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Prevalence and Molecular Identification of Head Lice Among Primary School Students And Refugee Camps in Zakho District, Iraq

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ABSTRACT

Head lice, scientifically referred to as *Pediculus humanus capitis*, lead to a condition known as pediculosis capitis, or head lice infestation. A survey was conducted to determine the prevalence of *Pediculus humanus* capitis infestation among children in 22 primary schools and two refugee camps situated in Zakho City, Kurdistan Region, Iraq. A total of 1,131 children underwent manual and individual examinations. Individuals were classified as positive for infestation when nits, immature lice, or adult lice were detected on their scalps. The overall prevalence of head lice was found to be 21.7%, with infestation rates of 25.5% in schools and 16.7% in camps. The majority of infested individuals were aged between 6 and 9 years, exhibiting higher infestation rates compared to those aged 10 to 12 years. Furthermore, infestation was significantly more common among females than males ($p < 0.001$). A notable correlation was identified between larger family sizes (7–14 members) and increased infestation rates in both environments ($p < 0.05$). In schools, a lower frequency of bathing was significantly linked to infestation ($p = 0.001$), although this correlation was not significant in camps ($p > 0.05$). Additionally, children whose mothers had lower levels of education were found to be more frequently infested. Statistically significant differences were also noted in both schools and camps. This study aimed to evaluate the prevalence of *P. humanus capitis* infestation among primary school children and in refugee camps, as well as to examine the association of prevalence with key factors.

INTRODUCTION

Head lice infestation, known as pediculosis, represents the presence of the obligate ectoparasite, Pediculus capitis, which exclusively targets the human scalp. These parasites can infest humans at any stage of life and feed on human blood. They do not infest other pests or animals, lack wings, and are incapable of jumping (Ali and Hama, 2018). Pediculosis is categorized as one of the six Epidermal Parasitic Skin Diseases and is informally classified under the umbrella of "Neglected Tropical Diseases" (Speare *et al.*, 2014)

The primary mode of transmission for head lice is through direct head-to-head contact. Typically, infestations occur among children during play, while transmission via objects is an uncommon occurrence and holds little epidemiological significance (Al-Marjan,2008). The host might not initially perceive the physical impact of head lice; however, it can take several weeks after the infestation for an individual to become aware of it. Symptoms of pediculosis include itching, or pruritus, which may accompany the infestation and, in certain instances, can be exacerbated by bacterial infections that arise when the skin is excoriated (Koch *et al*, 2001).

There exists considerable confusion regarding the significance of nits (which are dead eggs or egg shells) in the diagnosis of louse infestation. Typically, the diagnosis of louse infestation relies on the detection of nits, while the effectiveness of a treatment is determined by their absence from the hair. Nevertheless, it is important to note that not every person with nits harbors live lice, and the mere removal of visible nits from the hair does not automatically indicate that the individual is free from lice infestation (Albrecht, 2012). The life cycle of head lice consists of three distinct stages: the egg (nit), the nymph, and the adult. Typically, this cycle spans approximately three weeks (Bloomfield, 2002). Common itchy lesions can be seen at the site of feeding, potentially resulting in skin excoriations. Additionally, secondary bacterial infections caused by *Staphylococcus aureus* and *Streptococcus spp*. may develop on these skin lesions. Some patients have also exhibited local swelling of cervical lymph nodes, alongside a severe and chronic infestation that could result in anemia and hair loss (Özden *et al.*,2023). Secondary bacterial infections frequently occur in patients as a result of severe scalp itching. Recent studies indicate that head lice can carry and potentially transmit dangerous bacteria to humans, including *Acinetobacter baumanii*, *Rickettsia prowazekii*, and *Bartonella quintana* (Batır *et al.*,2024). Head lice deposit their eggs on the hair shaft, near the scalp, where the temperature is ideal for embryonic development (Karakus *et al*,2014). The present research sought to examine the prevalence of *P. humanus capitis* among children attending primary schools and residing in refugee camps within the Zakho district, as well as to identify the primary factors associated with the prevalence of this obligate human parasite.

