Visceral leishmaniasis (VL) is a systemic protozoan disease that is transmitted by phlebotomine sandflies and is endemic in eastern states of India, mainly Bihar, West Bengal and Orissa. However, epidemiological shift from east to west is taking place and an increased numbers of cases have been reported from natives of sub-Himalayan region of Uttararakhand and Himachal Pradesh. According to a World Health Organization (2013) estimate, 1.3 million new cases of leishmaniasis occur worldwide annually. Expansion of VL into new areas is one of the challenges in elimination of the disease from the region. Major risk factors of Leishmania distribution are socio-economic conditions, malnutrition, population mobility, and environmental and climate changes. The possible reasons for finding new foci of VL cases could be the improved monitoring system, high index of suspicion and subsequent improvement in diagnostic modalities, shifting of vector’s geographic range, development of favourable factors for vector expansion and leishmaniasis transmission.

We report four cases of VL diagnosed at a teaching tertiary care centre, located in the physiogeographic zone of Shivalik and Lesser Himalayas of the State of Himachal Pradesh over a period of four years. These patients had never visited VL endemic regions in their life. The clinical presentation was more or less classical in first two patients. However, in third patient secondary hemophagocytosis lymphohistiocytosis (HLH) was associated with VL. Another patient had an atypical presentation in the form of chronic diarrhoea and was unexpectedly diagnosed as a case of intestinal leishmaniasis through the identification of the Leishman-donovan (LD) bodies in duodenal and colonic mucosa. These are the first reports of VL from the non-endemic northwestern hilly region of Chamba, Himachal Pradesh and adjoining area of Basholi in Jammu and Kashmir.

Case reports

All the patients were native of Ravi River valley area situated at an average altitude of 996 m (3268 ft) above the mean sea level (Fig. 1). Among the four patients three were males and one female. The age distribution and year of presentation are given in Table 1. The duration of symptoms varied from 1–6 months. The presenting symptoms and signs are given in Table 1. The details of haematology and biochemistry parameters at admission are shown in Table 2. All the four patients were negative for human immunodeficiency virus infection. All the patients denied ever visiting any endemic area of VL. Patient number 1 and 2 had classical presentation of VL in the form of fever, hepatosplenomegaly and pancytopenia. Patient number 3, and 4 had atypical presentation which added to the diagnostic dilemma. In patient number 3, VL was found to be associated with secondary HLH. On haematological investigations pancytopenia was observed. Liver function tests showed transaminasemia. Bone marrow aspiration and biopsy revealed marked increase in histiocytes with a prominent hemophagocytosis. Amastigote forms of Leishmania donovani were seen both intracellularly and extracellularly (Fig. 2). The diagnosis of HLH was established, as 6 out of 8 diagnostic criteria (5 out of 8 are required) were fulfilled, viz. fever, splenomegaly, cytopenia, hyperferritinemia, hypertriglyceridemia and hemophagocytosis in the bone marrow. Patient number 4 presented with chronic diarrhoea. VL was not a differential diagnosis and was unexpectedly diagnosed as intestinal leishmaniasis through the identification of the LD bodies in duodenal and colonic mucosa (Fig. 3). The main pitfall in the diagnosis was that even with classical presentation the first diagnosis was always other than kala-azar in all of the cases. The treatment received by the patients and outcome is mentioned in Table 1.

DISCUSSION

Chamba district is topographically bounded by the Dhauladhar and Zanskar ranges, south of inner Himalayas. The district is bordered by Jammu and Kashmir in the
northwest and west, the Ladakh area of Jammu and Kashmir, and Lahaul and Bara Banghal in the northeast and east, Kangra in the southeast and Pathankot district of Punjab in the south. The temperatures in summer vary between 38°C (100°F) and 15°C (59°F), and in winter between 15°C (59°F) and 0°C (32°F). The average annual rainfall in the area is 785.84 mm (30.939 inch). The VL has already been reported in southeast area of Himachal Pradesh from the sub-alpine valley along the Sutlej River that leads to the mountain deserts of the tribal

Table 1. Patient profile of cases of visceral leishmaniasis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/ Gender</th>
<th>Year</th>
<th>Duration of symptoms in months</th>
<th>Clinical presentation</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>30/Female</td>
<td>2011</td>
<td>6</td>
<td>Fever, Exertional dyspnoea, Loss of appetite, Loss of weight, Hepatomegaly, Massive splenomegaly, Pancytopenia</td>
<td>Sodium stibogluconate</td>
<td>Expired</td>
</tr>
<tr>
<td>2.</td>
<td>33/Male</td>
<td>2013</td>
<td>2</td>
<td>Fever, Loss of appetite, Loss of weight, Moderate splenomegaly, Pancytopenia</td>
<td>Amphotericin-B</td>
<td>Recovered</td>
</tr>
<tr>
<td>3.</td>
<td>40/Male</td>
<td>2014</td>
<td>1</td>
<td>Fever, Hepatomegaly, Massive splenomegaly, Pancytopenia, Hemophagocytosis</td>
<td>Amphotericin-B</td>
<td>Recovered</td>
</tr>
<tr>
<td>4.</td>
<td>50/Male</td>
<td>2014</td>
<td>6</td>
<td>Fever, Chronic diarrhoea, Loss of weight, Hepatomegaly, Massive splenomegaly, Bicytopenia</td>
<td>–</td>
<td>Expired</td>
</tr>
</tbody>
</table>
district of Kinnaur and adjacent area of Shimla and Kullu
districts at an altitude of 924–2900 m above mean sea
level (Fig. 1). The current “hotspots” are at a distance of
> 600 km from previously identified “endemic” areas of
the state. The area has witnessed large scale deforesta-
tion, construction activities, urbanization, establishment
of new residential colonies, creation of townships, for-
mation of large water bodies in the form of dams and
lakes. A major multipurpose project, Ranjit Sagar Dam
has been built on the River Ravi. Further, hydroelectric
potential of the river has been exploited with the con-
struction of five large and numerous small hydroelectric
power projects. Creation of job opportunities has caused
migration of manpower from Bihar, Nepal, Jharkhand and
Uttar Pradesh. The six species of *Phlebotomus* and 15
species of *Sergentomyia* have been found in the northern
mountain ranges of the Himalayas (Himachal Pradesh is
situated in these ranges), with their distribution limited
to particular ecoclimatic zones7.

