The detection and differentiation of \textit{Entamoeba histolytica} and \textit{Entamoeba dispar} from diarrheic stool samples by real-time PCR, ELISA and Trichrome staining method

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\textbf{Introduction:}

\textit{Entamoeba histolytica} is an intestinal protozoan parasite that causes invasive amoebiasis in 40–50 million people, resulting in 40 000–100 000 deaths worldwide each year (1). The highest prevalence of \textit{Entamoeba histolytica} infection is found in countries in subtropical and tropical regions with low hygienic standards, and in countries with high population density. \textit{E. histolytica}, \textit{E. dispar} and \textit{E. moshkovskii} are morphologically identical but are different biochemically and genetically. Although a previous study showed \textit{E. moshkovskii} to be a non-pathogenic parasite, intestinal symptoms including diarrhea and other gastrointestinal disorders in individuals infected with this species have been reported (2). Also mild gastrointestinal symptoms has been reported in \textit{E. dispar} infected patients, but the symptoms are self-limited. The prevalence of \textit{E. dispar} is about ten times higher than \textit{E. histolytica} (3). Differential diagnosis of \textit{E. histolytica}, \textit{E. dispar} and, \textit{E. moshkovskii} which are three morphologically identical species of \textit{Entamoeba} genus, is essential both for treatment decision and public health knowledge. Although the distinction between these three species is of great importance, the methods developed for this purpose either are very time-consuming or not sensitive and specific enough. In this study, the efficiency of quantitative real-time PCR method in detection of \textit{E. histolytica} from stool samples has been tested.

\textbf{Material and Methods:}

Stool samples from amoebiasis suspected 306 diarrheic patients were collected. The samples were divided into three portions. Stool smears were stained with trichrome staining method and examined at least by 20 minutes by an experienced microscopist (4).

Until real-time PCR was performed all the stool samples were kept in -20$^\circ$C freezer. DNA extraction was performed to the stool samples by the method explained elsewhere (5). After DNA extraction real-time quantitative PCR (Artus
RealArt TM E.histolytica RG PCR Kit, Qiagen Diagnostics, Germany) was performed. In real-time PCR method, oligonucleotide primers and probes specific to ribosomal DNA was used. In every test, genomic DNAs from E. histolytica HM-1:IMSS, E. dispar SAW 760, and E. moshkovskii Laredo cells grown in axenic culture were used as positive controls.

To detect Entamoeba histolytica adhesin antigen in fresh faecal specimens a second generation monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (Techlab Entamoeba histolytica II antigen detection ELISA, Techlab Inc., Blacksburg, VA) was used. The test was performed according to the manufacturer’s instructions.

Results:

Sixty-eight of the samples were found to be positive by at least one of the methods. Real-time PCR and trichome staining method had been applied to all 306 samples. By trichrome staining method, Entamoeba histolytica/dispar, Entamoeba coli, Entamoeba hartmanii, Giardia lamblia, Dientamoeba fragilis, Blastocystis hominis were detected in 27, 4, 3, 1, 1, 1 stool samples respectively.

ELISA method could not be performed on 48 stool samples. In 258 stool samples 25 of them were found to be positive by ELISA method.

E. histolytica was found positive in 22 stool samples, by quantitative real-time PCR method. The lowest copy number was 5 copies/ml and the highest was 1.525 copies/ml.

The agreement ratio between trichrome staining method and ELISA method was 87%, while the agreement ratio between trichrome staining method and PCR was 83.1%. On the other hand, the agreement ratio between ELISA and PCR was 83.3%.

Conclusion:

The real-time PCR method was effective in differentiating E. histolytica from E. dispar and E. moshkovskii and also effective when parasite load was very low, the method could even detect five copies/ml (5). The real-time PCR method is an alternative tool in routine diagnosis and in epidemiological studies of amoebiasis. The correct detection and differentiation of E. histolytica from E.
dispar and E. moshkovskii will avoid unnecessary treatment of E. dispar - or E. moshkovskii-infected patients with antiamoebic drugs.

Acknowledgment:
This study was supported by the Hacettepe University Scientific Research Unit (project no: 05.01.101.002).
We thank Nejla Aydemir for her technical assistance and Dr. Graham Clark of the London School of Hygiene and Tropical Medicine for providing genomic DNAs of E. histolytica, E. dispar, and E. moshkovskii.

References: