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EFFECT OF LEPTIN RECEPTOR POLYMORPHISM ON EGYPTIAN CHILDREN WITH *ENTAMOEBA HISTOLYTICA* INFECTION

By

SOHA I. AWAD[1*], RAIDA S. YAHYA[2] and EMAN HAMED[2]

Department of Parasitology[1], and Department of Laboratories, Children Hospital[2], Faculty of Medicine, Mansoura University (*Correspondence: soawad@gmail.com)

Abstract

Entamoeba histolytica is a protozoan parasite that causes amebic colitis, an invasive disease responsible for as many as 100,000 deaths per year globally. Leptin signaling protects from amebiasis, with query molecular mechanism. This study aimed to evaluate the effect of leptin Receptor Q223R Polymorphism on Egyptian children with *Entamoeba histolytica* infection. After ethical clearance, blood and fecal samples were taken from one hundred children with *E. histolytica* and 100 children as control group.

All children were subjected to anthropometric assessment. *Entamoeba* infected group was further assessed for severity of symptoms, cyst load, and ELISA for differentiation of *E. histolytica* from E. dispar. Q223R LEPR polymorphism then performed for both groups. RR was higher in children with *E. histolytica* group while QQ and QR were higher in control group. Heavy cyst count, severe underweight, severe symptoms, and median leptin level was higher in RR group compared to QQ & QR groups. The highest risk of R allele was severe underweight, severe symptoms and high leptin level.

Key words: Egyptian children, *Entamoeba histolytica*, Leptin

Introduction

Diarrheal diseases are the second main etiology for death in children less than five years (Anteneh *et al*, 2017). Diarrheal induced deaths are directly related to malnutrition (Grenov *et al*, 2017). One of major cause of intestinal diarrhea in children in the developing countries is *E. histolytica* infection which results in millions of symptomatic children with the entamoebiasis *histolytica* yearly and more than 100,000 deaths annually (Victor *et al*, 2017). In Egypt, *E. histolytica* infection was reported (El-Naggar *et al*, 2006; Roshdy *et al*, 2015) particularly among school aged children (Curtale *et al*, 1998) and handicapped children (El Sherbini *et al*, 2008).

Leptin is a 16 kDa protein product of the LEP gene located on human chromosome 7 which originally work as a satiety signal, but now it was revealed its actions in immune modulation and gastrointestinal regulation (Tilg and Moschen, 2006; Yarandi *et al*, 2011; Facey *et al*, 2017). Leptin is important in immunity to *Entamoeba histolytica* (Duggal *et al*, 2011). Leptin induces intestinal mucosal epithelial cells proliferation and this antiapoptotic effect on enteroepithelium is needed for *Endameba histolytica* resistance (Woliński *et al*, 2003; Duggal *et al*, 2011). Leptin causes shift toward T helper 1 immunity and reverses immuno-suppression caused by starvation usually found in entamobiasis histolytica (Batra *et al*, 2010).

Leptin receptor is a member of cytokine receptors (Gorska *et al*, 2010). Leptin signaling has been linked to susceptibility to infectious diseases long time ago (Ozata *et al*, 1999; Farooqi *et al*, 2007; Mackey-Lawrence and Petri, 2012; Duggal *et al*, 2011).

The present study aimed to evaluate the effect of leptin Receptor Q223R Polymorphism on Egyptian children with *Entamoeba histolytica* infection.

Material and Methods

One hundred pediatric patients with *Entamoeba histolytica* infections were collected from Mansoura Children University Hospital, during the period between 1st of August 2017, and end of October 2017. Exclusion criteria: hyperthyroidism, pituitary diseases, chronic renal diseases, acute infection, and hematologic diseases, autoimmune liver cirrhosis, congenital liver disease, liver carcinoma, HIV infection, other parasitic disease, and obese patients. A total of 100 healthy cross matched children without symptoms of an acute or chronic infection were taken as a

control group.

