscript{3} Myasthenia gravis has been reported in the elderly patients presenting with falls and bulbar symptoms\textsuperscript{4,5} and children presenting with respiratory failure. However, respiratory failure as the first manifestation is unusual and not reported before in the elderly. We wish to recommend that myasthenia gravis should be considered in elderly with breathlessness and respiratory failure of obscure origin.

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References


Preliminary results of muscle diseases prevalence in patients from Jordan

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We reported 123 patients affected with a remarkable change in muscle strength and muscle weakness of variable clinical severity. The number of patients seen in all clinics during the months of July 2000 to September 2004 is shown in Table 1. The most common disease entities were muscular dystrophies (46 cases, 37.4%) followed by myositis diseases (25 cases, 20.3%) most of them were due to autoimmune diseases. Data demonstrated that motor neuron diseases represented the third common group of muscle diseases in this study (24 cases, 19.5%) whereas, mitochondrial myopathies were the fourth common group of muscle diseases (11 cases, 8.9%). On the other hand, type II atrophy which accounted for the fifth group was 8 cases (6.5%). However, metabolic myopathies (5 cases, 4.1%) included lipid storage diseases (2 cases, 1.6%) and glycogen storage diseases (3 cases, 2.4%). Furthermore, our data showed other rare causes of muscle diseases. Congenital muscular dystrophy (2 cases, 1.6%), end
stage muscle disease (1 case, 0.8%) and Stiffman syndrome (1 case, 0.8%). The muscle biopsy of congenital muscular dystrophy showed severe dystrophic findings by light microscopy. However, the ultrastructural findings confirmed the diagnosis in metabolic and mitochondrial myopathies. Collectively, these findings showed that the classification of muscle diseases in subtypes with a different clinical presentation is useful in clinical practice.

Muscle samples were obtained from the thigh, at a site in the lateral portion of quadriceps femoris by the needle method for the histochemical and electron microscopical investigations. The procedure is carried out under sterile conditions. The skin and subcutaneous tissue at the biopsy site were anesthetized by the local infiltration of 2% lidocaine. After anesthesia, a small scalpel incision, approximately 1 cm long using a size 15 blade, is made in the skin and deep fascia to facilitate the pathway of the needle. Upon removal of the needle from the muscle, the wound is closed over with pressure and skin Elastoplast. Frozen specimens were stored at -70°C until sectioning. For light microscopy, all sections were taken in series and stained with the following histological and immunocytochemical staining list: hematoxylin and eosin mATPase at alkaline and acidic pH; Nicotinamide adenine dinucleotide; gomori trichrome; phosphorylase; succinate dehydrogenase. However, double fixation with 2.5% glutaraldehyde followed by 2% osmium tetroxide of fresh samples, provides the optimal preservation for ultrastructural investigations of muscle samples from patients with mitochondrial and metabolic myopathies.

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References


Pregnancy outcome following exposure to orlistat, ramipril, glimepiride in a woman with metabolic syndrome

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Most babies are exposed to drugs in utero. Drugs given to mothers during pregnancy can affect the fetus by acting directly on the embryo and produce lethal, toxic or teratogenic effects such as altering placental function, changing the myometrial activity, altering the biochemical dynamics of the mother and by acting indirectly. The use of drugs during pregnancy is a complicated problem, especially for new drugs.

Orlistat, a new drug, has pancreatic lipase inhibitory property that decreases the absorption of ingested dietary fat. Data for the use of orlistat in treating obesity in pregnant women are not available currently. In case of hypertension and angiotensin converting enzyme (ACE) inhibitors in pregnancy,
the available data demonstrate that they are human teratogens if used in the second and third trimesters, and no harm is caused when their use is limited to the first trimester. However, no special report has been presented for ramipril use in pregnancy.

