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GLOBAL APPLICATION OF STUDYING COMPARATIVE ELECTROPHORETIC BEHAVIOUR OF *FASCIOLA HEPATICA* AND *FASCIOLA GIGANTICA*– A REVIEW

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ABSTRACT
The variation in electrophoretic patterns between two economically important species of *Fasciola*is important tools for identification of these species and also for vaccine designing. The aim behind the review is to encourage more young researchers to initiate work on this aspect of an economically cosmopolitan parasite. Disease biomarker discovery is generally carried out using two dimensional polyacrylamide gel Electrophoresis (2D-PAGE) to compare and identify differences in the protein expression patterns of two parasites. After 2D-PAGE fractionation and staining, the protein(s) of interest are removed, proteolytically or chemically digested and identified by mass spectrometry (MS). Although 2DPAGE separation provides excellent resolution, the need for protein staining and the subsequent sample handling limits the sensitivity of the overall approach. Protein profiling is expected to discover unexpected targets for drug design by determining the function of thousands of unidentified proteins still likely to be found in the genome of *Fasciola hepatica* and *Fasciolagigantica*. Electrophoretic Protein profiling is expected to multiply the number of known drug targets 100-fold. This will encourage the pharmaceutical industry to develop new drugs against fascioliasis. It will be also an indispensable tool for designing the species specific vaccine and for determination of molecular taxonomy of two parasites on more scientific grounds. This review will focus on research carried out globally to the field of paristoproteomic with special reference to *Faciola hepatica* and *Fasciolagigantica* by the applications of electrophoresis.

KEY WORDS: *Fasciola;* SDS-PAGE; paristoproteomic; Molecular taxonomy

INTRODUCTION

The availability of complete genome sequences for a large number of parasitic organisms has opened the door for large-scale proteomic studies to dissectboth protein expression/regulation and function. Electrophoresis is one of the innovative tools to exploit proteome - the genome operating system by which the cells of an organism react to environmental signals (Anderson and Anderson, 1996). The techniques include the development of activity-based probes and activity-based protein profiling methods to screen for pharmacological tools to perturb basic biological processes. The standard method for quantitative proteome analysis combines protein separation by high resolution (isoelectric focusing, SDS-PAGE) two-dimensional gel electrophoresis (2DE) with mass spectrometric (MS) or tandem MS (MSyMS) identification of selected protein spots. Important technical advances related to 2DE and protein MS have increased sensitivity, reproducibility, and throughput of proteome analysis while creating an integrated technology. By using 2DE with extended pH range and high-sensitivity protein identification by electrospray ionization and MSyMS, we have evaluated the potential of the 2DE-MS strategy to serve as the technology base for comprehensive and quantitative proteome analysis(Steven, 2000) .Two dimensional Electrophoresis (2DE) form the first generation proteome tool as host proteome responses such as post-translational modifications of host proteins (phosphorylation, glycolysylation, acetylation and methylation) in reaction to parasite invasion can be detected and identified (Patton, 2000).The present paper revises the application of electrophoresis in paristoproteomics of fasciolasps.

Fasciolosis

The two principal species of Genus *Fasciola*are *F. hepatica* and *F .gigantica* which represents two opposite ends of range of form. These flukes are cosmopolitan in distribution and are the cause for Fasciolosis(also known as Fascioliasis, Fasciolasis, distomatosis and liver rot), especially in sheep and cattle.*F.hepatica* and *F. gigantica* had been a true plague for sheep farmers before the anthelmintic era.Currently, this infection seems to be under control in sheep and in cattle, although it remains a major problem in some areas.Fascioliasis has always been well recognized because of its high veterinary impact but it has been among the most neglected diseases for decades with regard to human infection. However, the increasing importance of human fascioliasis worldwide has re-launched interest in fascioliasis (Mas-coma *et al.,* 2009). Recently, worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US$3.2 billion per annum. In addition, fasciolosis is now recognized as an emerging human disease: the World Health Organization (WHO).More recentfigures suggest that between 2.4 and 17 million peopleare currently infected with a further 91.1 million livingat risk of infection (Keiser and Utzinger, 2005). In Kashmir valley, prevalence of *Fasciola hepatica and Fasciola gigantica* is 9.96% and 23.92% respectively (Mir, 2008).

