Evaluating the clinical effect of interleukin-10 polymorphism on Egyptian children infected with chronic amoebiasis

Original Article

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ABSTRACT

Background: Since intestinal inflammation associated with chronic amoebiasis is regulated by interleukin-10 (IL-10), single-nucleotide polymorphism (SNP) at 1082, guanine (G) to adenine (A) alleles, are hypothesized to influence disease severity.
Objective: To investigate the potential link between IL-10-1082G/A gene polymorphism and diversity of clinical pediatric chronic amoebiasis.
Subjects and Methods: This case-control study included 100 selected children with chronic amoebiasis clinically complaining of chronic intermittent diarrhea (lasting > four weeks) alternating sometimes with constipation, and 100 healthy controls. Infected children were selected according to their routine investigations that included stool analysis, and bacterial culture-sensitivity method. Cases negative for bacterial infections, and positive for *Entamoeba* cysts were subjected to ELISA assay for detection of fecal *E. histolytica* adhesion antigen. Identification of polymorphism was performed using PCR amplification followed by restriction fragment length polymorphism (RFLP). The potential link of Il-10 polymorphism was assessed by correlating the detected genotype with diarrhea grading, weight, and cyst counts.
Results: A significant association (P<0.005) was recorded between IL-10 polymorphism and disease severity, weight, and cyst count. The G allele was associated with mild symptoms (47.1%; OR=3.8), normal weight (57.1%; OR=2.7), and high cyst count (50.7%; OR=3.5). In contrast, severe symptoms (43.4%; OR=2.7) and underweight (35%; OR=2.8), were independently associated with A allele.
Conclusion: These findings highlight the potential role of IL-10 gene polymorphism in influencing the clinical course of pediatric chronic amoebiasis. Further research should explore IL-10 levels and treatment outcomes to enhance our understanding of this potential link.
Keywords: Chronic amoebiasis; disease severity; Egyptian children; interleukin-10; polymorphism.

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INTRODUCTION

One of the widespread and significant health concerns impacting over three billion individuals worldwide is the presence of gastrointestinal protozoan parasites[1]. Children and infants constitute the majority of patients exposed to infections, and the predominant identified intestinal parasites included *G. lamblia*, and *E. histolytica*[2]. Amoebiasis is a global health problem, with higher prevalence in developing countries due to low sanitation and poor socioeconomic conditions[3]. Chronic amoebiasis often presents asymptomatically but may induce intermittent diarrhea, abdominal discomfort, and weight loss[4]. Timely diagnosis and proper treatment are crucial to prevent complications such as liver abscess or bowel perforation[5].

When assessing a child's health, weight plays a crucial role. To categorize a child's weight status and identify potential nutritional concerns, pediatricians utilize anthropometric measures, e.g., weight for height (WHZ). According to the WHO[6], three categories are classified. Normal weight refers to the child whose WHZ falls within the -2 to +1 standard deviation range of the WHO growth reference. This indicates adequate nutritional intake and growth patterns for their age and sex. Moderate underweight refers to children with WHZ values between -2 and -3 standard deviations. This signifies that they are lighter than expected for their height, potentially indicating insufficient calorie intake or nutrient deficiencies. Severe underweight refers to children whose WHZ falls below -3 standard deviations, and this serious condition indicates significant nutritional deprivation and stunted growth. It often necessitates immediate medical intervention and targeted nutritional support.

On the other hand, IL-10 is an anti-inflammatory cytokine produced by macrophages and T-helper type II (TH2) lymphocytes that down regulate inflammatory mediator production by stimulated immune cells and epithelial cells[7,8]. Intestinal inflammation associated with amoebiasis may be regulated by IL-10, known

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for its potent anti-inflammatory properties that reduce immune-mediated pathology caused by this pathogen; IL-10 dampens protective immunity but prevents immunopathology[9]. Among IL-10's anti-inflammatory actions is the inhibition of T cell responses to host intestinal flora, thereby decreasing the release of TNF-α, initially identified as an enhancer of chemotaxis, adherence, and intestinal epithelial injury by *E. histolytica*[10]. In addition, IL-10 helps the secretion of human colonic mucus, reducing the adherence of *E. histolytica* to intestinal cells[11].