Children or guardian of children were interviewed to take history & perform physical examination. Blood and fecal sample from each patient were taken by a trained technician. Severity of symptoms was assessed using Vesikari Clinical Severity Scoring System (Shim *et al*, 2016).

Anthropometric data (height, weight, upper arm circumference): stunted (height-for-age Z-score [HAZ] < −2), underweight (weight-for-age Z score [WAZ] < −2).

Stool examination: Direct smear, Formol-Ether concentration method, acid fast stain were used for Coccidea, Gomori's trichrome stain, Weber's trichrome stain for Microsporidia and agar plate culture for *Strongyloides stercoralis* (Garcia, 2007).

Differentiation of *E. histolytica* from *E. dispar* trophozoites in stool samples was detected by ELISA (*E. histolytica* Test Tech-Lab, Blacksburg, VA, USA) (Haque *et al*, 2000)

Leptin receptor polymorphism (Mohammadzade *et al*, 2014): Five milliliters of venous blood was drawn from each of the individuals and DNA was extracted using the QIAamp DNA Mini Kit (QIAgen Inc., Santa Clarita, CA). The PCR-restriction fragment length polymorphism (RFLP) based genotyping was carried out using gene-specific primers. The primer to amplify the Q223R region, forward 5'-GGCCTGAAGTGTTAG AAG AT-3′ and reverse primer 5'-CTGCT CTCTGAGGTG G GAAC-3. The amplified products were restricted with the specific restriction endonuclease, *MspI*, for Q223R polymorphisms. Digested products were as follow: undigested 642bp band showed A allele in the wild type homozygous genotype (QQ), 3 separate bands of 642, 469 & 173bp in heterozygous genotype (QR), and 2 separate bands of 469 &173bp showed G allele in the mutant homozygote genotype (RR).

Ethical consideration: Approval of the Institutional Research Board (IRB), Mansoura University was obtained. The informed consent from children guardians to participate in the study with a full right to withdraw was obtained with assurance of confidentiality and anonymity of the data.

Statistical analysis: Data were analyzed with SPSS version 21. Normality was first tested with the one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. Continuous variables were presented as Median (min-max) for non-parametric data. Two groups were compared with Mann–Whitney test while kruskalwallis test was used to compare medians of more than two groups. Significant variables entered into Logistic regression model using enter statistical technique to predict the most significant determinants and to control for possible interactions and confounding effects. Threshold of significance was fixed at 5% level (p-value). The results was considered: a- Non-significant when error probability was more than 5% ($p > 0.05$), b- Significant when error probability was less than 5% ($p \leq 0.05$), c- Highly significant when error probability was less than 0.1% ($p \leq 0.001$). The smaller the p-value obtained, the more significant are the results

Results

This study comprised of 100 children with *Entamoeba histolytica* infection (mean age 4.09±6.45 years) and 100 normal subjects (6.37 ± 8.80 years). Among those children with *Entamoeba histolytica*: 51% were normal, 34% with moderate malnutrition, 15% severe malnutrition. Regarding severity of symptoms: 40% mild symptoms, 35% moderate symptoms, 25% severe symptoms.

Thirty five percent of the infected children having the low cyst load, 25% were moderate, and 40% had high cyst load.

The details are given in tables (1, 2, 3 & 4) and figure (1).

Table 1: Comparison between *Entamoeba histolytica* children and control groups regarding parameters (n=100)

Variables	*Entamoeba histolytica* children	Control	Signifi-cance	p-value
Male Female	60 (60%) 40 (40%)	50 (50%) 50 (50%)	χ^2=2.02	0.155
Leptin polymorphism QQ RR QR	 40 (40%) 17 (17%) 43 (30%)	 57 (57%) 8 (8%) 35 (35%)	χ^2=7.04	0.03*
Leptin (ng/ml) Median (Min-Max)	8.20 (0.2-43)	1.10 (0.2-41)	Z=2.01	0.045*

*significant p <0.05

No significant difference was between children with patients and control regarding sexes distribution (p= >0.05). Significant difference was between pastients and control regarding Leptin polymorphism (p =< 0.05). Distribution of RR was higher in patients (17%) compared to (8%) in control while QQ & QR were higher in control (57%, 35%) compared to (40%, 30%) respectively in patients.

