Carboxylic Acids and Their Metabolic Enzymes as New Novel Biomarkers of Susceptible, Resistant Strains of *Biomphalaria alexandrina* and Snails Infected with *Schistosoma mansoni*.

Mona M. Mantawy[1], Naema Zayed Mohamed[1], Aza Fathy Arfa[1], Hanan F. Aly[1]

Abstract— Carboxylic acids play an important role in both aerobic and anaerobic metabolic pathways of both the snail and the parasite. Monitoring the difference between susceptible, resistance and the effects of infection by schistosome on Biomphalaria alexandrina carboxylic acids metabolic profiles represents a promising additional source of information about the state of metabolic system. In the present study , separation and quantification of fumaric , ,malic, succinic , pyruvic , lactic and propionic acids using ion-suppression reversed-phase high performance liquid chromatography (HPLC) to detect correlations between these acids in both hemolymph and digestive gland (DG) samples in a total of 100 B. alexandrina snails (40 infected and 30 for each susceptible and resistance) . In addition several enzymes representing different metabolic pathways were determined in these specimens. These enzymes included aspartate and alanine aminotransferases (AST and ALT), lactate dehydrogenase (LDH), glucose phosphate isomerase (GPI), phosphofructokinase enzyme (PFK), glucose-3-P dehydrogenase (G3PDH) ,glucose and glycogen . The results showed significant difference in organic acids ,enzyme activities ,glucose and glycogen level either in hemolymph or digestive glands of resistance and susceptible snails .In addition , significant difference in all the studied parameters in infected snail as compared to susceptible uninfected one . Some possible explanations of the underlying mechanisms were discussed.. These findings highlight the potential of metabolomics as a novel approach for fundamental investigations of host-pathogen interactions as well as disease surveillance and control.

Index Terms — carboxylic acid, liver function enzymes ,glycolytic enzymes , *Schistosoma mansoni*,

1 Introduction

Fresh water pulmonate snails of the genus Biomphalaria are best known for their role as intermediate hosts of the widely distributed parasite, *Schistosoma mansoni [1].* This parasite, one of the causal agents of the debilitating disease (intestinal schistosomiasis), infects 207 million people worldwide and puts an estimate of 779 million people at risk of acquiring infection [2 One of the keys to understand the present and future of Schistosoma mansoni infection in Egypt is to understand more about the snails that play an indispensable role in its transmission [3].

Parasitic helminths have an absolute dependency on carbohydrates for their energy source, and one of the key features of carbohydrate catabolism is the excretion of a wide range of carboxylic acids. However, most of them have low molecular absorptive and thus are poorly detectable compounds by photometric detection [4]. The depletion of energy sources and alterations in the carbohydrate metabolism caused by the trematode larvae on their snail intermediate host was thought to be similar to that observed during estivation in which food uptake ceases, water loss occurs, and gas exchange may also be affected.

All of these alterations exert an influence on the snail's metabolism which may be reflected in the concentration of metabolites resembling consumption of host metabolites by the developing parasites [5]. Becker [5] stated that starvation of Biomphalaria glabrata for 6 days was equivalent to 30 days' infection by S. mansoni. Several studies have demonstrated that infected schistosome vectors grow faster and have a greater soft tissue mass than their unparasitized counterparts [6]. This appears inconsistent with the starvation hypothesis. Moreover, it was recently reported that the adenylate energy charge of the digestive gland-gonad tissue complex (DGG), unlike in estivation, is affected very little by infection. Thus, the infected snail appears to undergo some degree of physiological adaptation to the parasitized state. The finding that snail hosts have the potential for regulating hemolymph carbohydrate level indicates that host metabolic responses to infection are compensatory and aimed at maintaining blood glucose in response to continuous utilization by developing parasite [7]. Biomarkers of diseases refer to cellular, biochemical, or molecular alterations that occur during diseases and could be measured in biological matrices, such as tissue, cells, or fluids [8,9]. Over the last several years, researchers have started to explore the new array based technologies to map biomarkers of diseases and to identify targets for drug design that promise to provide signatures that are characteristics for each disease state by taking snap shot of metabolism [10]. En-

[1]Therapeutical Chemistry Department , National Research Center , Dokki, Giza, Egypt. \
, Hanan F. Aly1* Therapeutical Chemistry Department , National Research Center , Dokki, Giza, Egypt. E.mail: alyhanan25@yahoo.com

hancement of our current understanding of a host's metabolic response to a parasitic infection is a promising approach for biomarker identification, yet its potential for diagnosis and disease surveillance has been under used [11].