The secretion level of IL-10 is genetically controlled by the IL-10 gene which contains several polymorphisms in the promoter region. The single-nucleotide polymorphism (SNP) at -1082 (G to A) is thought to influence IL-10 secretion and may influence outcomes in several disease states including infections such as amoebiasis[9,12-14]. A study suggested that IL-10 -1082 G allele is related to the increased expression of IL-10 cytokine gene, while the -1082 A allele is related to reduced expression of this gene[15]. Several studies indicated that certain genetic variations are linked to the regulatory function of IL-10 and its influence on the progression of diseases[15-17]. A changeover of allele G to allele A at -1082 bp (rs1800896) in the IL-10 gene was also linked to decreased IL-10 production[14]. Individuals with the homozygous G allele (-1082GG genotype), show elevated circulating IL-10 levels, heightened IL-10 mRNA expression, and increased IL-10 production[18]. Conversely, those with (−1082 AA and AG genotypes) are linked to reduced and moderate IL-10 cytokine generation, respectively[19].

Given this background, we conducted the present study to investigate the potential link between IL-10 -1082G/A gene polymorphism and chronic amoebiasis, and to correlate the findings with the severity of the disease.

SUBJECTS AND METHODS

This case control study was carried out at the pediatric outpatient clinic of Mansoura University Children Hospital and Department of Medical Parasitology, Mansoura University during the period between December 2022 and April 2023.

Study design: The study included children with chronic amoebiasis complaining of intermittent diarrhea (lasting > four weeks) and apparently healthy children. To confirm infection with pathogenic *E. histolytica*, ELISA assay of fecal *E. histolytica* adhesion antigen was conducted. Assessment of IL-10 gene polymorphism was conducted in both groups using RFLP-PCR.

Study population: A total of 100 children with suggestive complaints of chronic amoebiasis were recruited for the study. They were diagnosed clinically by the Pediatrician based on the complaint of intermittent diarrhea (lasting for > 4 w), sometimes alternating with constipation. Included were those with occasional complaints of distention, flatulence, and abdominal cramps. Exclusion criteria included recently vaccinated children with a live vaccine within 6 months before the study, children with autoimmune diseases, malignancy, known viral infections (HIV, viral hepatitis), positive stool bacterial culture for pathogenic bacteria. In addition, children who had received antibiotic therapy or immunomodulation therapy within three months before the study, those with nutritional disorders (above the 97th percentile and below the 3rd percentile)[20,21] and those with other parasitic infections were also excluded. The control group included 100 apparently healthy children, carefully selected from those attending for routine health screenings at Mansoura University Children's Hospital to match the case group in age and sex.

Clinical examination and collected samples: All participants underwent an interview to obtain their medical history by a specialized Pediatrist. A thorough physical examination was performed exclusively for the case group. The severity of symptoms was determined using the Vesikari Clinical Severity Scoring System[22] that categorizes the number of diarrhea attacks/day as follows: less than 7 (mild), 7–10 (moderate), and 11 or more (severe). Weight grading was determined based on growth parameters and their interpretation according to the WHO charts[20,21] in which moderate underweight is defined as a WHZ score of less than -2, while severe underweight is indicated by a WHZ score of less than -3. Three successive stool samples were collected, and a trained technician collected blood (~5 ml) from each participant for IL-10 gene polymorphism analysis.

Stool examination: Three successive stool samples were collected from each participant in a dry, clean, leak-proof plastic container following universal safety precautions and standard laboratory protocols. Fresh and formol-ether concentrated stool specimens were examined using saline and Lugol's iodine wet mount[23]. Other stains were used including acid-fast, Gomori's trichrome, and Weber's trichrome to diagnose cases infected with coccidia, and microsporidia, as well as agar plate culture for *S. stercoralis*[23].

Cyst count: The number of cysts/g of stool was calculated after formol–ether concentration technique using the formula N = S/(V × W); where N is the number of cysts/g of stool, S is the number of cysts counted on the slide, V is the volume of sample examined, and W is the stool weight (gm). Grading of cyst count was as follows: mild: 1-10 cysts, moderate: 11-100 cysts and severe: more than 100 cysts[24].