MATERIALS AND METHODS

A total of 1131 children were collected randomly from the first of October 2024 to the end of February 2025, from 22 primary schools (624 students) and 2 refugee camps (507 children) in the Zakho district, aged between six and twelve (1-6 grade), were involved in a pediculosis investigation. The assessment of head lice infestation was carried out through direct visual examination. Each child's head was thoroughly inspected using both scalp visual detection and fine-tooth combing, as both methods are sensitive, cost-effective, efficient, and easy to implement (De Maeseneer *et al.,*2000). Children were deemed positive for infestation if any signs of head lice were present, including adults, nymphs, or live or dead eggs. Participation in this study was voluntary. Nevertheless, participants were requested to respond to several related questions included in a specially designed questionnaire, which covered aspects such as gender, age, number of family members, maternal education, and frequency of showers or baths per week. A Chi-square test was utilized to examine the associations between infestation and the variables at the univariate level. The threshold for statistical significance was established at p less than 0.05 for all hypotheses tested in this research.

Extraction and Amplification of Head Louse DNA:

Head lice specimens were gathered from infected individuals and promptly preserved in 95% ethanol, subsequently stored at -20 ºC for future molecular analyses. The genomic DNA of the head lice was extracted from tissue samples utilizing an Addbio™ (South Korea) silica column-based extraction protocol, adhering to the manufacturer's guidelines. To accurately and genetically verify the genus identity of the parasite, the mitochondrial gene of the parasite was amplified using the PCR technique. Forward and reverse primers were sourced from (Al-Marjan *et al*,2022 and Ghavami *et al*,2022) and synthesized by Macrogen™, Huminizing Genomics (South Korea)

(Table 1). The primers employed in this study were specifically designed to amplify two mitochondrial gene markers: cytochrome b (*cytb*) and cytochrome c oxidase subunit 1 (*cox1*). The amplification reaction was prepared in a final volume of 50 µl, which included 10 µl of DNA, 10 pM of cox1 primers (POF and POR) or *cytb* primers (U8-F and U8-R), along with 25 µl of 2X master mix added to the reaction. The PCR process consisted of an initial denaturation cycle at 95 °C for 5 minutes, followed by 35 thermal cycles, each cycle configured as follows: 95°C for 30 seconds (denaturation); 52°C for 30 seconds (*cox1*), 55°C for 45 seconds (*cytb*) (annealing), and 72°C for 90 seconds (*cox1*), 60 seconds (*cytb*) (extension), concluding with a final extension step at 72°C for 7 minutes. A volume of 5 µl from each PCR product was analyzed through electrophoresis on a 0.75% w/v agarose gel at 75V for 45 minutes, followed by visualization under UV light. Six random samples of the cytb PCR product were dispatched for sequence confirmation via Sanger sequencing (Macrogen, South Korea).

Table 1: Primers used for the head lice genetic identification.

Primer Nam	Primer Sequences, 5′-3′.	Ref.
Cox1:POF	ATAGTTATGCCTGTAATAATAG	AL-Marjan, 2022
Cox1:POR	TGTTGGTATAAAACAGGATCAC	
Cytb: U8-F	GAGCGACTGTAATTACTAATC	Ghavami *et al.*, 2020
Cytb: U8-R	CAACAAAATTATCCGGGTCC	

Cytb DNA Sequence Analysis:

DNA sequences analyzed using the Sanger method in South Korea were subsequently processed with SeqTrace to produce high-quality consensus sequences. These sequences were identified through a BLASTn search against GenBank and aligned with MEGA X to assess the genetic relationships between the isolates and local head lice. Phylogenetic relationships were deduced using the Neighbor-Joining method with 1,000 bootstrap replications, which demonstrated the genetic similarity between local head lice isolates and global reference sequences.

RESULTS

A total of 1,131 individuals (624 students and 507 refugees) were subjected to the pediculosis investigation. Among these, approximately 21.75% (n=246) tested positive. The prevalence of pediculosis was greater among school pupils, reaching 25.8% (n=161 out of 624), in comparison to the refugee population, which had a prevalence of 16.7% (n=85 out of 507), as illustrated in (Table 2).

Table 2: Prevalence percentages of head lice cases among schools and refugee camps.