Table 2 : Relation between Leptin polymorphism and other parameters in *Entamoeba histolytica* children.

Variables	QQ (n=40)	RR (n=17)	QR (n=43)	Significance	P-value
Male Female	25 (62.5%) 15 (37.5%)	11 (64.7%) 6 (35.3%)	24 (55.8%) 19 (44.2%)	χ^2=0.575	0.750
Weight Normal Moderate underweight Severe underweight	31 (77.5%) 9 (22.5%) 0 (0%)	5 (29.4%) 4 (23.5%) 8 (47.1%)	15 (34.9%) 21 (48.8%) 7 (16.3%)	χ^2=31.83	<0.001*
Symptoms: Mild : Moderate : Severe	27 (67.5%) 9 (22.5%) 4 (10%)	5 (29.4%) 6 (35.3%) 6 (35.3%)	8 (18.6%) 20 (46.5%) 15 (34.9%)	χ^2=22.37	<0.001*
Cyst count: Mild : Moderate : Heavy	20 (50%) 9 (22.5%) 11 (27.5%)	6 (35.3%) 1 (5.9%) 10 (58.8%)	9 (20.9%) 15(34.9%) 19 (44.2%)	χ^2=12.53	0.014*
Leptin (ng/ml) Median (Min-Max)	0.9 (0.2-28)	22 (11.3-43)	1.1 (0.5-43)	KW=30.8	<0.001*

KW= kruskilwallis test

There was ignificant relation between leptin poly-morphism, weight, severity symptoms, cyst count and leptin level (p = <0.05). Severe underweight and symptoms were higher in RR group (47.1%, 35.3%) compared to (0%, 10%) in QQ respectively. Regarding cyst count, the heavy cyst count was higher in RR group (58.8%) compared to (27.5%, 44.2 %) in QQ and QR groups respectively also median leptin level was higher in RR groups compared with QQ and RR groups.

Table 3 : Relation between Leptin polymorphism alleles and other parameters in *Entamoeba histolytica* children

Variables	Q allele (n=123)	R allele (n=77)	Significance	P /Pc
Male Female	79 (64.2%) 44 (35.8%)	41 (53.2%) 36 (46.8%)	χ^2=2.38	0.123
Normal weight Moderate underweight Severe underweight	77 (62.6%) 39 (31.7%) 7 (5.7%)	25 (32.5%) 29 (37.7%) 23 (29.9%)	χ^2=27.38	<0.001*/ <0.001*
Symptoms: Mild : Moderate : Severe	62 (50.4%) 38 (30.9%) 23 (18.7%)	18 (23.4%) 32 (41.6%) 27 (35.1%)	χ^2=15.26	<0.001*/ <0.001*
Cyst count: Mild : Moderate : Heavy	49 (39.8%) 33 (26.8%) 41 (33.3%)	21 (27.3%) 17 (22.1%) 39 (50.6%)	χ^2=6.11	0.047*/ 0.09
Leptin (ng/ml) Median (Min-Max)	1.10 (0.2-43)	13.0 (0.5-43)	Z=5.56	<0.001*/ <0.001*

Z: Mann Whitney test, Pc= Bonforroni corrected P value (Number of comparison x P value).

There was severe underweight, severity of symptoms and heavily cysts load were associated with R allele (29.9%, 35.1%, 50.6%) compared to (5.7%, 18.7%, 33.3%) in Q allele group. Also, the median leptin level was higher with R allele.