Fecal detection of *E. histolytica* adhesion antigen: An ELISA assay was conducted utilizing the *E. histolytica*

II assay obtained from (TechLab, Blacksburg, VA, USA), following the manufacturer's instructions[25]. In a 96-microtiter well plate pre-coated with polyclonal antibodies for adhesion, a volume of 0.1 ml of a diluted stool specimen (diluted 1:1 in the provided kit diluent) was added to each well. Additionally, a single drop of conjugate clonal antibodies specific to adhesion from *E. histolytica*, coupled with horseradish peroxidase, was added to each well. Both positive and negative controls were included in every test. The plate was subjected to incubation and washed using an ELISA washer. Subsequently, substrate and stop solution were added, and the absorbance was measured at 450 nm using an ELISA reader. A sample was deemed positive if its optical density reading at 450 nm was 0.05 or higher, while it was considered negative if the reading was <0.05.

Assessment of IL-10 gene polymorphism[26]: Genomic DNA isolation was performed using the GFX blood DNA purification kit from Amersham Biosciences Buckinghamshire (UK). The IL-10 (-1082) polymorphism was assessed by PCR amplification in a thermal cycler (Techne Genius, UK), followed by digestion with a specific restriction enzyme. The primers used were 5' CCAAGACAACACTACTAAGGCTCCTTT3' and 5' GCTTCTTATATGCTAGTCAGGTA 3'. The XagI enzyme obtained from MBIFermentas (Vilnius, Lithuania), was used for digesting the products, resulting in digestion products of 280 and 97 bp (for A allele) and 253 and 27 bp (for G allele). Visualization was achieved through gel electrophoresis using a 10% polyacrylamide gel.

Statistical analysis: The Statistical Package for the Social Sciences (SPSS) version 21 was utilized for statistical analysis. Categorical variables were presented as numbers and percentages. Group comparisons were conducted using the Chi-square test. We assessed the genetic equilibrium by employing the Hardy–Weinberg equilibrium (HWE) model. This analysis aimed to ascertain the alignment of observed genotype frequencies in our study population with the anticipated frequencies based on the assumptions of a stable population. Univariate followed by multivariate logistic regression analysis was employed to predict independent predictors. Odds ratio (OR) and their corresponding 95% confidence intervals (CI) were calculated. A significance level of $P<0.05$ indicates statistical significance.

Ethical consideration: The study was approved by Institutional Research Board (IRB) at Faculty of Medicine Mansoura University with the ethical approval code: R. 17.09.54. Informed consent was obtained from mothers of all the participants before enrollment. Cases with proved infections were reported to the treating physician to apply appropriate treatment.

RESULTS

Fecal detection of *E. histolytica* adhesion antigen: Initially, 112 children proved positive for passage of *Entamoeba* cysts in their stools., and negative for bacterial infections. Twelve of these cases tested negative for fecal *E. histolytica* adhesion antigen and were subsequently excluded from the analysis. The exclusion was based on the concern that these cases might represent false positives, possibly due to infection with *E. dispar*. Consequently, the final analysis included the remaining 100 cases, all of which tested positive for stool antigen detection.

Socioeconomic and clinical data of both study groups: Distribution of gender, age, and residence are shown in table (1). Male distribution was 60% in the case group and 50% in the control group, while the female distribution was 40% in the case group and 50% in the control group. In terms of age, the majority of the population, comprising 74% in the case group and 78% in the control group, fell within the 2-12 years age range. The distribution based on residence indicated that 60% of cases and 55% of controls resided in rural areas, while 40% of cases and 45% of controls were in urban areas. Additionally, table (1) outlines the clinical data of studied groups. Regarding the weight it was 100% normal among controls while it was distributed as normal, moderate underweight and severe underweight among case group (48%, 29% and 23% respectively). In addition, severity of symptoms was graded as mild, moderate and severe (40%, 31%, and 29% respectively). Cyst count was graded as low, moderate, and high (23%, 33%, and 44%, respectively) among cases (Table 1).

Table 1. Socioeconomic and clinical parameters of the study groups.

