Clinico-laboratory profile of dengue patients returning from tropical areas to Poland during 2010–15

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ABSTRACT

Background & objectives: Dengue is a mosquito-borne viral disease, incidences of which have increased rapidly in the past decade. About 400 million new infections are recorded annually worldwide, and 40% of the human population lives in the areas at risk of dengue transmission. In this study, the clinical and laboratory profile of dengue diagnosed patients returning to Poland from tropical areas has been analyzed.

Methods: This is a retrospective study of 65 patients based on clinical symptoms and serological tests conducted in the Department of Tropical Parasitology of Institute of Maritime and Tropical Medicine (IMTM) in Gdynia, Poland during 2010–15. The diagnosis of dengue virus (DENV) infection was based on the detection of specific immunoglobulin M (IgM) and IgA antibodies performed by specific ELISA assays. The samples were considered as positive, if the absorbance was >10% above the cut-off value.

Results: Among 65 persons with dengue selected for the analysis, 59 patients were admitted to the hospital because of clinical symptoms of the disease; the six persons initially asymptomatic were hospitalized due to routine control treatment returning from tropical regions. Patients reported various destinations, purpose and duration of their travels. All of them had returned from dengue endemic regions and were positive for IgM antibodies against DENV. Common clinical symptoms observed were fever (ranging from 38 to 40°C in 43% of patients), weakness, headache and rash. Laboratory tests revealed elevated levels of transaminase activity, leukopenia and thrombocytopenia in 35.3, 24.6 and 20% of patients, respectively.

Interpretation & conclusion: The number of Polish travelers to subtropics and tropics increases every year. As cases of fever and other symptoms appear to be increasingly in Polish people returning from tropical regions of Asia, America and Africa, their screening and management should be taken seriously. The study also suggests that the disease might be more widespread than it was known previously.

Key words: Dengue; DENV; mosquitoes; Polish travelers; tropical areas

INTRODUCTION

Dengue is one of the most important arthropod-borne viral diseases; with around 40% of the world’s population living in areas of increased risk of dengue infection. Annually, 400 million new infections are recorded and about 5% of the patients present a severe form of dengue, with 25,000 deaths being reported each year1. Many reports show a dramatic increase in geographical range of the disease2–3. Before 1970, it was only reported from nine countries, but according to the World Health Organization (WHO), 2015 the disease is now present in ≥100 countries of the world, affecting predominantly children, including new cases in Europe (France, Croatia, Madeira in Portugal)4. The viral etiological agents of dengue are transmitted primarily by Aedes aegypti and Ae. albopictus mosquitoes (Diptera: Culicidae). The disease is caused by four distinct dengue RNA virus serotypes, i.e. DENV-1, DENV-2, DENV-3, and DENV-4 that belong to the Flavivirus genus of the Flaviviridae family. The fifth serotype, DENV-5 has been isolated in 2013; which is believed to be limited to the forest canopies of Southeast Asia; however, a possibility of transmission to other regions/countries can not be ruled out5. The clinical symptoms of dengue range from a simple febrile illness like fever, weakness, headache, muscle and joints pain, cough and throat pain to hemorrhagic fever or shock.

The increase in incidence is partially attributed to geographic expansion of Ae. aegypti mosquitoes, which results in co-circulation of all four dengue serotypes in urban areas, and increasing severity of the disease6. Dengue is considered as the most rapidly spreading mosquito-
borne viral disease in the world, because of a rising number of persons traveling greater distances; viraemic travelers play crucial role in the spread of disease as they may carry dengue strains beyond the national borders.

In 2015, Centers for Disease Control and Prevention (CDC) described a new case definition of dengue-based on: (i) clinical symptoms, (ii) laboratory tests, and (iii) epidemiological inquiry. The former classification of clinical forms of dengue has been abandoned and replaced by three recognized forms, viz. dengue, severe dengue and dengue-like illness.