Study Field	No. of Examination	Positive cases	Prevalence %
School	624	161	25.8
Camps	507	85	16.7
TOTAL	1131	246	21.75

Prevalence of Pediculosis and Associated Risk Factors:

To assess the risk factors that are closely linked to the spread of head lice infestations, data were collected and categorized into various groups, including age, gender, family size (number of members), frequency of bathing per week, and the educational status of mothers. Analysis using chi-square (χ^2) values and p-values revealed a significant relationship between age and infestation rates in both schools ($\chi^2 = 11.47$, $p = 0.001$) and camps ($\chi^2 = 9.18$, $p = 0.002$), with the younger

cohort of children (ages 6–9 years) exhibiting higher infection rates. Gender also showed a notable correlation, as females had a greater prevalence than males in both studied environments (schools: χ^2 = 10.69, p = 0.001; refugee camps: χ^2 = 9.26, p = 0.002). Additionally, family size emerged as a significant risk factor (schools: χ^2 = 5.23, p = 0.022; camps: χ^2 = 9.45, p = 0.002), with children from larger families (7–14 members) being more frequently affected. The frequency of bathing per week was significantly correlated with infestation in schools (χ^2 = 10.41, p = 0.001), but not in camps (p = 0.675), indicating that hygiene may have a more pronounced impact in school settings. Lastly, the educational level of mothers was significantly associated with lice prevalence in both schools (χ^2 = 9.60, p = 0.002) and camps (χ^2 = 11.32, p = 0.001), as children whose mothers lacked formal education exhibited higher infestation rates (Table 3**).**

Table 3: The role of several risk factors in the prevalence of head lice infestation.

Category	No. of Examined Individuals	Positive Individuals		X^2 (p-values)	
		Schools	Camps	Schools	Camps
Age/ years				11.47 (0.001)	9.18 (0.002)
6-9	654	120	54		
10-12	477	41	29		
Gender				10.69 (0.001)	9.26 (0.002)
Male	477	57	19		
Female	654	104	60		
Family Size/ No.				5.23 (0.022)	9.45 (0.002)
2-6	583	107	49		
7-14	548	54	36		
Bath Frequency/ Week				10.41 (0.001)	0.917 (0.675)
1-3	926	142	71		
4-6	205	19	14		
Mother Education				9.60 (0.002)	11.32 (0.001)
Yes	381	24	53		
No	750	137	32		

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Randomly extracted DNA from six head lice (Fig. 1) was sequenced to facilitate the genetic identification of the isolated parasite. After obtaining the *cytb* sequences, these were compared against the GenBank database. Phylogenetic relationships were deduced using the Neighbor-Joining method with 1,000 bootstrap replications, which demonstrated the genetic similarity between local head lice isolates and global reference sequences. The resulting sequences were submitted to the GenBank database and were assigned the following accession numbers: PV904816, PV904817, PV904818, PV904819, PV904820, and PV904821

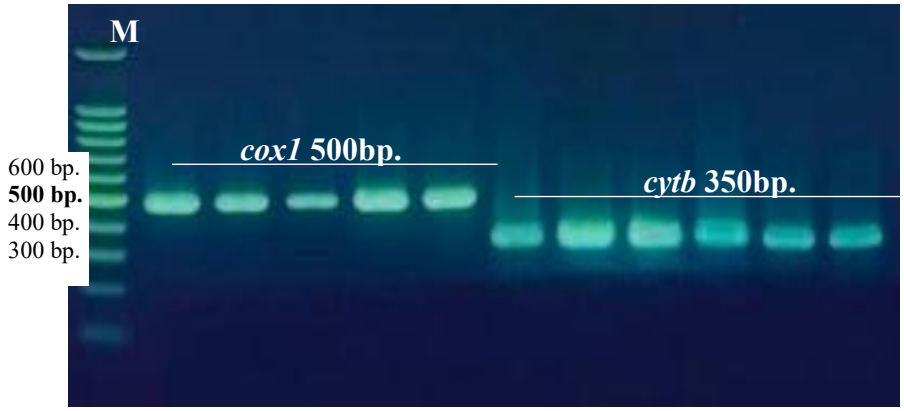

Fig. 1: Gel-electrophoresis analysis of the *cytb* and *cox1* genes amplification. Expected sizes were obtained; *cox1*: 500bp, and *cytb*: 350bp. Lane M:5 µL DNA marker (Addbio^{TM}). Around 5µL of both amplified genes were loaded in the gel wells