Combining several biomarkers have been shown to improve the discriminatory capability considerably. Recent developments in the field of metabolomics now provide the tools to go one step further; identify profiles of metabolites that together serve as a biomarker [12]. The present study was initiated from this assumption and designed to identify a biomarker profile that could distinguish uninfected susceptible , resistant from infected snails . Organic acids are important component of parasite metabolism and participate in both catabolic (e.g glycolysis) and anabolic (e.g gluconeogenesis) pathways . Pyruvate and lactate are indicators of glycolytic processes under aerobic conditions, while fumarate , succinate and malate are indicators of the tricarboxylic acid cycle . The presence of ketone bodies , such as β- hydroxybutrate and acetoacetate as well as of fatty acids , such as acetate and propionate , are indicative for lipid metabolism [13].Organic acid play a central role in parasite of S. mansoni metabolism which serve as indicators of various metabolic reactions since they represent important components of energy and parasites metabolism . Thus , they may indicate the use of carbohydrates as an energy source in the flow of aerobic and anaerobic transition , the replacement of glucose through gluconeogenesis , and of protein via glucogenic amino acids or metabolism of lipids on a smaller scale via fatty acids and ketone bodies[14]. A profile of selected carboxylic acids was done using the malic, pyruvic, and fumaric acids as representatives of carbohydrate intermediary metabolism of both aerobic and anaerobic pathways which are directly linked to energy production and facultative metabolic ways in both the snail and the parasite [13-15]. Whereas, acetic and oxalic acids represent the end product of carbohydrate metabolism [14,15]. Studies on such acids as biomarkers in ~~Biomphalaria~~ snails after infection with ~~S. mansoni~~ may be important in developing rational methods to diagnose infection, control, and even eradicate medically important snails that transmit parasitic diseases to humans and animals [16]. So, the present study aims to clarify the differences in carboxylic acids and their metabolic enzymes representing different pathways in hemolymph and digestive glands of resistance , susceptible and infected snails .modify the header or footer on subsequent pages.

2 . MATERIAL AND METHODS

Biological materials were obtained from the Medical Malacology Laboratory, Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. A total of 100 snails of an Egyptian strain, including 40 infected cases and 30 susceptible controls and 30 resistant were used in this study.

2.1 Snail maintenance

Snails were maintained in plastic trays, each containing 10 snails and 1 L of aerated tap water (26±2˚C), replaced twice a week, and fed boiled fresh lettuce and blue green algae [17]. Aquaria were cleaned weekly for removal of feces and dead snails [18]. Laboratory reared ~~B. alexandrina~~ reaching 6-7 mm in shell diameter was exposed to ~~S. mansoni~~ miracidial infection according to Massa et al. [16]. Harvesting of the snails was done as follow: group 1 (G1) included 30 susceptible snails .Group 2 (G2) was 30 resistant snails; and group 3 (G3) was 40 snails of 2 weeks after infection. All snails have the same shell diameter and were maintained in the same manner and harvested at the same time .

2.2 Digestive gland extraction

The DG tissue extract samples were prepared by dissection free from the snail body then carboxylic acid extraction was done using 50 % Ringer`s solution (Carolina Biological Supply,USA). Centrifugation of the extract was done at 250 g for 15 min to collect the supernatants which were stored at -20 °C until use [16].

2.3 Extraction and separation of the organic acids

The organic acids were extracted immediately from the centrifuged hemolymph using Bond – Elut ®columns (SAX – anion exchange-quartenary amine, manufactured by Analytichem International, Habor City,USA) as described by Lian et al. [19].Under vacuum, the columns were activated by consecutive washes with 1 ml of 0.5M HCL, 1 ml of methanol and 2 ml of HPLC-grade water. They were loaded with 200µl of hemolymph and 2ml water. The columns were disconnected from the vacuum pump and 250µl of 0.5 M sulphuric acid were applied to elute the organic acids retained on the matrix. The eluate was centrifuged at 1200g for 5 min and the supernatant stored at -70 °C until analysis by high performance liquid chromatography.

The liquid chromatography (HPLC-system Milton Roy-Analyst 7800) was performed at room temperature using a BIORAD-Aminex ion exclusion HPX-78H column (300x7.8mm) designed specifically for the separation of organic acids. The separation column was protected by a BIORAD-Aminex HPX-85 guard column. The mobile phase was sulphuric acid (0.5mM) delivered at a flow rate of 0.8 ml/min. The elution profile was determined at 210nm. The elution profile was determined at 210nm. The injection volume of each sample was 100µl.

Pooling snails was used throughout the study, and the mean of three trails for each HPLC analysis was done for each sample. The conversions to part per million concentrations (ppm).The concentration (ppm) of each acid in snail DG was calculated by multiplying the sample solution concentration interpolated from calibration curve (I) (ppm) by the original sample volume (V) (ml) and division of the product by the mass of the snail DG (M) (g) and for hemolymph, carboxylic acid concentration (µg/dl) was calculated by multiplying I times V times 100, and division of the product by the hemolymph volume (HV) (ml) according to Massa et al. [16].

.2.3. Enzyme assays

Aspartate and alanine aminotransferases (AST and ALT):
AST and ALT were measured according to the method of Reitman and Frankel [20],using dinitrophenylhydrazine as colour reagent.
Lactate dehydrogenase (LDH):
Lactate dehydrogenase was measured according to the method of Babson and Babson [21], which based on the reduction of pyruvate to lactate by incubation with LDH in the presence of coenzyme NADH.
Glucose phosphate isomerase (GPI):
GPI was measured according to the method of King [22].
Phosphofructokinase (PFK):
PFK was measured according to the method of Zammit et al. [23].
Glucose-3-P dehydrogenase (G3PDH):
Was estimated according to the method of Voet and Voet [24].
Measurement of glucose:
Glucose was measured calorimetrically according to the method of Trinder [25].
Measurement of glycogen content:
glycogen content was estimated by the method of Nicholas et al. [26],

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2.4 Statistical analysis

Statistical analysis is carried out using SPSS computer program ,post hoc and Student –T-test , where significance level at $p \leq 0.05$

3 RESULTS

Table 1 and Fig 1 A represents the level of carboxylic acid in hemolymph of susceptible and resistance snails of ~~Biomphalaria alexandrina~~ . Significant increase in carboxylic acids ; Fumeric and Malic acids ,while significant decrease in succinic acids in hemolymph of susceptible snail as compared to resistance one . However , Pyrouvic , lactic and Propionic acids showed insignificant change in hemolymph of both susceptible and resistance snails. Concerning concentrations of organic acids in digestive gland , significant decrease was recorded in Fumeric , Succinic, Lactic and Propionic acids in digestive glands of susceptible snail as compared to resistance . While Malic and Pyrovic acids showed significant increase and insignificant change respectively.

Table 2 and Fig 1B demonstrate comparison between organic acids levels in hemolyph and digestive glands of infected snails . It was noticed that , insignificant change in Fumeric and Malic acids levels in both organs of infected snail, while ,significant decrease in Succinc and Propionic acids in hemolyph of infected snails as compared to digestive organ . On the contrary , significant increase was observed in Pyrouvic and Lactic acids of hemolyph of infected snail as compared to digestive organ($P \leq 0.05$).

Table 3 declared glucose , glycogen and some hepatopancreas enzymes representing different metabolic pathways in susceptible , resistance and infected ~~Biomphalaria alexandrina~~ snail. Significant increase was recorded in amino acids pathway;AST and ALT in hepatopnacreas of susceptible snail as compared to resistance one . Infected snails showed significant inhibition of tranaminases enzymes either as compared to susceptible or resistance snails. Glycolytic enzymes ; PFK, GPI and G3PDH demonstrated significant increase in susceptible snails than resistance . Although , infected snails showed significant increase in the all enzymes representing glycolytic pathway. In contradictory Kreb's cycle enzyme ; LDH showed significant inhibition in infected snail and significant increase in susceptible as compared to resistance ~~Biomphalaria alexandrina~~ snails. In addition, glucose level showed significant increase in susceptible as compared to resistance snails .However ,glycogen level exhibited significant decrease in susceptible as compared to resistance ~~Biomphalaria alexandrina~~ snails , while significant increase was recorded in infected snails.

Table 4 representing the Relative activities of lactate, pyruvate, succinate, fumarate and malate in haemolymph and digestive gland of susceptible and resistant ~~B. alxandrina~~ snails. It was noticed that ,higher Lactate /Pyruvate ,Succinate/ Fumarate and Fumarate / Malate ratios in digestive gland and haemolymph of resistant snails as compared to susceptible one . In addition , AST / ALT and ALT / LDH exhibited higher ratio in digestive glands of resistant snails than susceptible one (Table 5).

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4 DISCUSSION

Fresh water pulmonate snails of the genus *Biomplalaria* are best known for their roles as intermediate hosts of the widely distributed parasite, *Schistosoma mansoni*. The major physiological and metabolic mechanisms of snail- chistosome relationships that are crucial for survival and growth[1].

Pooling of each 10 snails was used throughout the study and the mean of 3 trails for each HPLC analysis was done for each sample. Van Saun [27] encouraged the use of pooled samples and stated that, though some variation may be masked, pooled samples may provide an economic alternative to traditional metabolic profiling, as most of the important measures of metabolic status showed minimal differences between pooled and individual samples and found them to be statistically equivalent. He stated that the real challenge of using pooled samples is interpretation. Empirically one can interpret pooled samples by determining how far they deviate from the midpoint of the reference range for the control [28]..