In Poland, diagnosis of dengue is available in selected centers of tropical medicine since 2005. There is no specific obligation to report dengue to sanitary-epidemiological service; instead, there is only a general requirement to report “viral hemorrhagic fevers, including yellow fever”. It seems that it might be worth considering in the future to add dengue as a specific infection among other viral hemorrhagic fevers to the list of obligatory reported infectious diseases in Poland. Consequently, there is no reliable official data on the number of Poles suffering from dengue, while in consideration of increasing interest in foreign travels to tropical countries regarded as dengue endemic areas, we can expect an increase in dengue incidence to occur.

The aim of this retrospective study was to analyze the clinical and laboratory profiles of dengue patients returning to Poland from different tropical and subtropical destinations and to identify the predictive factors of severe dengue infections that enable better clinical management in the hospital units.

**MATERIAL & METHODS**

Results of serological tests for the presence of IgM and IgG antibodies performed during the years 2010–15 on blood samples of 192 patients with suspected dengue received from the Department of Tropical and Parasitic Diseases, University Center for Maritime and Tropical Medicine (UCMTM), and analyzed for dengue antibodies positivity in the laboratory of the Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine (IMTM), Medical University of Gdansk. Based on the data, an Excel spreadsheet was prepared for patients whose results were found positive for either of the investigated antibody classes. In case of borderline results in either antibody class, accompanied by a negative result in another antibody class, the data were rejected unless the blood samples were collected again and an increase in antibody titer was observed.

Laboratory diagnosis of dengue fever was performed during the study on all patients with suspected dengue fever, in the Department of Tropical Parasitology for detection of specific immunoglobulins using Dengue IgM ELISA and Dengue IgG ELISA kits (IBL International, Hamburg, Germany). In both tests, samples were considered as positive if the absorbance was >10% above the cut-off value and negative if the absorbance was <10% below the cut-off value. Samples with an absorbance value of 10% above or below the cut-off value were treated as equivocal (according to kit’s instruction).

No molecular tests were performed on samples collected from patients, therefore, it was not possible to confirm the presence of viral nucleic acid and serotype differentiation. Tests for confirming the presence of antigen NS1 DENV was either not performed.

For dengue case definition, the CDC criteria of 2015 were used. Due to technical limitations, for laboratory confirmation, anti-DENV IgM were determined in patients’ sera by an immunoassay validated in travelers returning from a dengue endemic area without ongoing transmission of another flavivirus (*e.g.* West Nile virus, Japanese encephalitis virus, Yellow fever virus) or clinical evidence of co-infection with any of the flaviviruses. None of the patients reported recent immunization against yellow fever, tick-borne encephalitis or Japanese encephalitis.

Using the documentation archive of the UCMTM, a database was created including patient’s age, sex, number of days of hospitalization, diagnosis presented in the hospital discharge card, country or countries of destination reported in the medical history and duration of the journey, symptoms of the disease and results of laboratory investigation and imaging tests. It also included clinical symptoms of the disease like fever, hepatomegaly, bleeding signs, etc. laboratory results (hemoglobin, hematocrit and platelets) and results of imaging assays. The dengue cases were divided into dengue fever, severe dengue and shock in the course of the disease.

**RESULTS**

Based on the retrospective review of the results of serological tests of 192 patients with suspected dengue, 65 (33.8%) patients were selected for further analysis. All of them were positive for IgM antibodies against DENV and all of them had returned from regions of dengue endemic zones and free from transmission of other flaviviruses. None of these patients reported a recent vaccination against flaviviruses.

In the analyzed group, 46 (70.7%) patients were men and 19 (29.2%) were women; and the mean age of pa-
Patients was 38 yr (Range 13 to 75 yr). The majority of the patients returned from Asia [29 (44.6%)]—India, Afghanistan and Pakistan; followed by Africa [19 (27.6%)]; South America [5 (7.6%)]—Brazil, Argentina, Bolivia and Peru; and Middle America [3 (4.6%)]—Cuba and Dominican Republic Mexico. The data for 9 (13.8%) patients were uncertain, i.e. the exposure to infection probably occurred in the Far East (Thailand, Vietnam, Laos, Cambodia, Korea and China), South and Central America. Mean duration of the stay was 32.78 months, ranging from one week to 10 yr. After excluding nine missionaries, usually travelling for long stays, the mean duration of stay was 16 months.