Variable	Cases (No.=100)	Control (No.=100)
Gender		
Male	60 (60%)	50 (50%)
Female	40 (40%)	50 (50%)
Age (years)		
2-12	74 (74%)	78 (78%)
> 12-16	26 (26%)	22 (22%)
Residence		
Rural	60 (60%)	55 (55%)
Urban	40 (40%)	45 (45%)
Weight		
Normal	48 (48%)	100 (100%)
Moderate underweight	29 (29%)	-
Severe underweight	23 (23%)	-
Symptom severity		
Mild	40 (40%)	-
Moderate	31 (31%)	-
Severe	29 (29%)	-
Cyst count		
Low	23 (23%)	-
Moderate	33 (33%)	-
High	44 (44%)	-

Polymorphism of the gene encoding IL-10: Figure (1) showed digestion products of 280 + 97 bp and 253 + 27 bp for A and G alleles, respectively. Table (2) presented significant differences between cases and control groups regarding IL-10 polymorphism (P<0.05). Distribution of GG was higher in the cases group (55%) compared to 30% in control group, while AA and AG were higher in control group (25%, and 45%) compared to 15%, and 30% in the cases group, respectively. There was also significant relation between IL-10 polymorphism, weight, severity of symptoms and cyst count (P<0.05). Severe underweight and severe symptoms were higher in AA (40%, and 53.3%) respectively compared to 20% for both in GG group. Regarding cyst count, heavy cyst count was higher in GG group (56.4%) compared to 26.7%, and 30 % in AA and AG groups respectively.

In table (3), the relation between IL-10 polymorphism alleles (A and G) and clinical parameters in cases group showed that severe underweight and severity of symptoms were higher in A allele (35%, 43.4% respectively) compared to 22.1, and 22.9% respectively, in G allele group. In contrast, high cyst count was associated with G allele (50.7%) compared with 28.3% in A allele group.

In table (4), logistic regression analysis of independent predictors of G and A alleles showed that normal weight (OR=2.7), mild symptoms (OR=3.8) and high cyst count (OR=3.5), were independently associated with G allele. Following a multivariate regression analysis and adjustment for confounding factors, the highest risk of G allele were mild symptoms (OR=2.8) and high cyst count (OR=3.2). Severe underweight (OR=2.8), and severe symptoms (OR=2.7) were independently associated with A allele. However, this relation was not found in multivariate regression analysis.

Table (5) revealed that the cases and control subjects did not exhibit departure from the Hardy-Weinberg equilibrium, with no significant differences between observed and expected genotype frequencies.

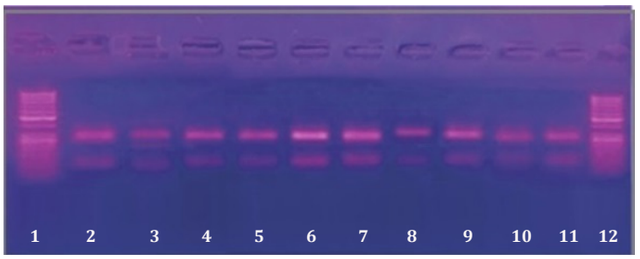

Fig. 1. Digestion products for IL-10 gene polymorphism AA, AG, and GG alleles in children complaining of chronic amoebiasis. **Lanes 1, and 12:** DNA size marker (50-1000 bp); **lanes 4, 5, 8 and 9:** homozygous wild-type AA (280, and 97 bp); **lanes 2, 6 and 7:** heterozygous-genotype AG (280, 253, 97, and 27 bp); **lanes 3, 10 and 11:** homozygous-genotype GG (253, and 27 bp).

Table 2. Relation between IL-10 polymorphism (AA, GG, AG) and other parameters in amoebiasis cases group.

Variable	Study groups			Statistical analysis
	Cases (n=100)	Control (n=100)		
IL 10 poly				
AA	15 (15%)	25 (25%)		X^2 = 12.85
GG	55 (55%)	30 (30%)		P = 0.002*
AG	30 (30%)	45 (45%)		
	IL-10 gene polymorphism			
	AA (n=15)	GG (n=55)	AG (n=30)	
Weight				
Normal	5(33.3%)	35 (63.6%)	10 (33.3%)	X^2=10.01
Moderate underweight	4 (26.7%)	9 (16.4%)	11 (36.7%)	P = 0.04*
Severe underweight	6 (40%)	11 (20%)	9 (30%)	
Symptom severity				
Mild	3 (20%)	29 (52.7%)	8 (26.7%)	X^2=10.97
Moderate	4 (26.7%)	15 (27.3%)	12 (40%)	P = 0.027*
Severe	8 (53.3%)	11 (20%)	10(33.3%)	
Cyst count				
Low	7 (47.6%)	9 (16.4%)	7 (23.3%)	X^2=11.41
Moderate	4 (26.7%)	15 (27.3%)	14 (46.7%)	P = 0.022*
High	4 (26.7%)	31 (56.4%)	9 (30%)	

*: Significant (P <0.05).