Survey of Phlebotomid sandflies from the Himalayan
region, India has established the presence of *Phleboto-
mus longiductus*, *P. major*, *Sergentomyia bailyi*
and *S. montana* (Diptera: Psychodidae) at an altitude of
1570 m in the Tissa region of Chamba district 7–8. While under-
standing the ecosystem of phlebotomine vectors it has
been observed that VL vector *P. argentipes* produced high
clusters around waterbody and dense forest in non-en-
demic sites. These conditions are preferred as oviposi-
tion and breeding places and favour the development of
vector9. Their role as a vector for transmission of VL, in
this particular region needs to be delineated by demon-
strating similar strains of *Leishmania* in both the vector
and the human host. Environmental changes are occur-
ring simultaneously during last two decades. Climate
change (systematic temperature increases by 0.01–0.04
°C per year) is likely to increase the burden of infectious
diseases in particular, to vector-borne tropical diseases
in the Indian subcontinent, VL is mainly caused by *L. donovani*
and its transmission is assumed anthroponotic through the bite
of the sandfly species *P. argentipes*11. We interpret ap-
pearance of VL in this non-endemic area as an indication
of autochthonous transmission as there is no travel his-
tory of the reported cases to endemic regions. However,
what favours imported VL vis-à-vis indigenous transmis-
sion in non-endemic areas is low frequency of cases
among children. It is still not clear whether the parasite
can come from intrusion into sylvatic cycle or introduced
by migrant population. These immigrants could have been

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient No. 1</th>
<th>Patient No. 2</th>
<th>Patient No. 3</th>
<th>Patient No. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g%)</td>
<td>6.4</td>
<td>4.6</td>
<td>6.7</td>
<td>7.5</td>
</tr>
<tr>
<td>TLC (cells/mm³)</td>
<td>2000</td>
<td>1300</td>
<td>600</td>
<td>2100</td>
</tr>
<tr>
<td>Polymorphs</td>
<td>31</td>
<td>44</td>
<td>41</td>
<td>45</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>66</td>
<td>49</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2</td>
<td>5</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Platelets (per mm³)</td>
<td>99,000</td>
<td>26,000</td>
<td>36,000</td>
<td>134,000</td>
</tr>
<tr>
<td>ESR (mm 1st hour)</td>
<td>–</td>
<td>60</td>
<td>102</td>
<td>60</td>
</tr>
<tr>
<td>Total (mg/dl)</td>
<td>1.6</td>
<td>1</td>
<td>1.35</td>
<td>0.31</td>
</tr>
<tr>
<td>Direct (mg/dl)</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>147</td>
<td>194</td>
<td>256</td>
<td>24</td>
</tr>
<tr>
<td>ALT (IU)</td>
<td>130</td>
<td>86</td>
<td>151</td>
<td>8</td>
</tr>
<tr>
<td>Alkaline phosphatase (KAU)</td>
<td>689</td>
<td>86</td>
<td>475</td>
<td>152</td>
</tr>
<tr>
<td>Total (g/dl)</td>
<td>6.2</td>
<td>5.5</td>
<td>7</td>
<td>7.1</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.5</td>
<td>1.9</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>26</td>
<td>49</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9</td>
<td>1.1</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Serum ferritin (mg/ml)</td>
<td>–</td>
<td>–</td>
<td>18,500</td>
<td>–</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>–</td>
<td>–</td>
<td>274</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 2: Bone marrow aspiration smear shows (a) Macrophages with engulfed erythroid cells and lymphocytes (hemophagocytosis); and (b) Megakaryocyte with engulfed LD bodies (Giemsa, ×1000).

Fig. 3: Photomicrograph showing macrophages with abundant intracytoplasmic and extracellular bodies of leishmania: (a) Duodenal biopsy; and (b) Colonic biopsy (H and E, ×1000).
infected with *L. donovani* outside this region. The fact that all our patients had contracted the disease indigenously is suggestive of a local vector and probably a zoonotic reservoir. Concerns of potential animal reservoirs in India are rising as evident by the increasing seroprevalence of rK 39 antigen tests in animals\textsuperscript{12}. Domestic animals as reservoir hosts in VL transmission have been indicated in a recent study in Nepal\textsuperscript{13}.

Furthermore, the genetic makeup of *Leishmania* isolates of this region should be studied and compared with other Indian isolates.

REFERENCES