Declared purposes of the travels were tourism (for 17 (26.1%) patients and business trips for 23 (35.3%) persons. Among the persons travelling for business reasons were missionaries (9), regular soldiers, physicians and nurses, journalists, participants of charitable missions, workers of touristic agencies and sport trainees. One person reported a mixed purpose of the travel.

Out of 65 hospitalized patients, 59 showed signs and symptoms of dengue while six patients were asymptomatic and hospitalized due to routine control treatment. The mean time of returning from the tropics to the onset of symptoms was eight days; the duration of hospitalization was 2 to 21 days.

The most common symptom was fever defined as body temperature ≥38°C. It was present in 43% of the patients ranging from 38–40°C. Sub-febrile but elevated temperature ranging from 37.4–37.8°C was observed in 4.6% of the patients. During hospitalization, the fever lasted for another 2.3 days on average. Among other symptoms and ailments, weakness (43%), headache (21.5%) and rash (18.4%) were common. The clinical symptoms appearing in patients with dengue are presented in Table 1.

Laboratory tests revealed thrombocytopenia, defined as number of blood platelets (PLT) <140 x 10⁹/l, in 20% of patients with the lowest PLT value of 28 x 10⁹/l. Leukopenia, defined as WBC <4 x 10⁹/l, was present in 24.6% of patients and the lowest number of leukocytes was 0.52 x 10⁹/l. Activity of transaminases, i.e. serum glutamic oxaloacetic transaminases (SGOT) and serum glutamic pyruvic transaminases (SGPT) was elevated in 35.3% and 21.5% of the patients, respectively. The mean concentration of creatinine was 0.94 mg/dl with the highest value of 1.61 mg/dl and was above the reference value in 12.3% of patients. D-dimers were elevated in 35.3% of patients with the average value of 1131.94 μg/dl and the highest value of 6691 μg/dl. Results of laboratory tests of patients with dengue are compiled in Table 2.

![Table 1. Spectrum of clinical symptoms appearing in dengue patients](attachment:table1.png)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever ≥ 38°C</td>
<td>28 (43)</td>
</tr>
<tr>
<td>Subfebrile temperature 37–38°C</td>
<td>3 (4.6)</td>
</tr>
<tr>
<td>Weakness</td>
<td>28 (43)</td>
</tr>
<tr>
<td>Headache</td>
<td>14 (21.5)</td>
</tr>
<tr>
<td>Rash</td>
<td>12 (18.4)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>10 (15.5)</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6 (9.2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (9.2)</td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (7.6)</td>
</tr>
<tr>
<td>Pain behind the eyes</td>
<td>4 (6.1)</td>
</tr>
<tr>
<td>Cough</td>
<td>4 (6.1)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>4 (6.1)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>3 (4.6)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

![Table 2. Compilation of laboratory tests results of dengue patients](attachment:table2.png)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference value</th>
<th>Average value in patients</th>
<th>Range</th>
<th>No. of patients with results below/above the reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (10⁹/l)</td>
<td>140 – 400</td>
<td>190.6</td>
<td>28–346</td>
<td>13 (20)</td>
</tr>
<tr>
<td>WBC (10⁹/l)</td>
<td>4 – 11</td>
<td>5.19</td>
<td>0.52–10.6</td>
<td>16 (24.6)</td>
</tr>
<tr>
<td>SGOT (U/l)</td>
<td>&lt;33</td>
<td>64.01</td>
<td>8–411</td>
<td>23 (35.3)</td>
</tr>
<tr>
<td>SGPT (U/l)</td>
<td>&lt;40</td>
<td>50.82</td>
<td>9–356</td>
<td>14 (21.5)</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>60 – 80</td>
<td>55.71</td>
<td>52–72</td>
<td>2 (3)</td>
</tr>
<tr>
<td>INR</td>
<td>0.8 – 1.2</td>
<td>1.12</td>
<td>0.87–2.26</td>
<td>7 (10.7)</td>
</tr>
<tr>
<td>D-dimers (μg/l)</td>
<td>&lt;500</td>
<td>1131.94</td>
<td>275–6691</td>
<td>23 (35.3)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5 – 0.9</td>
<td>0.943</td>
<td>0.37–1.61</td>
<td>8 (12.3)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages.