Table 3. Relation between IL-10 polymorphism alleles (A and G) and other parameters in cases group.

Variable	A allele (n=60)	G allele (n=140)	Statistical analysis (X^2 test)
Weight			
Normal	20 (33.3%)	80 (57.1%)	Normal vs moderate: *P* = 0.009*
Moderate underweight	19 (31.7%)	29 (20.7%)	Moderate vs severe: *P* = 0.018*
Severe underweight	21 (35%)	31 (22.1%)	Normal vs severe: *P* = 0.001*
Symptom severity			
Mild	14 (23.3%)	66 (47.1%)	Mild vs moderate: *P* = 0.002*
Moderate	20 (33.3%)	42 (30.0%)	Moderate vs severe: *P* = 0.004*
Severe	26 (43.4%)	32 (22.9%)	Mild vs severe: *P* < 0.001*
Cyst count			
Low	21 (35%)	25 (17.9%)	Low vs moderate: *P* = 0.005*
Moderate	22 (36.7%)	44 (31.4%)	Moderate vs severe: *P* = 0.01*
High	17 (28.3%)	71 (50.7%)	Low vs severe: *P* <0.001*

Pc: Bonforroni corrected *P* value (Number of comparison x *P* value), *: Significant (*P*<0.05).

Table 4. Logistic regression analysis of independent predictors of G and A alleles.

Variable	Univariate regression			Multivariate regression	
	β	*P*	COR (95% CI)	*P*	AOR (95% CI)
Weight					
Normal	0.997	0.008*	2.7 (1.3-5.7)	0.071	2.3 (0.9-5.5)
Moderate underweight	0.033	0.935	1.03 (0.5-2.3)	0.421	1.5 (0.6-3.6)
Severe underweight (r)	-	-	1	-	1
Symptom severity					
Mild	1.343	0.001*	3.8 (1.8-8.3)	0.027*	2.8 (1.1-6.8)
Moderate	0.534	0.158	1.7 (0.8-3.6)	0.297	1.5 (0.7-3.4)
Severe (r)	-	-	1	-	1
Cyst count					
Low (r)	-	-	1	-	1
Moderate	0.519	0.189	1.7 (0.8-3.6)	0.828	1.1 (0.5-2.5)
High	1.255	0.002*	3.5 (1.6-7.7)	0.007*	3.2 (1.4-7.6)
Constant			-0.59		
Model X^2			25.2, *P* <0.001*		
% correctly predicted			76%		
Independent predictors (A Allele)					
Weight					
Normal (r)	-	-	-	-	-
Moderate underweight	1.161	0.243	1.1 (0.6-4.2)	0.331	1.3 (0.7-5.1)
Severe underweight	1.321	0.001*	2.8 (1.2-4.2)	0.432	1.5 (0.9-6.4)
Symptom severity					
Mild (r)	-	-	-	-	-
Moderate	1.221	0.345	1.2 (0.5-5.8)	0.231	1.1 (0.9-4.2)
Severe	1.643	0.009*	2.7 (1.1-4.2)	0.216	1
Cyst count					
Low (r)	-	-	1	-	1
Moderate	0.672	0.235	1.1 (0.8-3.6)	0.218	1.2 (0.7-3.4)
High	0.985	0.12	0.93(0.01-8.7)	0.17	2.2 (0.85-6.4)
Constant			-0.64		
Model X^2			28.4, *P* =<0.001*		
% correctly predicted			79%		

r: Reference group; **β:** Model value; **OR:** Odds ration; **COR:** Crude OR; **AOR:** Adjusted OR, **CI:** Confidence interval. ***:** Significant (*P*<0.05).

Table 5. Hardy-Weinberg equilibrium in cases and control subjects.