The importance of the measured carboxylic acids could be easily supported by the previous work of Massa et al. [16] who proved that infection with *S.mansoni* caused a significant reduction in the concentrations of acetic, fumaric, malic, and pyruvic acids in the digestive gland (DG) but not the hemolymph of *Bimophalaria glabrata (B. glabrata*) snails compared to uninfected snails. The significant reduction of certain carboxylic acids in the DG of *B. glabrata* patently infected with *S .mansoni* suggested that the infection stimulates reduced production or increased utilization by the snaisl tissue and /or the significant reduction of certain carboxylic acids in the DG patently infected with *S. mansoni* suggests that these acids are utilized by the sporocysts and cercariae in the snail tissue. in addition, infection stimulates reduced production or increased utilization by the snail tissue [16].

Table 1 : Carboxlic acids concentrations in hemolymph and digestive gland of susceptible and resistance *Biomphalaria alexandrina* snails

Organs		1) groups	Mean± SD		P value
Hemolymph	Fumeric	S	3.4082	.46435	0.02
		R	1.5076	.80550	
	Malic	S	3.8196	0.13265	0.000
		R	1.8767	0.02255	
	Succinic	S	0.7506	0.05305	0.000
		R	1.8303	0.03445	
	Pyrovic	S	7.4669	0.19895	0.23
		0) R	6.8170	0.76925	
	Lactic	1) S	0) 6.2997	0.78250	0.36
		2) R	1) 7.3077	0) 1.49870	0)
	Propionic	3) S	2) 4.3878	1) .32831	1) 0.102
		4) R	3) 2.9709	2) 1.11405	2)
Digestive gland	Fumeric	5) S	4) 2.5345	3) 0.35675	3) 0.007
		6) R	5) 3.5942	4) 0.01325	4)
	Malic	7) S	6) 7.1751	5) 0.09285	5) 0.000
		8) R	7) 1.3284	6) .29670	6)
	0) Succinic	9) S	8) 3.5809	7) 1.35280	7) 0.037
		0) R	9) 5.9799	8) 0.01098	8)
	1) Pyrovic	1) S	0) 3.1791	9) 1.22415	9) 0.35
		2) R	1) 2.4350	0) 0.01590	0)
	2) Lactic	3) S	2) 4.1512	1) 0.06630	1) 0.000
		4) R	3) 5.1459	2) .00000	2)
	3) Propionic	5) S	4) 5.8877	3) .50400	3) 0.001
		6) R	5) 8.2096	4) .01325	4)

Organic acids concentrations in digestive glands are expressed in mg/g tissue , while in hemolymph organic acids are expreesed in ug/dl.

Table 2 : Significance levels between carboxylic acids in hemolymph and digestive glands of infected *Biomphalaria alexandrina* snail .

	Organs	Mean± SD		P value
Fumeric	Hemolymph	2.4579	1.19560	0.296
	Digestive gland	3.0643	0.62279	
Malic	Hemolymph	2.8481	1.06761	0.333
	Digestive gland	4.2517	3.20836	
Succinic	Hemolymph	1.2904	0.59267	0.000
	Digestive gland	4.7804	1.56800	
Pyrovic	Hemolymph	7.1419	.61583	0.000
	Digestive gland	2.8070	.87499	
Lactic	Hemolymph	6.8037	1.20340	0.003
	Digestive gland	4.6486	.54643	
Propionic	Hemolymph	3.6793	1.06858	0.001
	Digestive gland	7.0486	1.31108	

Organic acids concentrations in digestive glands are expressed in mg/g tissue , while in hemolymph organic acids are expreesed in ug/dl.

In the present study, separation and quantitative determination of the 6 carboxylic acids were done by the ion-suppression reversed-phase HPLC which has 2 significant features; the use of extremely diluted perchloric acid in water as the mobile phase decreases the total analysis time and provides good separation of the samples that facilitates simultaneous determination of all the acids of interest. The separation technique was successfully adapted to be used on hemolymph and DG of tissue samples. Separation was achieved successfully, and it was possible to separate and quantify the 6 acids at the same time in one sample of hemolymph and in one sample of tissue extract with lesser retention time than those in other papers .Without need for solid phase extraction before HPLC analysis, the used technique was a convenient, easier, faster, more accurate, and economical method of carboxylic acid separation[14,16 and 29]. In the present study, the order of concentration of the carboxylic acids in the heamolymph of susceptible group was pyruvic > lactic > propionic> malic > fumaric> succinic, while those of resistant group was Lactic > pyruvic > propionic >malic > succinic > fumaric. The order is different from that found in the previous study of Massa et al. [16].