Our Toxicology Information and Follow-up Service, Karadeniz Technical University, Trabzon, Turkey is a counseling service for pregnant and lactating women and their health professionals. We provide information on the teratogenic risks of drugs depending on available data. We follow up women throughout pregnancy and lactation period. In addition, we perform periodic checks of all babies. Among the 659 cases followed by our center for drug exposure in pregnancy, we have one pregnant woman with metabolic syndrome that used orlistat and ramipril in addition to glimepiride, thiocolchicoside, simvastatin, metformin, ciprofloxacin and aspirin, which were prescribed by her physicians who were unaware of her pregnancy. By presenting this case, our objective is to call attention on the use of multiple and new drugs in pregnancy in controlling the metabolic syndrome of the mother.

Our patient, a 33-year-old, gravida 7, para 3 Caucasian woman, has suffered hypertension, type II diabetes mellitus, hypercholesterolemia, and morbid obesity (body mass index (BMI) = 42 kg/m²) for 5 years and urolithiasis for 6 months. She has a history of 3 induced abortions with her own decision after exposure of multiple drugs (ramipril, glimepiride, and metformin) in her previous pregnancies. She is currently married to a second cousin (third lane consanguineous marriage). Due to her irregular menses, the patient was not aware of her pregnancy until the 8th week. During the first 7 gestational weeks of her seventh (present) and unplanned pregnancy, she used orlistat (360 mg/day), ramipril (5 mg/day), glimepiride (2 mg/day), thiocolchicoside (4 mg/day), simvastatin (20 mg/day), metformin (1700 mg/day), ciprofloxacin (1000 mg/day) and aspirin (100 mg/day). Except for ciprofloxacin and thiocolchicoside, she used these drugs continuously. After the diagnosis of pregnancy at the 8th week, all the drugs were stopped. Methyldopa (750 mg/day) and insulin therapy were started. In the last trimester, the dosage of methyldopa was increased to 1000 mg/day. In the outpatient follow-up visits, blood glucose levels and arterial blood pressure were not optimal. She was hospitalized 3 times during pregnancy period to regulate blood glucose level and arterial blood pressure. In the hospitalization periods, her serum glucose level varied between 70-210 mg/dl, and her arterial blood pressure was between 120/85-160/100 mm Hg. The insulin dose was increased to 12 IU 4 times daily (regular insulin 12 IU 3 times and neutral protamine Hagedom (NPH) insulin 12 IU once a day) in the last trimester. Her glycosylated hemoglobin (HbA1C) was found to be 8 and 8.5% in the follow-up in the second and third trimesters. The patient could not exercise. At the beginning of her pregnancy, she weighed 110 kg, and at the end of her pregnancy, she weighed 131 kg. The results of triple marker screening and amniocentesis were normal in the 17th week. Ultrasonographical examinations were made monthly between the 8th-28th weeks and weekly during the 29th-37th weeks; and all were found normal. Umbilical Doppler ultrasonography was normal when checked in the 18th, 32nd, 33rd, 34th, and 37th weeks. The patient delivered a female infant (3470 gr, 51 cm with APGAR scores of 5-7 at 1 and 5 minutes) at the 38th week with an uncomplicated normal spontaneous vaginal delivery. The baby had no minor or major congenital malformations.

Maternal high BMI is associated with adverse reproductive outcomes, including some birth defects. Data for the use of new drugs for treating obesity in pregnant women are very few. While there was no available report regarding orlistat use in literature, there was only one report of 2 cases with good fetal outcomes about sibutramine use in pregnancy. Our case was exposed to 8 drugs during pregnancy due to obesity, metabolic syndrome, urolithiasis and urinary tract infection. Of these drugs, only aspirin at the dose used by this case seems safe in pregnancy.