Genotypes	Observed	Expected	Statistical analysis
Control			
AA	25	23	X^2 = 0.95
GG	30	50	*P* = 0.32
AG	45	27	
Cases			
AA	15	9	X^2 =1.6
GG	55	42	*P* = 0.2
AG	30	49	

DISCUSSION

The apparent paradox in the protective function of IL-10 becomes evident when studying systemic infectious disease models, in these cases, IL-10 suppresses protective immunity while preventing immunopathology[27]. The behavior of many gastrointestinal infections differs from these systemic infections. For example, in *Helicobacter hepaticus*, IL-10-deficient mice actually experience more severe infections and higher disease burdens[28]. In such infection model, the disease manifests due to the inability to generate a protective population of T regulatory cells and becomes evident after a 20-day period during the adaptive immunity phase[29]. A polymorphism within the promoter region of the IL-10 gene at position -1082 has been associated with change in IL-10 production[30].

Therefore, we designed this study to evaluate the impact of IL-10-1082 G/A polymorphism on *E. histolytica* infection. To the best of our knowledge, based on extensive research, our study is the first to address this particular issue. We recorded severe underweight, and severe symptoms higher in AA (40%, 53.3%) compared to 20% for both in GG group. Besides, cyst count in our study was higher in GG group (56.4%) compared to (26.7%, 30%) in AA and AG groups respectively. This may be explained by the reported increased susceptibility of IL-10-deficient mice to early amoebic infection. Reports indicated that one potential mechanism could involve mucus production. The epithelium of IL-10-deficient mice shows impairments in the synthesis of Muc2, a crucial component of human colonic mucus that typically restricts *E. histolytica* adherence to host cells[31,32].

Moreover, in IL-10-deficient mice, the epithelium may display an increased tendency toward apoptosis. The resulting cellular remnants have the potential to act as a source of nutrients for the amoeba, as observed *in vitro*[33]. Another interesting possibility is that the tissue phase trophozoites benefit from an IL-10-deficient proinflammatory state. For instance, TNF-α, a proinflammatory cytokine, is recognized for its role in promoting *E. histolytica* chemotaxis, adherence, and trophozoite-induced epithelial damage[10]. All of these factors explain the severe presentation of chronic amoebiasis associated with AA genotype and the mild presentation associated with GG genotype in our study.

These findings align with a prior study indicating that heightened IL-10 levels may reduce the risk of amoebiasis[9]. Another recent study demonstrated that IL-10 gene polymorphism provided protection against a high burden of *S. mansoni* in children[13]. Contrary to our results, another study found that the correlation between HIV-1 susceptibility and IL-10 gene polymorphisms was insignificant[34]. Furthermore, the -1082 AA IL-10 genotype, associated with lower IL-10 production, has been linked to the presence of invasive pulmonary aspergillosis[35]. This may be explained by the fact that the role played by IL-10 and IL-10 gene polymorphism may differ between viral, fungal and protozoal infections due to the complex interplay between IL-10 and the immune system.

It appeared from our study that the G allele may be associated with a mild clinical course of amoebiasis since the regression analysis showed independent association with G allele i.e., normal weight (OR=2.7), mild symptoms (OR=3.8) and high cyst count (OR=3.5). Following a multivariate regression analysis and adjustment for confounding factors, the highest risk of G allele were mild symptoms (OR=2.8) and high cyst count (OR=3.2).

In conclusion, the A allele of IL-10 -1082 G/A SNP is linked to a more aggressive presentation of *E. histolytica* infection, characterized by severe symptoms and significant underweight. Conversely, the GG genotype appears to confer a level of protection against severe amoebiasis. However, one drawback of our study is the absence of correlation between the genotype and IL-10 levels. Another limitation is the lack of investigation into the relationship between IL-10 polymorphisms and treatment outcomes. These aspects should be considered in future research.

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Author contributions: Hamouda MM proposed the study design, collected the literature, performed the parasitological work, and drafted the manuscript. Yahya RS performed the clinical examination, and revised the work critically for important intellectual content. Hamed E helped in laboratory investigations and statistical analysis. Awad S conceived the study, curation of data, helped in drafting the work, edited and revised it critically for important intellectual content. All authors approved the final version for publication.

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