Application of Comparative Electrophoretic Study of FasciolaSps

The need for basic laboratory research on Fasciolosisis stronger than ever. Recent advances in technology, particularly in the so-called post-genomics arena, have created opportunities for the identification of proteins expressed by Fasciolidparasites.Electrophoresis is the global proven technique to open new vistas in human, veterinary, and laboratory animal medicine. It provides the increasedresolution and precision in the inference of results. The new conceptual approach of electrophoresis suggested for parasitoproteomicswill help to increase the knowledge of immune responses to different parasite species, in addition to the creation of a proteomic database with a holistic view ofhost–parasite interactions, based on evolutionary concepts of host immune responses to a parasite. This new methodological approach offers a new way not only to discover drugs and vaccines but also to study host–parasite interactions, such as characterizing proteins whose function is implied in the behavioral manipulation of host in many taxa. In addition, it will open the way to reconstructing the molecular phylogeny of proteins such as those involved in the host immune response and to determine their level of conservation during evolution (Biron,2005). Electrophoretic type of study will increase the knowledge of manipulative strategies and open the way to create protein databases of *Fasciola*sps based on a manipulative chart. It's utility in exploringknowledge regarding Fasciolosisis pivotal. Thus electrophoresis help to broaden the vision regarding different parameters pertaining tofasciolosis, important ones are enlisted and discussed below:

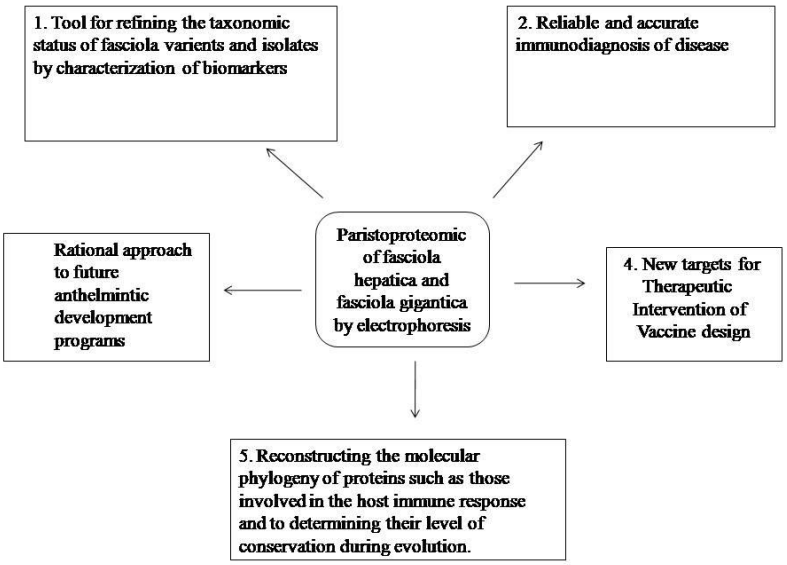

Figure-1. Potential application of parasite oproteomic of Fasciolasps.

Electrophoretic pattern as tool for refining taxonomy status of Fasciolasps

To discriminate the two principle species of fasciolaand their isolates and varientsof different areas is important for number of areas of research, particularly in defining taxonomy and monitoring transmission in epidemiological investigation.Much of the current knowledge of *Fasciola*spptaxonomy has stemmed from numerous observational and morphological studies. However, conventional methods of detection and differentiation of*Fasciola* do not accurately reflect the full diversity of *Fasciola* spp. Moreover, the identification of fasciolasps by morphological distinction is quite unreliable(Ashrafi et al.,2006). A recent study investigated the extent of genetic variability among *Fasiola*collected from different host sps and geographical localities (Lin *et al*, 2011).

Comprehensive protein characterization by electrophoresis using more variable markersalong with proteomic analysis of *Fasciol*a species can be used to refinethe taxonomic status of the "intermediate*Fasciola*" andto assess its potential as a zoonotic agent.Nevertheless, molecular genetics studies over the past two decades have added significantly to our understanding of *Fasciola*taxonomy, genetics, and contributed to the development of advanced approaches for the accurate identification and differentiation of *Fasciola* spp. Importantly, these molecular methods have facilitated the identification of the hybrid "intermediate *Fasciola*". However, presently there is no molecular diagnostic method. Somatic and E\S antigens of two spshave been contrasted using SDS PAGE.which provides the baseline to distinguish between two on electrophoretic basis.Differences between *F. hepatica* and *F. gigantica*somatic proteins have been noticed. *F. gigantica*has 11 major protein bands with molecular weights of 18, 22, 24, 33, 36, 42, 46, 57, 60, 62 and 68 kDa, whereas *F. hepatica* has proteins characterized by 8 distinct bands with molecular weights of 18, 22, 24, 33,36, 42, 46 and 62 kDa.(Meshgi *et al.,* 2008). Potential intraspecific variation within *F. hepatica* was investigated by Agarose gel electrophoresis of RAPD-PCR products of *Fasciola hepatica* isolates from Iran. 52 *F. hepatica* isolates

wereanalyzed by RAPD, using 2 primers. An estimate ofintraspecific variability of *F. hepatica* from two regionsin Iran was achieved, using RAPD-PCR which clearly demonstrated that genetic heterogeneityexists within *F. hepatica* in Iran.

2.Electrophoretic pattern as tool for diagnosis of fasciolosis:

The diagnosis of fasciolosis is generally carried out via coprologicalexamination, andimmunological technique like ELISA but such tests have many disadvantages. Pseudofasciolosis is the potential for misdiagnosis in such tests.Moreover, coprological diagnosis of fasciolosis is possible from 8-12 week post-infection (WPI) .Though ELISA can recognize *F. hepatica* specific-antibodies since 2-4 week post-infection, thus providing early detection of the infection but there is some possibility of cross-reactivity with the schistosomiasis antibodies. Moreover, in many human infections, the fluke eggs are often not found in the faeces, even after multiple faecal examinations.Furthermore, eggs of *F. hepatica*, *F. gigantica* and *Fasciolopsisbuski* are morphologically indistinguishable.In recent years, sodium dodecyl sulphatepolyacrylamide gel electrophoresis (SDS-PAGE) andWestern blotting procedures have initiated a new era inimmunodiagnosis which greatly reduced cross-reactionThese techniques were used as a verifying test inthe diagnosis of viral and bacterial infections at first, butlately these techniques have been used in the field ofparasitology(Sarimehmetoúlu, 2002).

Thus electrophoresis ensures sensitive potential tool for serodiagonosis of fasciolosis. Electrophoretic bands of 21 and 25KDA derived from metacercarial antigens (MAg) of *Fasciolagigantica* enhance the sensitivity and specificity of test for early diagnosis of fasciolosis. It can be used as promising alternative to conventional method of faecal egg detection .This will encourage early chemotherapy to save animal prior to damage in the form of traumatic hepatitis (Velusemy *et al,* 2006).The 8 kDa protein of *F.hepatica*obtained by gel electrophoresisisis suggested as one of the diagnostic antigens in human fascioliasis without cross-reaction with other human trematodiasis (kwangsig *et al,* 2003). A 28-kDa cysteine proteolytic enzyme extracted from *F. gigantica*by electrophoresis has a potential use for the serodiagnosis of ruminant fasciolosis as a supplement to the usual coprological methods. (Benjamin, 1995).The 17-kDa *F. hepatica* excretory secretory antigen is an excellentcandidate for the immunodiagnosis of acute and chronic fascioliasis. Purification of this antigen by electrophoresis and its application to quantitative serologic tests will permit further analysis of its predictive value to evaluate cure (Hillyer, 1988).

3. Electrophoresis as tool to purify and characterize detoxifying enzymes as target for evaluation of drugs

Numerous antifluke drugs have been developed which rapidly are metabolized intocompounds having fasciolicidalactivity,but the *Fasciola* possess numerous detoxifying and antioxidant enzymes which suppress the activity of such drugs to kill adult and juvenile parsites.Such enzyme include superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and glutathione(GSH). Glutathione S-transferase (GST) represents the major class of detoxification enzymes from helminth parasites such as *Fasciola hepatica* and *F. gigantica*and it is a candidate for chemotherapeutic design. Therefore, GST enzyme of *Fasciola* spp. was regarded as reliable target for evaluation of drugs such as triclabendazole (C14H9Cl3N2OS). GST purified fraction as a 26kDa (MW) band can be obtained on sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDSPAGE).The general inhibition of *Fasciola*spp. GSTs in the microgram range bytriclabendazole may help explaining the mode of action of this chemotherapeutic agents in vitro against the same parasite.Comparison of the effect of powder and bolus of triclabendazole in solutions containing purified GST has revealed inhibition concentration (IC50) 8.36 and 9.05 µg/ml for *Fasciola hepatica* GSTs and 7.20 and 10.80 for *F. gigantica*GSTs (Farahnak,2006)A TCBZderivative, designated compound alpha, has provento be highly active against immature and adult*F.hepatica* (Fairweather 2005) and has recently beenshown to disrupt the tegument of flukes from a TCBZ-resistant isolate (McConville et al. 2007).Other experimental fasciolicides such as artemetherand OZ78 also have activity against TCBZ-resistantflukes (Keiser *et al.,* 2007).These studies have been carried by first purifying antigens by SDS PAGE.

4. Electrophoretic pattern as tool for vaccine design

Triclabendazole (FASINEX) is considered as the most common and widely used drug due to its high efficacy against adult and juvenile flukes in animals. Recently, a new fasciolicide was successfully tested in naturally and experimentally infected cattle in Mexico. This new drug is called compound Alpha and is chemically very much closed to triclabendazole(Ibarra et al. 2004)A TCBZ derivative, designated compound alpha, has proven to be highly active against immature and adult *F. hepatica* (Fairweather 2005) and has recently been shown to disrupt the tegument of flukes from a TCBZ-resistant isolate (McConville *et al.,* 2007). But long term veterinary use of Triclabendazole has caused appearance of resistance to *Fasciolahepatica.* Robertset *et al.* (1996) proposed that *F.gigantica* adults contain an antigen that stimulate a protective response and there is no analogue to that antigen in *F.hepatica*.Thus an alternative or additional advance in the control of animal fasciolosis will be provided by the development of anti-Fasciola vaccines against species specific antigens .Vaccines for neglected parasitic diseases are of paramount importance.The