Good control of diabetes mellitus throughout gestation is important for an optimal maternal and infant outcome. Oral antidiabetics are not the treatment of choice during pregnancy. Carefully designed insulin therapy provides better control of mother’s blood glucose, thereby preventing fetal and neonatal complications that occur with this disease. In our case, optimal glucose levels could not be achieved in spite of using glimepiride and metformin in the first 7 gestational weeks before the diagnosis of pregnancy, and insulin therapy after the pregnancy diagnosis. Recent reports have shown that even in western countries, perinatal mortality was 5 times, and neonatal mortality was 15 times greater in the offspring of diabetic women. These high results may be the consequence of poor medical and social care prior to conception as well as during the perineonatal period. There are limited data on the use of glimepiride and metformin in pregnancy. In a clinical study, the prevalence of congenital malformations was found to be 52% for a patient group exposed to metformin and 15% for the control group. There was no available data for the use of glimepiride in pregnant animals, and there was only one human case report documenting persistent hypoglycemia in a newborn of a mother who used the drug until delivery. Our case had used glimepiride during the first 6 gestational weeks. Since insulin is still the treatment of choice for
diabetes in pregnancy, insulin was started in our case when the pregnancy was diagnosed. But, she had been exposed to both glimepiride and metformin up to the time of that diagnosis. In addition to oral antidiabetic drugs, the patient was also exposed to orlistat for obesity during first 7 gestational weeks. There was no report on the use of orlistat in pregnancy in humans.2 Teratogenic studies have been conducted in rats and rabbits at doses up to 800 mg/kg/day. Neither study showed embryotoxicity or teratogenicity. This dose is 23 and 47 times the daily human dose calculated on a body surface area basis for rats and rabbits. However, the manufacturer does not recommend the use of orlistat during pregnancy. The patient was also exposed to simvastatin. There are limited data on the use of this drug during human pregnancy.2 Based on animal data and limited human experience, simvastatin exposure does not appear to present a significant risk for the baby. A case report of a diabetic, hypertensive and obese woman exposed to another new statin indicated a normal fetal outcome.3

The management of chronic hypertension in pregnancy is one of the most controversial areas, despite the relative frequency of the condition (1.5-2%). Association between chronic hypertension and increased risk for morbidity and mortality to both mother and fetus is undisputed; stillbirth, placental abruption, intrapartum growth retardation and hypoxic effects of superimposed pregnancy-induced hypertension are common in fetus. There are many reports showing that ACE inhibitors are safe if taken during the first trimester, and among these drugs, fetal exposure to captopril and enalapril in the second and third trimesters has been found to be associated with teratogenicity and severe toxicity in the fetus and newborn.2 Angiotensin converting enzyme inhibitors may cross the human placenta in pharmacologically significant amount. Angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists should be discontinued due to fetotoxicity. The use of ACE inhibitors during pregnancy was reported to be associated with poor fetal outcomes, including oligohydramnios, renal tubular dysplasia, cranial malformations, and fetal death. Our case was exposed to ramipril, an ACE inhibitor, which was prescribed for treatment of chronic hypertension. No report about ramipril use in human pregnancy has been located, and no teratogenic effect was found in animals. Since the ACE inhibitors share the same mechanism of action, ramipril might cause the same malformations as the other ACE inhibitors. When the patient’s pregnancy was diagnosed, this drug was also stopped and methyldopa, which is considered to be safe for pregnant women2 was started.

Our case was exposed to ciprofloxacin and thiocolchicoside for treatment of urinary tract infection and urolithiasis. No case of thiocolchicoside exposure in pregnancy was reported in either humans or animals. While there are some studies that concludes that the use of ciprofloxacin in pregnancy does not appear to be associated with an increased risk of major congenital malformations, some other studies have concluded that fluoroquinolones should be considered as contraindication or at least not the first choice during pregnancy, and physicians should be encouraged to select safer alternatives.

To our knowledge, this is the first case in the literature that has evaluated the effects on the fetus of using orlistat, ramipril and thiocolchicoside during pregnancy, in addition to 5 other drugs in the same case in the first trimester. The baby had no congenital anomaly and that case may contribute to the very limited knowledge regarding human exposure to at least 3 prescribed drugs (orlistat, ramipril and thiocolchicoside) for which no previous literature is available.

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References

Randomized trial of ceftriaxone prophylaxis in elective cesarean section

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Cesarean section is an essential operation, which is widely practiced and its rate has increased steadily and may reach up to 25% in some centers. Maternal morbidity related to infections after cesarean section was 8-fold higher than vaginal delivery and endometritis was the most common of these complications. The reduction of endometritis by two third to three quarters justifies a policy of administering prophylactic antibiotic to women undergoing elective or non elective cesarean section. Controversy still exists on the benefit and the choice of prophylactic antibiotic in elective cesarean section, and the best prophylactic regimen has yet to be described. The study was performed to investigate the efficacy of ceftriaxone in decreasing the frequency of postoperative infection and related morbidity in elective cesarean section.

Patients planned for elective cesarean section at New Halfa Teaching Hospital, Eastern Sudan during the period September 2003 to April 2004 were enrolled in the study. Those who received antibiotics within the last 2 weeks had any visible infection, elevated temperature, allergic to antimicrobials or if they did not wish to participate in the study were excluded.

Patients were randomized in 2 groups; the study group to receive a single dose of ceftriaxone 1 gm intravenously at anesthetic induction or no prophylaxis (control group). After verbal consent, a complete history was taken in standard questionnaire and physical examination was performed. The outcome examined were the incidence of: 1) Postoperative febrile morbidity, defined as an oral temperature of 38.5°C or more on 2 occasions at least 4 hours apart excluding the first 24 hours. 2) Postoperative infections, which include: a) Endometritis (fever, uterine tenderness and abnormal lochia). b) Wound infection. c) Pelvic abscess. d) Peritonitis (elevated temperature, tachycardia, abdominal distension and pain with guarding and rigidity aggravated by moving and breathing with absent bowel sounds at the onset of paralytic ileus). e) Other febrile morbidity, namely, urinary tract infection, chest infection and malaria.

Once febrile morbidity was identified, the patients were examined to localize the potential source of infection (tonsils, breasts, chest abdomen and pelvis). Urine analysis followed by urine for culture and sensitivity if the result of examination was suggestive of infection, total white blood cells count, blood and cervical swabs were sent (Mackonkey agar media) for culture and sensitivity. Blood film (Giemsa stained) were taken by finger pricks to confirm or to exclude malaria.

The policy in New Halfa Teaching Hospital is to treat post-cesarean febrile morbidity (endometritis, peritonitis and pelvic abscess) with triple antibiotics (ampicillin/cloxacin 500 gm every 6 hours, gentamicin 80 mg every 8 hours, metronidazole 500 mg every 8 hours for 7 days). If no response, these drugs were changed to antibiotic guided by the result of the culture sensitivity.

Data were entered in microcomputer for analysis using Statistical Package for Social Sciences. The X² test, students, t-test and Fischer’s exact test were used where applicable. A p value of <0.05 was considered significant.

During the period of this study there were 920 vaginal deliveries and 287 cesarean sections (23.7%), 34.8% of them were elective while 65.2% were emergency. There was one maternal death due to septicemia following emergency cesarean section.

Hundred patients planned for elective cesarean section for various reasons (repeated scars, cephalo-pelvic disproportion and others), were enrolled to the study, 50 patients in each group (study and control). The 2 groups were well matched at the enrollment and there were no statistical differences in the admission variables, Table 1. The incidence of postoperative febrile morbidity was not significantly different between the study and control groups (2% versus 4%, p = 0.5). There were 2 (2%) cases of endometritis, one in each group.

There was no patient in any group suffered wound infection or peritonitis. While one patient (2%) in the control group developed other febrile

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>30.8 ± 6.3</td>
<td>29.9 ± 6.2</td>
</tr>
<tr>
<td>Parity</td>
<td>1.9 ± 1.9</td>
<td>2.4 ± 2.04</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.2 ± 10.6</td>
<td>65.8 ± 12.2</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>38.7 ± 0.90</td>
<td>38.5 ± 1.09</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>37.08 ± 0.17</td>
<td>37.1 ± 0.18</td>
</tr>
<tr>
<td>Preoperative hemoglobin, (%)</td>
<td>69.6 ± (8.8)</td>
<td>71.1 ± 8.3</td>
</tr>
</tbody>
</table>

SD - standard deviation
*p >0.05 in all the values
morbidity not associated with endometritis (malaria), none of the study group developed this complication \((p = 0.5)\).

