## CHAPTER - I

### INTRODUCTION

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INTRODUCTION

Amoebiasis, the infection by protozoan parasite *Entamoeba histolytica* is a major health problem in several developing countries\(^1\). The infection leads to a high degree of morbidity and mortality. Next to malaria, amoebic infection has been considered parasitic cause of death in the world\(^2\). An estimate indicated that 480 million people carried *E. histolytica* in their intestinal tract. Amongst these, 48 million (i.e. 10\%) had invasive amoebic disease\(^3\). However, incidence of amoebiasis varies place to place depending upon social and economic conditions and nutritional norms of community. In India, 5-58 percent of the individuals suffering from gastro-intestinal symptoms have been reported to harbour *E. histolytica* in the colon.\(^4,5,6\)

Amoebic disease includes conditions caused by the invasion of the tissues of man by the pathogenic amoebae. The parasite may live commensal in the lumen of the bowel without appreciable tissue invasion or may act as a pathogen and invade tissues\(^7\). The parasite restricts itself usually to colon and caecum but may spread occasionally to extra intestinal sites. The most common metastatic site is liver where it leads to the production of amoebic liver abscess. In addition to being a potentially lethal disease, invasive amoebiasis has important social and economic consequences. Temporarily incapacitating infections are frequent in adults
during the wage earning years resulting in several weeks of hospitalization.

There exists a delicate balance between the host immunity and the clinical spectrum of the disease. Though, the wide spectrum of pathological lesions and clinical disease has been attributed to the interplay of host immune system and the virulence characteristic of the parasite, the parasite exists as being invasive (pathogenic) and non invasive (non pathogenic). The virulence character of amoebae has primarily been checked by infecting the experimental laboratory animals. However, it has been observed that amoebae isolated from asymptomatic carriers were in many instances as virulent as those from symptomatic cases. It was Sargeaunt and his colleagues who reported that invasive isolates of E. histolytica can be differentiated by electrophoretic mobilities of isoenzymes (Zymodemes). Basically electrophoretic mobilities of 4 isoenzymes i.e. L-malate: NADP+ oxidoreductase (oxalo-acetate decarboxylating) (ME), glucose phosphate isomerase (GPI), phosphoglucomutase (PGM) and hexokinase (HK) in lysates of amoebic isolates have been used to classify amoebic isolates into 23 zymodemes. Of these 23 zymodemes, nine have been associated with clinical evidence of tissue invasiveness. These (II, VI, VII, XI, XII, XIV, XIX, XX and XXI) zymodemes have been labelled as "Pathogenic Zymodemes". The various pathogenic zymodemes seem to have different geographical distribution. In India,
mainly zymodeme XIV has been associated with the clinical
status of disease\textsuperscript{14,15}; though isolated studies\textsuperscript{16} indicated
the existance of other pathogenic zymodemes in various parts
of India. However, detailed investigation of the prevalence
of pathogenic zymodemes from a large number of \textit{E. histolytica} isolates in India needs to be carried out. It
has been also suggested that additional enzyme systems
should be examined to broaden the zymodeme
classification\textsuperscript{17,18} and certain physicochemical properties
such as stability, electrophoretic mobilities etc. of \textit{E. histolytica} isolates.

The changing of electrophoretic mobilities of
amoebic isolates from pathogenic to non pathogenic or vice
versa is extremely important for understanding of the
etiology of symptomatic amoebiasis. There are contradictory
reports on the stability of zymodemes of amoebic isolates.
Some workers\textsuperscript{19,20,21} documented that pathogenic or non
pathogenic behaviour as determined by isoenzyme
electrophoretic patterns is not a stable and inherent
property of a given amoebic isolate. They suggested that
variations in isoenzyme electrophoretic mobilities depend on
growth conditions, media components, and the presence or
absence of bacteria in the medium. The other group of
workers\textsuperscript{22,23}, however, never observed shift of zymodemes of
\textit{E. histolytica} from non pathogenic to pathogenic or vice
versa in culture conditions. In recent years, changes in
zymodemes has been postulated in vivo and in vitro by genetic exchange of the parasite. These investigators observed the phenomenon of genetic exchange by mixing of two clones of amoebic population of different electrophoretic mobilities resulted "a progeny" different than the parent clones. Such an explanation is more or less speculative. It is well known that amoebic parasite divides by binary fission not by meiosis. This rules out the possibility of exchange of "genomes" of E. histolytica by any well known genetic exchange theoretical mechanisms.

In addition to isoenzymes of E. histolytica, the cell associated molecules responsible for target cell lysis may provide some lead to differentiating among pathogenic and non pathogenic amoebae. Invasive/non invasive amoebic isolates may differentially express these molecules. Enhanced expression of 29 KDa cell associated amoebic protein has been observed in pathogenic amoebic isolates. Similarly there exists epitopic differences on 170 KDa cell associated amoebic protein of pathogenic and non pathogenic amoebic isolates. However, a 96 KDa adhesin has only been found on pathogenic population of amoebae. Several other surface associated amoebae proteins with molecular mass of 210, 160, 90, 70, 50 and 24 KDa has been suggested to be adhesive molecules of amoebic trophozoites which mediate attachment of amoebae to erythrocytes. Investigations of these molecules, in depth, may provide a marker to differentiate virulent and non virulent amoebae.
There are numerous antiamoebic compounds available for the treatment of amoebiasis but most of them are quite toxic\textsuperscript{29}. With zymodeme classification of parasite into pathogenic and non pathogenic, there are suggestions to modify the treatment of asymptomatic and symptomatic amoebic patients. Because more than one zymodeme is responsible for a pathogenic condition, drugs, must be such which are effective against a wide spectrum of zymodemes. Thus, in depth investigations in relation to susceptibility of pathogenic or non pathogenic amoebae to anti-amoebic compounds are warranted.

The present study was undertaken with the following aims and objectives:

**AIMS AND OBJECTIVES**

(i) Isoenzyme characterization of *E. histolytica* isolates from symptomatic cases and asymptomatic carriers of amoebiasis.

(ii) Investigation of "additional enzymes" to characterize amoebic isolates.

(iii) To assess antigenic variations of amoebic isolates belonging to different zymodemes.

(iv) *In vitro*, assessment of anti amoebic compounds on the amoebic isolates belonging to different zymodemes.