Imaging studies revealed pleural effusion (1.5%), hepatomegaly (25%) and splenomegaly (18.8%). None of the patients had ascites. Mean systolic blood pressure was 121 mmHg, ranging from 90 to 146 mmHg. Values lower than 110 mmHg were present in 12.5% of the patients, although none of them had hypotension <90 mmHg. Altered mental state was found in 4.6% of the patients.

In two patients, tests were conducted twice. These patients had fever after their return from South Asia. Initially, IgM antibodies were not detected in these patients, but they were positive for IgG antibodies. In one of them IgM antibodies against DENV appeared and IgG antibody titer increased ≥ 4 times, which confirmed the disease. In the other patient, IgM antibodies remained negative and IgG antibody titer was unchanged; therefore the disease was excluded.
Furthermore, in 61 patients out of the 192, IgG antibodies were detected against dengue while IgM remained negative. About 57.3% (35) of them were missionaries. In this group, there were nine women (14.7%) and the mean age was 44 yr. The purpose of travel was mostly business (72.1%), although 3.4% of persons declared it as touristic, and there were missing data for 24.5% of the persons. Total 15 persons from this group reported fever ranging from 38–40°C. Other diagnosis explaining fever as a cause of the disease, indicated pharyngitis and laryngitis (1 person), schistosomiasis (2), skin ulceration (1), tropical malaria (1), gastrointestinal tract infection (1), pneumonia (2), reactive arthritis (1), and amoebic liver abscess (1). The diagnosis of five patients was not possible, and their body temperature normalized spontaneously.

DISCUSSION

According to the European Centre for Disease Prevention and Control, 1796 dengue cases were recorded in the years 2010–14 in Europe, out of which 1510 were laboratory confirmed. About 78.8% of the cases were imported from Asia and 10% from South America. Seasonality was observed with increase in number of infections between June and October. The report also showed the number of diagnosed cases in Poland with two cases in 2008, four in 2009, six in 2010 and five in 2011 and 2012. There is no official data on the number of Poles travelling to tropical areas. Patients returning from the tropics are usually diagnosed in one of the two centers of tropical medicine, including the reference center situated in Gdynia. However, the patients are often admitted also to other healthcare units: Outpatient clinics or departments of infectious diseases. During the years 2002–11 in the Hospital Ward of Tropical Diseases and Zoonoses in Warsaw, 22 patients with the clinical course of the disease were hospitalized. This data shows insufficient attention paid to this disease, despite the increase in recreational and occupational international travels, in tropical and subtropical destinations. In this study a clinico-laboratory profile of 65 patients is presented who were hospitalized in the Department of Tropical and Parasitic Diseases, UCMTM in years 2010–15, and diagnosed with dengue in accordance with the CDC guidelines of 2015, based on epidemiological history, clinical symptoms and available serological tests. The results showed that, majority of them had returned from Asia, however attention is drawn also to a large group of patients returning from Africa, reflecting a geographical expansion of dengue viruses. The most common symptoms were fever and weakness.

In many studies concerning dengue patients, the fever is a most common symptom, present in as many as 98% of the cases. In UCMTM, some hospital treatments were given, to patients without complaints or with subclinical and oligosymptomatic symptoms, due to routine control reasons, after their return from tropical areas. According to CDC definition, the severe dengue was present in four patients—One patient with extravascular fluid accumulation in the pleural cavity, two patients with gastrointestinal tract bleeding, and three patients with deterioration of consciousness and altered mental state. Severe damage to organs or shock was not found in any of the patients.

Some reports quite often mention lymphadenopathy, although none of the patients analyzed in the present study showed this symptom. In a study in Hong Kong, lymphadenopathy was found in only 16% of cases in comparison to 50% in other reports. This difference was attributed to casual physical examination which can also be an explanation to the cases described in this study.