Regarding the order of concentration of the studied acids in the DG of susceptible and resistant snails, the obtained date showed that an order of malic >propionic > lactic > succinic > pyruvic > malic respectively. These variations in the level of the studied organic acids between susceptible and resistant snails could be helpful to suggest the critical importance of these acids for the success of host-parasite relationship This could be confirmed through considering the recent work of Abou El-Seoud et al. (2010) who found that carboxylic acids investigated in both the haemolymph and tissue samples could be used as a potential diagnostic biomarkers to discriminate between infected and non-infected ~~Biomphalaria alexandrina~~ (~~B.alexandrina~~) snails. Moreover, the difference between

Table 3 : Levels of glucose , glycogen and some hepatopancreas enzymes representing different metabolic pathways in susceptible , resistance and infected *Biomphalaria alexandrina* snails

Parameters	Group	Mean± SD		Infection (3)	P value
ALT	S (1)	3.0933 (2,3)	0.61330	0.90±0.05 (1,2)	0.01
	R(2)	1.4600 (1,3)	0.08000		
AsT	S	1.1033 (2,3)	0.08386	0.30±0.02 (1,2)	0.001
	R	0.6400 (1,3)	0.05568		
PFK	S	2.1667 (2,3)	0.21962	6.0±0.05 (1,2)	0.001
	R	0.6773 (1,3)	0.12428		
GPI	S	8.9100 (2,3)	0.77019	11.05± 1.00 (1,2)	0.000
	R	2.0727 (1,3)	0.28601		
G3PDH	S	2.3867 (2,3)	0.40278	3.34±0.05 (1,2)	0.005
	R	0.8507 (1,3)	0.24948		
LDH	S	17.9200 (2,3)	2.51597	5.45± 0.9 (1,2)	0.004
	R	8.7267 (1,3)	0.80532		
Glucose	S	36.6667 (2,3)	2.87591	45.00±2.10 (1,2)	0.000
	R	16.4267 (1,3)	0.68973		
Glycogen	S	1.5683 (2,3)	0.11514	0.70±0.03 (1,2)	0.001
	R	2.7900 (1,3)	0.22605		

All enzymes are expressed in µ mole /mg protein / min. Glucose and glycogen are expressed in mg /g tissue used .Statistical analysis is carried out using SPSS –computer program and analysis of variance is carried out using post hoc.Mean ± SD of 3 replicates significant at $p < 0.05$.

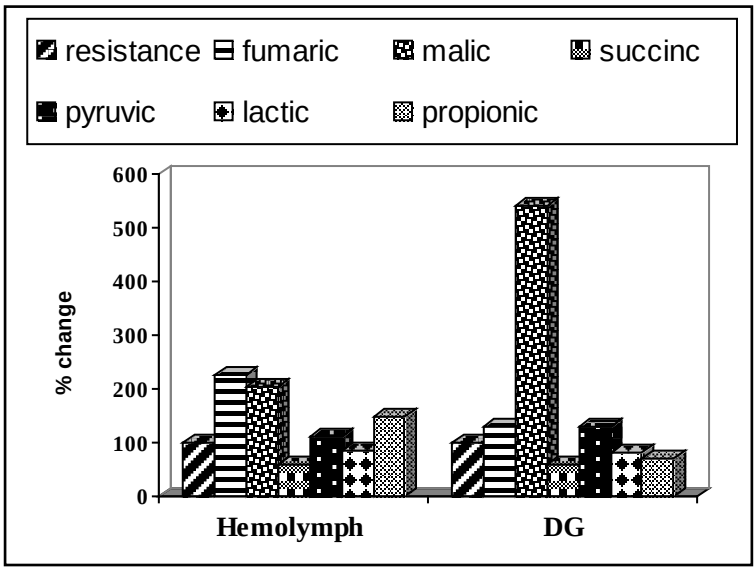

Fig 1 : (A) Percentage change of carboxylic acids inhemolymph and digestive gland of resistance and susceptible ~~Biomphalaria alexandrina~~ snails