DISCUSSION

The results of this research indicate a significant occurrence of head lice infestations among children attending primary schools and residing in refugee camps in Zakho city. The total infestation rate recorded in schools was 25.8%, whereas a lower rate of 16.7% was observed in the refugee camps, yielding an average prevalence of 21.75% . These findings highlight an ongoing public health issue, particularly in communal settings where close interactions and inadequate hygiene facilities are prevalent. The increased prevalence among schoolchildren, in contrast to those living in refugee camps, may be linked to various factors, such as overcrowded classrooms, the absence of regular screening initiatives, and restricted access to effective treatment options in educational institutions. The findings presented are in alignment with those documented by (Al-Marjan, 2008), where a prevalence rate of 25.5% among schoolchildren was noted. This resemblance, despite the geographical disparities, underscores that pediculosis capitis continues to be a prevalent and unresolved concern within educational settings throughout the Kurdistan Region. In contrast, the prevalence observed in Cham Mishko Camp was 36.2%, which is considerably higher than the overall camp rate of 16.7%. This suggests the possibility of localized outbreaks or varying levels of access to hygiene and health education across different sectors of the camp. While this figure is lower than the previously cited rate of 36.2% reported by (Alberfkani, and Mero,2020), it nonetheless emphasizes the ongoing challenge of head lice infestations in displacement contexts. The discrepancies between the current results and those reported by Alberfkani and Mero may be attributed to temporal variations, slight improvements in hygiene practices, or heightened awareness stemming from earlier infestations. Significantly, (Ali and Hama,2018) documented a notably lower infestation rate of 1.12% within the Sulaimaniyah refugee camps, which they attributed to inadequate sanitation and limited availability of hygienic services. This observation suggests a possible variation in methodologies or population dynamics across different regions. On a larger scale, Nakhostin and his associates reported an infestation rate of 33.17% in Garmsar city, Iran, which surpasses the findings of the current study. This reinforces the notion that head lice infestations are not exclusive to displaced populations but can also be prevalent in stable urban environments (Nakhostin *et al.*,2022). In a similar vein, global data compiled by (Falagas *et al.*,2008) indicated prevalence rates ranging from 3.6% to 61.4% across various nations in the Americas, particularly in Brazil, Cuba, Argentina, and the USA, with higher rates

observed among females. These international statistics highlight a significant variation in prevalence, shaped by cultural, socioeconomic, and environmental factors. In the current investigation, it was determined that head lice infestations are more common among children aged 6 to 9 years, with a prevalence rate of 14.67%, in comparison to those aged 10 to 12 years. This trend based on age is consistent with the results reported by (Ali and Hama,2018), who found a prevalence of 15.11% in children aged 6 to 12 years in Zakho, thereby reinforcing the notion that younger children are typically at a greater risk. The heightened susceptibility in this age demographic may be linked to their close physical interactions during play, a limited understanding of hygiene practices, and a greater tendency to share personal items such as combs or hats. However, contrasting findings have emerged from other research. For example, Amin and colleagues in Kalar identified the highest infestation rate (23.35%) in slightly older children aged 10 to 11 years, indicating that local environmental or behavioral factors could alter the risk dynamics (Amin *et al*,2019). Additionally, noted that the prevalence among students aged 6 to 7 years was 23.23%, surpassing that of older age groups (Tawfeeq, 2020). Furthermore, the highest rate (43.30%) was recorded in the 8 to 10 age group in Sulaimaniah-Iraq, underscoring regional variations in infestation trends (Haama,2020). These findings collectively support the assertion that early primary school children are at an increased risk due to various behavioral and developmental factors. The differing results across regions and age categories imply that age is a significant yet context-dependent factor in the transmission of head lice. In terms of gender, the present study uncovered a distinct disparity, with females exhibiting a notably higher infestation rate (25%) in comparison to males (15.93%). This finding aligns with the results of a survey conducted in Kirkuk, Iraq, where the infestation rate was 13% for females versus 4.44% for males (Aziz,2025). Likewise, in Al-Kut, Iraq, reported infestation rates of 21.8% in females and 13.3% in males, further emphasizing that females are disproportionately affected (Muri and Rhadi,2022). This gender difference may stem from factors such as longer hair, frequent close social interactions, and a greater likelihood of sharing hair accessories among females. Additionally, it is plausible that cultural grooming practices contribute to this heightened risk, particularly in school and communal environments. The current study indicated that head lice infestation is significantly more prevalent among girls (25%) than boys (15.93%), a trend that is widely corroborated by findings across various regions and populations. **(**Moradiasl *et al*,2018) reported a similar trend in Iran, where the infestation rate was 12.44% in girls compared to 0.71% in boys (Moosazadeh *et al.,*2021). This gender disparity is likely attributable to behavioral and biological factors, including longer hair in girls, closer social interactions, and the use or sharing of personal grooming items. In Libya, (Halila,2024) discovered that 10% of girls were infested compared to 4% of boys, reinforcing the idea that females are more susceptible to head lice. A comparable pattern was observed in Nigeria, where the prevalence among females was 21.6%, significantly exceeding the 9.0% observed in males (Abah *et al.*,2023). The findings presented are consistent with the current research, indicating that female students consistently demonstrate higher infestation rates across various sociocultural environments. Conversely, certain studies have revealed significantly large gender disparities. For example, one investigation noted an infestation rate of 2.9% in girls compared to merely 0.6% in boys, which closely corresponds to the gender difference observed in this research, albeit to a lesser degree (Motovali *et al.*,2008). The persistence of this trend across numerous studies highlights the necessity for gender-specific prevention measures, which should

