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. [1]

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. [6]

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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Contribution study of visceral leishmaniasis in Syria

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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 immunoflourescent 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

(clinically sighting the *Leishmania* parasites extracted from bone marrow aspirates).

We studied the cases admitted to 11 hospitals in major cities. Different diagnostic methods were applied on the bone marrow aspirates obtained from Daraa'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 Daraa 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

Table 1 - Cases of visceral leishmaniasis reported during the period 1993 - 2003 in 11 major cities in Syria.

Geographic region	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Damascus	0	0	0	0	0	0	1	0	0	0	0
Homs	0	0	1	0	1	0	0	0	0	0	0
Hama	1	0	0	0	0	0	0	0	0	0	0
Tartous	3	0	0	3	2	0	0	0	0	0	0
Lattakia	4	1	3	8	12	7	12	4	1	14	14
Idlep	1	1	1	0	1	26	26	18	20	15	2
Aleppo	0	0	0	1	1	7	4	6	6	8	9
Al-sweida	2	0	1	1	0	1	0	0	0	0	0
Daraa	5	22	9	12	13	4	14	2	2	1	1
Damascus suburb	1	0	0	0	0	0	0	0	1	0	0
Hasakeh	0	0	0	0	0	0	1	2	0	0	0
Total	**17**	**24**	**15**	**25**	**30**	**45**	**58**	**32**	**30**	**38**	**36**

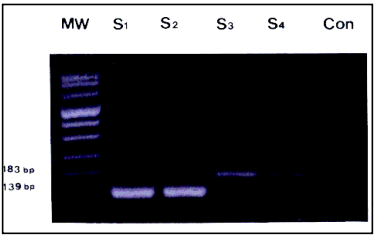

Figure 1 - Detection of amplification products using 4% gel and ethidium bromide staining. The 139 bp fragment corresponds to *Leishmania* amplified DNA and the 183 bp fragment corresponds to internal control amplified DNA. MW - molecular weight standers, S1, S2, S4 - positive samples of visceral leishmaniasis, 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.[5] Indeed, Idlep and Lattakia Governorates 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,[6,7] it was used to diagnose suspected patients who presented the clinical symptoms of VL but its parasite could not be demonstrated by biological tests,[2,3] which shows the parasites microscopically, in stained smears, *in vitro* cultivation and indirectly by serological means,[3] whereas the PCR test depends upon the detection of *Leishmania* DNA in VL suspected samples. The kDNA was composed of up to 10000 copies of approximately 800 bp minicircles.[8] 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.[1] 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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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