Other symptoms mentioned in the literature include damage to the liver, central nervous system and myocardium. Liver damage was described mostly after shock and in many cases as a result of elongated hypoperfusion and hypoxia rather than a direct effect of the dengue virus. The neurological symptoms such as encephalopathy or seizures are very rare and present in about 1% of cases. Such patients complain of headache or somnolence. Infection can be detected by serological or polymerase chain reaction (PCR) testing of cerebrospinal fluid.

In the present study, changes in mental state were found in three cases, although testing of cerebrospinal fluid or imaging tests of head was not performed in any of them. Alarming symptoms that can precede deterioration of a patient’s condition include abdominal pain, constant vomiting, accumulation of extravascular fluid, bleeding from mucosa, liver enlargement > 2 cm, increase in haematocrit and a sudden drop in the number of platelets. In none of the patients treated in UCMTM did the disease progress to death.

In this study, laboratory confirmation of dengue cases was based on detection of IgM antibodies against DENV in patient returning from a dengue endemic area, without any ongoing transmission or active infection with other flaviviruses, and a recent vaccination against flaviviruses. The viral load or serotypes of the virus have not been specified; diagnosis was based on serological tests in the context of clinical symptoms and epidemiological data.

According to the kit’s information, in the first infection a slow increase in IgM class antibody level is detected. IgG antibodies are detected at low titer by the end of the 1st week after the infection. It may result from an
among the patients hospitalized in Gdynia, one
7) with mortality rate of 12% despite intensive health
ening symptom of dengue. It develops within 24–48 h
Typical variations in laboratory tests include leukopenia,
Malaria is a serious disease caused by Plasmodium,
DS-135-2002 (562 persons), in which antibodies against
tained diagnosed diseases, after malaria and dengue in-
be present in patients recently vaccinated against tick-
been present only 2.1% had symptoms of dengue. The obtained re-
(including rickettsial, typhoid and paratyphoid). The analysis was based on data collected from
received disease symptoms after their return from the trop-
they refer only to patients admitted to UCMTM, whereas
This retrospective study was based on data available from
actual hospital stay in tropical regions. Some of these patients were
patients positive for IgG antibodies against dengue and pa-
sionaries and volunteers returning after several months
A significant group of UCMTM patients were mis-
In Poland, dengue diagnosis was not available until
admitted not in an emergency, but on routine basis, often
their holidays stay in Poland. This probably reflects in
Nowadays, the increasing possibilities of translocation of people
other causes of elevated body temperature, including P. falciparum
It is difficult to compare a group of dengue patients with
 mononucleosis, rickettsia infections, typhoid and paratyphoid. The analysis was based on data collected from
17,000 ill travelers22.
In this study, out of 15 patients with fever who were
negatively diagnosed with dengue, 10 had other causes
malaria22. This refers to a large group of patients who
of elevated body temperature, including P. falciparum malaria. The data of this study do not constitute an ac-
prise of stay in tropical regions. Some of these patients were
value of 6691 μg/dl.
Plasma leakage is the most common and life-threat-
ening symptom of dengue. It develops within 24–48 h
and leads to a shock (maximal risk between Days 3 and
Ancillary laboratory results were obtained in 1 of the patients,
patients with dengue of benign clinical symptoms. In some
patients with dengue of benign clinical symptoms. In some
laboratories17. Typical variations in laboratory tests include leukopenia,
thrombocytopenia (values ≤ 100 x 10^9/l in 55% of pa-
tients), elevated activity of SGPT, usually 2–5 times above
the upper reference value, rarely 5–15 times18.
The above mentioned variations were observed in this
study also, i.e. leukopenia was present in 25% of patients,
thrombocytopenia in 20% of patients, and elevated activ-
ity of transaminases in 35.3% (SGOT) and 21.5% (SGPT)
of patients. However, it was not clear why in the present
patients; increased values of SGOT were more common
than SGPT. About 23 (35.3%) patients showed elevated
D-dimers with the highest value of 6691 μg/dl.