include education on personal hygiene and the discouragement of sharing hair-related items among female students. The current research identified a greater occurrence of *Pediculus humanus capitis* infestation among children from larger families, specifically those consisting of 7-14 members, in contrast to smaller families (4-6 members). This observation indicates a potential link between head lice infestation and family size. Such a correlation may arise from increased physical interactions and shared living resources within larger households. Our findings are consistent with results from various prior studies. This previous approach was corroborated by several earlier investigations, which suggested a significant connection between the number of family members and the prevalence of head lice infestation, as proposed by (Zahirnia *et al.,*2021 and Al-Zanbagi and Al-Hashdi,2025). Similarly, it is concluded that family size, along with other household factors such as sanitation and room density, significantly influences infestation rates (Hajiloie *et al.*,2021). Additional corroborative evidence is provided, who documented a 66.23% infestation rate in children from large families, suggesting a significant link between household density and the spread of parasites (Abed,2024). In the study, the most elevated infestation rate (40.13%) was similarly noted in families comprising more than six individuals (Saghafipour *et al.*,2017).

Furthermore, Alberfkani and Mero reported that families with over ten members exhibited the highest global rates of pediculosis, reaching 74.5%, thereby reinforcing the current study's assertion that overcrowded households play a crucial role in the transmission of lice. Also, validated this trend, noting increased infestation rates (1.75%) in families with more than six members when compared to those in smaller households (Ali and Hama,2018). In contrast, no statistically significant difference was found between family size and infestation, suggesting that other factors such as hygiene behavior, Parental education, and access to treatment might moderate the impact of family size (Nazari *et al*,2006 and Değerli *et al.*,2013). Moreover, a weak correlation was suggested between family size and head lice infestation, highlighting that family size by itself may not reliably predict the risk of infestation without taking into account other interacting factors (Firoozfar *et al*,2019). Although there are some discrepancies in the literature, the present study suggests that a larger family size is consistently linked to a higher incidence of lice infestation, particularly in settings where hygiene resources are scarce and physical closeness is prevalent, such as in refugee camps or low-income households. In the present study, a significant relationship was found between the frequency of showering and the occurrence of Pediculus humanus capitis among schoolchildren. Children who indicated bathing 1-3 times per week exhibited significantly higher infestation rates compared to those who bathed more often (4-7 times/week), especially in school settings (p = 0.001). This result reinforces the hypothesis that inadequate personal hygiene, particularly infrequent hair washing, heightens the risk of head lice infestation. In line with our findings, we established a significant correlation between infrequent bathing and elevated infestation rates (Moosazadeh *et al.*,2015). Likewise, in Erbil, Iraq, noted that a weekly hair washing was notably linked to a 6.7% infestation rate, whereas daily washing resulted in considerably lower rates, underscoring the protective benefits of regular hygiene practices (Al-Marjan *et al.*,2022). Furthermore, a study revealed that the prevalence of lice was 43.5% among individuals who washed their hair once a week, in contrast to a mere 0.7% among those who washed it twice a week, thereby affirming the inverse relationship between the frequency of hair washing and lice prevalence (Kassiri and Gatifi,2016). These trends further corroborate the