There were 2 (4\%) babies with low Apgar score (< 8) at 1 and 5 minutes in the study group versus 3 (6\%) in the control group \((p = 0.64)\). There were 2 perinatal deaths; one in each group, due to respiratory distress syndrome (control) and second died due to septicemia complicated imperforate anus (study).

The total incidence of postoperative febrile morbidity was 3\% without significant statistical difference between the two groups; this figure is near to the incidence of postoperative febrile morbidity when ceftriaxone was compared with ampicillin/cloxacinil in the Central Sudan.7 Thus, postoperative infections morbidity following low-risk cesarean section cannot be reduced by ceftriaxone prophylaxis.

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References


Retropubic space hemorrhage: An unusual complication of cesarean section

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Hemorrhage during cesarean section is usually from the uterus. Massive bleeding from the parietes is an unusual case. We report a very rare case of hemorrhage in the space of Retzius during elective cesarean section. The bleeding was a venous ooze and was eventually controlled with tamponade. Tamponade is the first approach to bleeding from the space of Retzius and it usually controls the bleeding. Factor VIIa is a single coagulation factor manufactured by recombinant cell technology and has been found to be useful in controlling hemorrhage in some surgical patients.

A 34-year-old female, gravida 3, para 2 with history of 2 previous cesarean sections, was booked in the antenatal clinic in our hospital at 32 weeks of gestation. She had undergone 2 cesarean sections for big babies (4.9 kgm and 5.2 kgm) in the past. She was healthy with no significant family history of diabetes mellitus. Abdominal examination revealed subumbilical midline vertical scars from previous cesareans. Oral glucose tolerance test was performed in view of her past recurr of having big babies and the results were as follows: fasting 6 mmol/l and 2 hours postprandial was 10 mmol/l. She was advised to follow diabetic diet and the subsequent glucose profile was normal. She was booked for an elective cesarean section at 38 weeks gestation. She was counseled for a subumbilical midline incision in view of her previous 2 subumbilical midline scars, but she refused. Cesarean section was carried out under spinal anesthesia through a suprapubic transverse incision at her request. A baby girl weighing 3330 gms was delivered without difficulty and uterine wound hemostasis was satisfactory. At the time of closure some bleeding was noted in the retropubic space, which appeared to be venous bleeding. An attempt was made to control the bleeding with simple pressure. This procedure only deteriorated the bleeding for there was deepening of the bleeding space and hemorrhage became heavy. An attempt to control the bleeding by putting stitches also failed. As there was no identifiable arterial bleeder, the
space was eventually packed with hemostatic surgical cellulose (oxidized regenerated cellulose, Ethicon) and a large wet pack. The bleeding was controlled and the rectus sheath was closed with interrupted sutures. Part of the pack was brought out through an opening in the rectus sheath and the skin. The wound was closed and a urinary catheter was left in situ. We estimated the blood loss to be approximately 2000 ml. The patient was a transfused 4 units of matched blood. Clear urine was draining after the cesarean section. Coagulation profile carried out intraoperatively and a few hours after transfusion were normal. She was started on cefuroxime (Glasgow Smith Kline, United Kingdom) and metronidazole. The pack was left in situ and was removed after 48 hours under general anesthesia. Since there was no further bleeding after pack removal the rectus sheath and skin were closed again and the catheter was removed soon after. She was discharged home after another week of hospital stay in good condition. On further follow up, after 6 weeks she remains well with no complaints.

Hemorrhage from retropubic space is an uncommon complication usually reported after bladder neck buttress operations. Foley catheter tamponade is usually used to control such a bleeding. Although sometimes, this method may not work. Ottolenghi and Sesenna described a rare case of hemorrhage in the space of Retzius after normal delivery, which was treated with tamponade as other methods failed. Another case of a male patient involved in a traffic accident who also responded to the same method of treatment was reported by a Polish authors. Tamponade appears to help control bleeding from retropubic space. Another treatment option could have been recombinant FVIIa. Recombinant FVIIa has been considered as a universal hemostatic agent, prompting its use in the management of severe uncontrolled surgical bleeding in patients without pre-existing coagulopathies. However, it is costly and not readily available in many units.