The study included 753 persons, out of which anti-
bodies were detected in blood sera of 149 persons; in
43.6% of them “early antibodies” were detected, although
only 2.1% had symptoms of dengue. The obtained re-
results were unclear, including negative results of tests for
the presence of NS1 protein that were conducted in 49
persons16. Another similar study presented molecular
analysis of blood samples collected from 48 patients who
had previously been tested for the presence of IgM anti-
bodies against dengue with positive results in 10 persons.
The three serotypes identified were—DENV-1, –2 and
–3. The negative results of the molecular studies were
explained by the time that elapsed since the onset of the
disease, resulting in elusive viremia accompanied by the
presence of antibodies21.

In differential diagnosis of fever in patients returning
from tropical regions, dengue is in second position after
malaria. This refers to a large group of patients who
reported disease symptoms after their return from the trop-
ics and were included in the GeoSentinel study. The re-
main ing diagnosed diseases, after malaria and dengue in-
cluded mononucleosis, rickettsia infections, typhoid and paratyphoid. The analysis was based on data collected from
17,000 ill travelers22.
In this study, out of 15 patients with fever who were
negatively diagnosed with dengue, 10 had other causes
of elevated body temperature, including P. falciparum malaria. The data of this study do not constitute an accu-
rate survey of the subject of dengue in Poland, because
they refer only to patients admitted to UCMTM, whereas
overall number of cases of dengue, as a self-limiting fe-
brile illness treated individually by patients, without any
medical help or in outpatient clinics, is probably higher.
It is difficult to compare a group of dengue patients with
a severe dengue group due to the small size of the latter.
This retrospective study was based on data available from
medical documentation.

A significant group of UCMTM patients were mis-
sionaries and volunteers returning after several months
of stay in tropical regions. Some of these patients were
admitted not in an emergency, but on routine basis, often
without obvious disease symptoms, for check-up during
their holidays stay in Poland. This probably reflects in
the analyzed group as the presence of asymptomatic pa-
tients positive for IgG antibodies against dengue and pa-
tients with dengue of benign clinical symptoms. In some
patients, whose symptoms can be attributed to the post-
dengue syndrome, a high titer of IgG antibodies against
dengue was found. However, analysis of this group is
beyond the scope of the presented study.
The dengue cases in children and pregnant women
were not analyzed; as they are rarely admitted/treated in
UCMTM.

CONCLUSION

The increasing possibilities of translocation of people
between continents and countries, resulting in a rising num-

unspecific reaction, for example, because of the cross re-
actions with antigens of other flaviviridae. As IgG titer
may rise rapidly if subsequent infection occurs, the test
should be repeated for verification after several weeks16.

It is recommended to collect blood serum twice, al-
though very often it is not possible. Cross reactivity may
be present in patients recently vaccinated against tick-
borne encephalitis, yellow fever or Japanese encephali-
tis; in such cases, a confirmation of dengue requires neu-
tralization tests conducted in BSL3/4 laboratories17.

Literature shows the presence of acute kidney injury
(AKI) in patient groups of different sizes, for example in
about 3% of patients AKI was a result of shock, but it
was also present in the mechanisms of rhabdomyolysis,
glomerulonephritis and acute tubular necrosis20. In the
group analyzed in IMTM, the highest concentration of
creatinine was 1.61 mg/dl with an average value of 0.943
mg/dl. The concentrations of this parameter above the
reference value were present in 8 (12.3%) patients, al-
though AKI was not found in any of the patients.

In Poland, dengue diagnosis was not available until
2005. In 2010, attempts were made to evaluate the pre-
ence of antibodies in patients hospitalized in UCMTM,
to estimate the usefulness of diagnostic tests and to in-
vestigate the risk of the disease in Polish travelers.

The study included 753 persons, out of which anti-
bodies were detected in blood sera of 149 persons; in
43.6% of them “early antibodies” were detected, although
only 2.1% had symptoms of dengue. The obtained re-
results were unclear, including negative results of tests for
the presence of NS1 protein that were conducted in 49
persons16. Another similar study presented molecular
The authors declare that they have no conflict of interest.

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