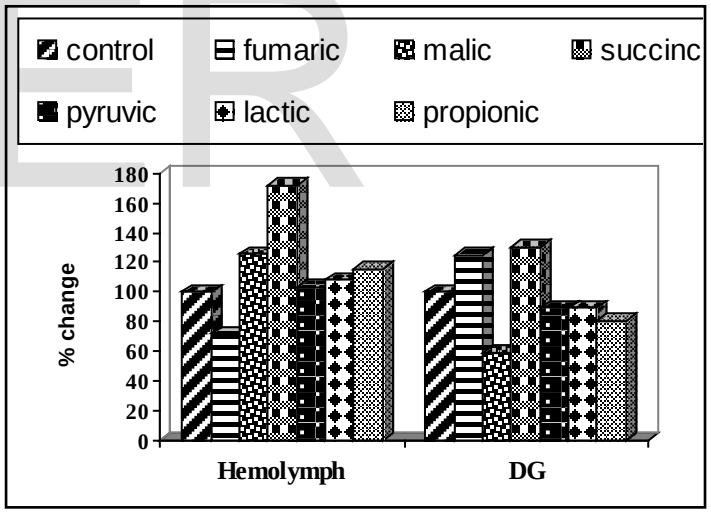

Fig 1: (B) Percentage change of carboxylic acids in hemolymph and digestive gland of susceptible control and infected ~~Biomphalaria alexandrina~~ snails.

Table 4 : Relative activities of lactate, pyruvate, succinate, fumarate and malate inhaemolymph and digestive gland of susceptible and resistant *B. alxandrina* snails

Resistant		Susceptible		Acids
Digestive gland	Haemolymph	Digestive gland	Haemolymph	
2.11	1.07	1.30	0.84	Lactate Pyruvate
1.66	1.21	1.14	0.22	Succinate/ fumarate
3.10	0.80	0.35	0.89	Fumarate / Malate

Table 5: Relative activities of AST, ALT and LDH enzyme activities in digestive gland of susceptible and resistant *B. alxandarina* snails

Resistant Digestive gland	Susceptible Digestive gland	Enzymes ratio
4.5	1.3	AST / ALT
0.89	0.48	ALT / LDH

haemolymph and DGG concentrations of the measured acids within susceptible and resistant snails could be used to suggest that haemolymph and tissue metabolic rates are different. The change in the acid concentrations during the studied course of susceptibility, the following was reported[24]. Reduction in the acid concentrations from that of the susceptible and during infection is explained by their possible use as metabolites by the developing schistosome sporocysts and cercariae [24]. These larval stages inhabit the intertubular spaces of the DG and cause mechanical and lytic damage to the di-

gestive gland cells of the hepatopancreas [24]. The damage probably results in a leakage of the acids, which are in turn utilized by the larval schistosomes. Moreover, increased metabolic activity associated with the presence of larval trematodes may accelerate the use of the acids by host cells in the digestive gland as well [24], the site of active intermediary metabolism in these snails is associated with the mitochondria of the digestive gland cells [19].

The trial to test the carboxylic acids as a potential diagnostic biomarker was one of the aims of this study. HPLC analysis, which is less expensive and more rapid than the previously mentioned techniques, succeeded in stating that these acids could be used as diagnostic biomarkers to detect whether the snail is susceptable or not . In addition, all of the studied acids in both the hemolymph and tissue samples could be used as a potential diagnostic biomarker except for the lactic acid in hemolymph. The key challenges in the anti-parasitic drug discovery are target selection and identification of appropriate small molecules as potential legends' for these targets. The search for biomarkers that might be useful in the drug discovery and development is an active area of research, and the complexity of disease and drug effects means that biomarker combinations will be more accurate [28].

In the present work, malic acid recorded the most varied concentration between susceptible and resistant snails which is in good agreement with the previous work of Abou El-Seoud et al. [30] who reported that malic acid was the most sensitive acid that could discriminate between control and infected snails and they recommended further assessment on their validity for usage on field studies to diagnose schistosomal infection of ~~B.alexandrina~~ snails and to state the stage of infection, and that assessment of these acids in earlier prepotency diagnosis is required to be investigated.

elative concentrations of the measured acids (Table 4) showed that haemolymph of susceptible snails recorded a lactate / pyruvate ratio of 0.84 compared to a ratio of 1.07 in the haemolymph of resistant snails which reflects the rate of glycolysis in susceptible snails. This could be supported through considering the work of El-Ansary [31] who reported different lactate / pyruvate in *B.alexandrina* snails parasitized by *S.mansoni* parasite. A lower ratio of lactate / pyruvate in susceptible snails could reflect a highly active lactate oxidase enzyme [31].

Mollusk-Parasite relationships represent a vast field of research. The dynamic interaction between mollusks and their trematode parasites leads to a state of co-existence or to incompatibility, where the trematode is either destroyed and eliminated by the host snail defensive responses or fails to develop because the host is physiologically unsuitable (Van der knaap and Loker , [32]. There is a high degree of specificity of shistosomes to their intermediate snail hosts. Resistant snails possess a natural defense system which protects them against ~~S.mansoni~~ larvae, which generally die one to three days after penetrating the snail [33,34 and 35] . Although susceptible snails are able to protect themselves against disease transmitting agents, ~~S.mansoni~~ appears to be able to avoid the snail´s defense system, thus allowing its own development in the host. Several studies on snail-Schistosome interaction have shown that susceptibility to infection may be the result of genetic factors present in the genome of both the vector snail and the parasite [36,37 and 38].