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References


Condylomata acuminata in infants and young children. Topical podophyllin an effective therapy

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Condylomata acuminata, an infection caused by human papilloma virus, has become one of the most common sexually transmitted disease in adults. Correspondingly, the incidence of anogenital warts among children is rising. Although its relationship to child abuse remains controversial, many cases of anogenital warts in children probably represents autoinoculation, vertical transmission or nonsexual transmission. Still, anogenital warts can be the only manifestation of child sexual abuse and that human papilloma virus typing does not provide a definite evidence for or against sexual abuse. Prospective surveys have documented perinatal transmission of human papilloma virus at oropharyngeal and genital sites in as many as half of infants delivered vaginally. Reports of subclinical infection of neonates delivered by cesarean section and of congenital condylomata strongly support the possibility of ascending infection. The potentially long incubation period of human papilloma virus also confounds the picture. Some investigators believe that the appearance of warts before the age of 2 years is suggestive of perinatal transmission and appearance either at birth or within the first week of life, is a diagnostic of perinatal transmission. The existence of multiple treatment modalities reflects the fact that there are no effective or direct antiviral. Several treatment options are available for condylomata acuminata in adults, none have been studied for the treatment in children. Most of the treatments mentioned are painful and traumatic for children, some even requiring general anesthesia with its associated risk. Whatever method is used, there will be failure and recurrences.

The present work was conducted to evaluate this condition among Iraqi children and to report the experience with podophyllin as a safe, effective, mode of therapy. Condylomata acuminata was assessed in 18 patients attending the Department of Dermatology and Venereology in Baghdad Teaching Hospital, Baghdad, Iraq during the period.
January 1996 to January 2000. A full history was taken from their parents regarding the presence of obvious genital warts and behavioral abnormalities. Possible sexual abuse on these children was carefully investigated. Examination of the affected patients was conducted. Careful searching for signs of sexual abuse was carried out as follows: fresh and old bruise location are noted, as well as unusual scars, burns or wounds, funnel shaped anal area, signs of rectal or genital infection or injury. The age of the patients ranged from 6-84 months (23.66 ± 20.30 months) while the duration of the disease ranged from 3-8 months (5.83 ± 1.24 months). Eleven female infants were mostly affected compared to 7 male infants with a female - male ratio of approximately 1.6:1. During treatment, podophyllin in tincture benzoin (15%) was applied by the attending physician once weekly in the Department of Dermatology and Venereology of Baghdad Teaching Hospital, Baghdad, Iraq. The surrounding skin of the warts was covered with white soft paraffin to protect it from the splashed paint. Using cotton tipped applicator, freshly prepared paint was applied to the warts and the parents were asked to wash off the area after 6 hours. Follow up was carried out for several months. Examination of the female infants revealed typical cauliflower condylomata acuminata in the genital area of 2 patients in the anal and perianal area in 5 patients, while in both areas in 3 patients. One girl had skin colored papular form of condylomata acuminata in natal cleft and perianal area. All males had only anal condylomata acuminata. The infants parent and other members of family denied having genital warts apart from one girl whom the mother had verruca vulgaris on her fingers. Sexual abuse could not be ascertained in any patient.

The result of treatment with 15% podophyllin in tincture benzoin was effective after 1-3 applications (1.77 ± 0.73 months) once weekly. All genital warts had disappeared during follow up and there was no signs of relapse. No expected side effects such as irritation were reported by the parents. The treatment was well tolerated by the patients.

The incidence of genital and perianal warts in infants and children is increasing worldwide. This probably reflects on the increase in the prevalence of genital warts in the general population. Our work was comparable with this increase as to the frequency of genital warts among infants and young children. Since there was no history of sexual abuse in these affected patients, we believe that their genital warts was a result from direct contact with other members of the family, who are either not aware of the presence of warts or have latent infection.

Podophyllin seems to be the drug of choice for all patients who responded quickly to this mode of therapy. This experience with podophyllin in this age group has not been reported in any published literature before.

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