Table 4 : Logistic regression analysis of independent predictors of R allele

Independent predictors	Univariate regression			Multivariate regression	
	β	P-value	Crude or (95%CI)	P-value	AOR (95%CI)
Weight Normal (r) Moderate underweight Severe underweight	- 0.829 2.31	- <0.014* <0.001*	1 2.3 (1.2-4.4) 10.1 (3.9-26)	- 0.379 <0.001*	1 1.4 (0.6-3) 7.4 (2.5-21)
Symptom severity Mild(r) Moderate Severe	- 1.06 1.39	- 0.003* <.001*	1 2.9 (1.4-5.9) 4.04 (1.9-8.7)	- 0.004* 0.005*	1 3.5 (1.5-8.1) 3.9 (1.5-10)
Cyst count Mild(r) Moderate Heavy	- 0.184 0.797	- 0.643 0.02*	1 1.20 (0.5-2.6) 2.22 (1.1-4.3)	- 0.841 0.514	1 1.1 (0.4-2.8) 1.3 (0.6-2.9)
Leptin Median (Min-Max)	0.055	<0.001*	1.06 (1.03-1.1)	<.001*	1.03 (1.0-1.1)
Constant Modelχ^2 % correctly predicted	-2.54 61.3, P =<0.001* 75.5%				

(r): reference group, AOR: adjusted OR, CI: confidence interval.

Regression analysis showed no associated with R allele, moderate & severe under weight (OR=2.3,10.1), moderate & se-vere symptoms (OR=2.9,4.04), Severe cyst count (OR=2.22) & high leptin level (OR=1.06). Multivariate regression analysis & adjusting confounding factors, highest risk of R allele were severe underweight (OR=7.4), moder-ate and severe symptoms (OR=3.5,3.9) & high leptin level (OR=1.06).

Discussion

In this study, leptin level was higher in RR groups compared with QQ and QR group (higher with R allele). This is agreed with Howard et al (2010) who stated that single-nucleotide polymorphisms in leptin receptor as substitution of Adenine by Guanine at nucleotide 668 result in substitution of glu-tamine by arginine at codon 223 in exon 6 (Q223R) of the LEPR gene which encodes the extracellular portion of leptin receptor. This change can lead to change in signaling and function of leptin receptor and result in high serum leptin level.

The RR was higher in patient's children than controls. Also, heavy cyst count and severe symptoms were higher in RR group (R allele) compared to in QQ group. This agreed with several authors who claimed that mutation (Q223R, A to G) in leptin re-ceptor gene at 223aa position has been found to be associated with the 4 fold in-crease in susceptibility to *E. histolytica* in-fection (Duggal *et al*, 2011; Guo *et al*, 2011; Verma *et al*, 2014). Leptin role in resistance to infectious agents had been studied in lep-tin and leptin receptor–deficient mice and human, revealing marked susceptibility to infection (Farooqi *et al*, 2007). This muta-tion leads to higher leptin secretion which result in too robust proinflammatory re-sponse leading to exacerbation of tissue damage, allowing further invasion of *E. his-tolytica* into the colonic epithelium (Ralston and Petri, 2011; Mack-ey-Lawrence and Petri, 2012). This mutation led to attenuation of anti-apoptotic effect of leptin which pro-tect intestinal epithelial cells from *E. histo-lytica* induced apoptosis (Marie *et al*, 2012).

In this study, severe underweight were as-sociated with higher leptin level and R al-lele. In contrast to other studies who claimed that leptin levels in malnourished children were lower than the well-nourished ones (Moore *et al*, 2002; Schaible and Kaufmann, 2007). This can be explained by the fact that

the malnourishment among children caused by *E. histolytica* infection, raise leptin level. The R allele is also associated with severe colonic pathology, which resulted in the marked malnutrition.

Conclusion

The presence of R223 allele in homozygous form is a significant predictor of *Entamoeba histolytica* higher load and morbidity in form of malnourishment and severe symptoms. It can be speculated that the polymorphism Q223R act as diagnostic marker for *Entamoeba histolytica* morbidity.

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Explanation of figure

Fig. 1: Q223R LEPR polymorphism: from left DNA marker, then 1st 3 lanes QR, 4th lane QQ, 5th & 6th QR, 7th RR, 8th, 9th QQ, 10th QR, 11th to 13th QQ, 14th QR, 15th QQ, 16th DNA marker.