Previous studies have demonstrated that the digestive gland is the snail organ that provides the best conditions for the development and multiplication of the parasites [39, 40 and 41] , where there is a very good food supply for the sporocysts [42, 43 and 44] .In case of ~~Schistosoma mansoni~~ , the larvae can multiply to reach a weight equivalent up to 50% of that of ~~Biomphalaria glabrata~~ digestive gland [44] .For a successful host-parasite complex, the host must have high levels of energy metabolites to sustain normal metabolic activities during the developing of the parasites[44 and45] .

Many aspects of the host-parasite interaction remain to be clarified. When the parasites arrives the interfollicular connective tissue of the digestive gland, they are bathed in the haemolymph taking up large amount of free amino acids and carbohydrates for growth, energy metabolism and production of cercariae [46], and in exchange release products of their intermediate metabolism into the host´s body [17].The significant difference in carboxylic acids in susceptible and resistant snails , was explained by different reports , [18,19,21], as they that metabolite correlations do not necessarily correspond to proximity in the biochemical network as it is noted that neighboring metabolites and directly interacting metabolites in the metabolic network may have little or no correlation. It is not because they are not related, but because the variance in the enzymes that control them also affects them in equal amounts and different directions. This is what happens to most of the metabolite pairs and is the consequence of the systemic nature of metabolic control. However, most high correlations may be due to either stronger mutual control by a single enzyme or variation of a single enzyme level much above others Abou Elseoud et al. [30]. Also, metabolites in chemical equilibrium will have nearly perfect positive correlation. As a consequence, metabolites with negative correlation are not in equilibrium. In this case, the correlation does not originate from the enzyme that catalyzes the equilibrium reaction, as the metabolites have very small response towards it [16].

Consistent differences in both the hemolymph and tissue carbohydrate metabolite correlation profiles of the studied groups represent a promising additional source of information about the state of a metabolic system. However, their interpretation in terms of the underlying biochemical pathways is not straight forward and largely defies an intuitive analysis. These findings highlight the potential of metabolomics as a novel approach for fundamental investigations of host-pathogen interactions as well as for disease surveillance and control.

Identifying enzymes involved in specific metabolic pathway detected only in ~~S. mansoni~~ infected snails may allow the use

of certain enzyme inhibitors as target chemotherapeutic control agents. Alteration in the metabolism or metabolic status may also be used as a control measure without killing snails to keep balanced ecology [30].

The accumulation of organic acids as end products of energy metabolism is a common feature among parasitic helminthes [47]. Their concentrations in tissue and haemolymph were studied in infected snails and compared with those of non-infected snails (resistant & susceptible strains). In this context, organic acids may serve as indicators of various metabolic reactions. Thus, they may indicate the use of carbohydrates as an energy source in the flow of aerobic and also during anaerobic because they can be correlated without the help of kreb's cycle by a sequence of reactions and of protein via glucogenic amino acids [48], or metabolism of lipids on a small scale via fatty acids and ketone bodies [49]. Using such data, one can then evaluate in a broader manner the physiological processes that permit the snail to survive with or without pressure of infection.

One of the main methods of studying the evolutionary formation of metabolic forms is comparative analysis of activity and regulatory peculiarities of enzymes and polyenzymatic systems in the phylogenetic series of animals. Sufficiently numerous investigations of the adaptive changes in glycolytic enzymes in different types of invertebrates were reported [31]. It has been shown that the enzymatic activity of lactate dehydrogenase (LDH), an enzyme catalyzing the final step in glycolysis is reduced in the resistant snails to a considerable extent [31].

The LDH functions are partially fulfilled by alanine aminotransferase (ALT) performing mutual transformation of pyruvate and alanine[50]. A second transamination enzyme, aspartate aminotransferase (AST) is connected to a great degree with oxidation metabolism additionally filling the pool of the Krebs cycle metabolites.

Whereas levels of LDH, ALT and AST have been measured in a number of marine invertebrates[31]. Little information is available on the activity levels or patterns of these enzymes in fresh water snails. The present study demonstrates that AST, ALT and LDH were detected in susceptible and resistant snails. AST activity show high significant difference between the susceptible and resistant snails. The distribution of ALT activity presents an other picture. Susceptible snails have the highest level than the resistant which has the lowest ALT activity.