application of proteomics has significantly improved our knowledge of *F. hepatica* secretory proteins and thus has widened the panel of antigens available for diagnostics and vaccine discovery (Jefferies *et al.,* 2001; Morphew *et al.,* 2007; Robinson *et al.,* 2008). Proteomics is also a particularly valuable tool for studying changes in parasite protein expression; for example, proteomic analysis of *F. hepatica* following in vitro culture with TCBZ-sulphoxide has led to the identification of fluke proteins (including a 70 kDa heat-shock protein) that are specifically upregulated in a TCBZ-resistant isolate (Brennan et al. 2007).Although a range of vaccines against *F. hepatica* infections have been developed by several laboratories (McManus and Dalton, 2006) none are commercially available at present. However, since the incidence of animal fasciolosis is increasing worldwide and anthelmintic resistance continues to spread, it is more likely that a vaccine will offer a level of protection that is comparable with the existing anti-fluke drugsand, hence, become more financially attractive to pharmaceutical companies.Tegumental proteins unique to one species are now beginning to be identified and these may be suitable vaccine targets as in Schistomiasis.

5. Electrophoresis as tool for reconstruction of phylogeny of*Fasciola*sps

The origins, patterns of diversification, and biogeography of fasciolids are all poorly known. Molecular phylogenetic studies will help us to better understand the origins, radiation, evolution, and patterns of host use of theseimportant trematodes(Lotfy M W,2008).Phylogenetic tree of Fasciola enzymes have been been constructed by various researchers. Thephylogenetic data (using cathepsin L and GST) also suggest that the *F. gigantica* and *F. hepatica* species separated approximately 19 million years ago, around the time that the ancestors of modern-day pecoran lineagesdiverged. Theseobservations are consistent with both co-adaptation and co-speciation of the parasitic genes with the parasite's host. (Irving, 2003).Recent phylogenetic, biochemical and structural studies indicate thattrematodecathepsins exhibit overlapping but distinct substrate specificitiesdue to divergence within the protease active site. The developmentallyregulated expression of these proteases correlates with the passage ofparasites through host tissues and their encounters with different host macromolecules (Stack, 2011).

The analysis and characterizing the profile of cathepsin L proteases secreted by adult *F. hepatica* by two-dimensional gel electrophoresis (2-DE) and MS is used to determine the relative importance of the various cathepsin L groups to parasite virulence and adaptation. A phylogenetic analysis of 24*F. hepatica* and eight *F.gigantica*full-length sequences revealed that theseseparatedinto five well supported clades that arose by a series of geneduplications The two initial gene duplications separated thecathepsins isolated from the infective newly excysted juvenile parasites(Clade 3, FhCL3 and Clade 4,FhCL4) from three clades expressed in the adult worm stage(clades FhCL1, -2, and -5). Following this, geneduplicationled to the separation of the adult clades FhCL1 andFhCL5 fromclade FhCL2. The phylogenetic tree also showedthat the *Fasciola*clade FhCL1 has undergone the greatestexpansion and is represented by three distinct subclades: FhCL1A, FhCL1B, and FhCL1C.It is noteworthy that all clades contain sequences from both*F. hepatica* and *F. gigantica*. However, subclades FhCL1Aand FhCL1B are composed exclusively of *F. hepatica*sequences, whereas clade FhCL1C contains only one *F. hepatica*cathepsinL.This phylogenetic analysis, therefore, indicates that the early duplicationevents in the cathep-sin L gene family occurred before the speciation of the *F.hepatica*and *F. gigantica*fasciolids and that expansion ofsubcladesFhCL1A/FhCL1B and FhCL1C occurred after thesegregation of these twospecies. Irving *et al.* (12) made asimilar observation and suggested that divergence of theFhCL1 clade reflected adaptation of the 'temperate' *F.hepatica*and the "tropical" *F. gigantica*to different host species.(Robinson,et al.2008)The samplepreparation of cathepsin L was done by 2DElectrophoresisand thus depicts its indispensable role for determining phylogeny of fasciolosis.

CONCLUSION

The electrophoretic behavioural study is the most cost-effective and simplest way in the field of parasitoproteomic to characterize and compare proteomes of *Fasciola hepatica* and *Fasciolagigantica*. Though, some studies have shown thelimitations of the current approach to parasitoproteomicsbut no one can deny that 2DE is the most accessible andefficient proteomic tool for a laboratory.Many parasitologists are betting heavily on proteomic studies based on electrophoresis to explain comparative protein profile of*Fasciola hepatica* and *Fasciolagigantica* and, thus, to contribute to the control of fasciolosis.

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