It is seen from the presented data that AST and ALT activities considerably changes within susceptible and resistant strains. Goromosova [51] compared the ratio of transaminases activities (AST& ALT) of invertebrates and with the view point of ecology, they showed that mollusks can be subdivided into the groups. The first group includes the species living the attached life and adapted to anaerobic conditions. The AST/ALT ratio of which fluctuates within 0.1 – 0.3. Low AST/ ALT values (0.4 – 1.0) are also characteristic of facultative anaerobes. Some higher level of AST/ALT (1.0 – 1.7) is observed in a number of mollusks inhabiting a sufficient oxygenated environment (the second group). The third group unites oxyphilic and mobile molluscs with AST / ALT ratio of 2.0 and higher.

Based on these observations, activities of transaminases (AST&ALT) can serve as an index of the metabolic degree or a relative role of the aerobic and anaerobic pathways in the energetic metabolism of the studied strains. Comparative analysis of the AST / ALT ratio of the studied strains showed that, the susceptible snails have the lowest AST / ALT ratio. They can be classified as aerobes but highly adapted to the anaerobic conditions with AST / ALT ratio around 1.7. Resistant snails is extremely oxyphylic, unable to withstand under anaerobic conditions (AST / ALT ratio of 4.5) (Table 5). These results are in accordance with those of Nabih and El- Ansary [50],who proved through measuring the Michaelis constants (Km) of succinate oxidase and fumarate reductase of fresh water snails, susceptible and resistant to schistosoma infection that ~~B. alexandrina~~ and ~~B. truncates~~ as specific molluscan hosts for ~~S. mansoni~~ and ~~S. haematobium~~ respectively are aerobes and that lactate is the major end product of glycolysis. In the resistant snails Km (succinate) / Km (fumarate) Value led them to suggest that these snails species are facultative anaerobic adapted to hypoxia through succinate production (Table 4) .

In our presented data, activity of AST in resistant strains and activity of ALT in the susceptible snails confirmed the previous observation of Nabih and El- Ansary [50], since AST can provide Krebs cycle intermediates which in turn favour the succinate production in resistant snails, while highly active ALT can be correlated to lactate production through transforming alanine to pyruvate. These results led us to express a supposition about a regulatory role of AST of oxidative metabolism, while ALT participates in the regulation of glycolysis.

A comparison of the ALT activity with LDH level will illustrate their great role in the energetic metabolism during hypoxia. Goromosova and Tamozhnyaya [52] observed a very low ALT/LD (0.04- 0.1) in sponges, hydroids. A somewhat higher ratio (0.2- 0.6) in a number of slow mobile gastropods and attached bivalve molluscs .ALT/ LDH ratios recorded for the susceptible snails confirmed the adaptive capabilities of the susceptible snails to anoxic conditions .

The present study clarify , significant increase in glycolytic enzymes PFK, GPI and G3PDH were observed in infected and susceptible snails , while low significant levels in resistant snails of B. alexandrina . The enhancement in the activities of glycolytic enzymes in infected and susceptible snails could be attributed to increase metabolic activities of infected and susceptible snails to compensate the inhibition of host Kreb 's cycle caused by the parasitic infection (as indicated by lowering LDH level in infected snail). The increase in anaerobic glycolysis might be attributed to activation of PFK due to decreased citrate formation , provision of energy lead to inhibi-

tion of Kreb's cycle , and decreased NAD/NADH ration due to inhibition of mitochondrial oxidation which favors conversion of pyruvate to lactate (Tielns , 1997 and Hamed et al., 2004). Lower LDH activity in infected and resistance snails revealed the aerobic –anaerobic switch induced in infected state by developing parasite (Tielens et al., 1994) . Lower activity in LDH in the direction could be easily correlated to the crabtree effect of schistosme (Tielens , 1997), through which lactate is accumulated and glycogen depleted confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis (Kuser et al., 2000). These findings explained and confirmed the present results through the observed significant reduction in glycogen concentrations in infected and susceptible snails as compared to resistant one due to their degradation into glucose to produce energy and enhanced glycolysis which leads to significant increase in glucose level in infected and susceptible snails.

4 Conclusion

In conclusion, consistent differences in both the hemolymph and tissue carbohydrate metabolites profiles as well as some enzymes representing different pathways of resistant , susceptible and ~~B. alexandrina~~ infected with ~~S. mansoni~~ were identified with a HPLC and colorimetric end point methods as these strategies allowed the prediction of infected and uninfected susceptible and resistance snails. These findings highlight the potential of metabolomics as a novel approach for fundamental investigations of host-pathogen interactions as well as disease surveillance and control.

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