findings of the present study. contributed an essential environmental perspective by indicating that the presence of bathrooms had a significant impact on lice prevalence. Children residing in homes lacking bathroom facilities exhibited a considerably higher infestation rate of 78.1% compared to those living in homes equipped with bathing amenities, which had an infestation rate of 60.7%. This suggests that not only the frequency of washing but also the accessibility to hygiene infrastructure is crucial in mitigating head lice transmission, particularly in contexts of displacement or within underserved communities (Soleimani *et al.*,2017).

Additional support is provided, which identified a notable correlation between personal hygiene and head lice infestation within an Islamic boarding school. Students who engaged in more frequent hair washing exhibited significantly lower odds of infestation, thereby reinforcing the significance of hygiene practices in managing pediculosis (Khasanah *et al.*,2022). While the present study established a significant association in school settings, this relationship did not reach statistical significance in camp environments (p = 0.675). This variation may be linked to external factors present in camp settings, such as restricted access to water, bathing supplies, and general sanitation, which could diminish the effectiveness of individual hygiene efforts. The aforementioned studies underscore the contextual relevance of environmental conditions when analyzing personal hygiene behaviors. Similarly, demonstrated that educational interventions targeting parents (Najjari *et al.*,2022). A statistically significant correlation was identified between the educational attainment of mothers and the incidence of Pediculus humanus capitis in school-aged children, observed in both educational institutions (p = 0.002) and refugee camp environments (p = 0.001). Children whose mothers had attained lower educational levels demonstrated increased rates of infestation, underscoring the essential influence of parental knowledge especially that of mothers on the prevention and management of lice. This perspective is robustly corroborated by prior studies. It has been established that parents with higher educational qualifications typically exhibit enhanced awareness regarding the prevention, identification, and treatment of head lice, thereby rendering them more adept at safeguarding their children (Jahnke *et al.*,2008). There has been a notable decrease in infestation rates, from 8.4% to 3%, following the implementation of a structured awareness program. This illustrates the direct influence of parental knowledge on health outcomes, as evidenced by, the discovered that higher levels of maternal education were associated with reduced lice prevalence among primary school girls in Qom Province. The studies corroborate the current observations regarding the significance of maternal education in lice infestation prevention, likely attributable to enhanced hygiene practices, health literacy, and a heightened awareness of early infestation signs (Saghafipou *et al.,*2017). Additional support is provided by, who indicated that lower maternal education levels considerably raised the likelihood of head lice infestation, with an adjusted odds ratio of 2.46, thereby affirming a robust statistical correlation that emphasizes the impact of maternal education on both preventive actions and prompt treatment within households (Abed,2024 and Moosazadeh *et al.*,2021).

Conclusion

The findings of this study indicate that head lice infestation (*Pediculus humanus capitis*) continues to be a significant public health issue among primary school children and refugee populations in Zakho, located in the Kurdistan Region of Iraq. The overall infestation rate of 21.75% underscores the necessity for ongoing monitoring, particularly in school environments where the prevalence was notably higher. Various

socio-demographic factors including age, gender, family size, bathing frequency, and maternal education were found to be significantly linked to infestation rates, suggesting that both environmental and behavioral factors are crucial in the transmission of head lice. Additionally, molecular analysis utilizing mitochondrial gene markers (*cytb and cox1*) effectively confirmed the genetic identity of the parasite and demonstrated a high level of similarity with strains reported globally.

Declarations:

Ethical Approval: The current research received approval from the Ethics Committee at the College of Medicine, University of Zakh (UOZ33, JAN2025).

Competing Interest: The Authors declare that there are no financial or personal conflicts of interest that could have influenced the results of this research.

Availability of Data and Materials: The data used in this study are available on request from the corresponding author.

Authors Contributions: The researcher, Hanna Showkat Hadi, designed the study, collected and analyzed the data, conducted genetic analysis using MEGA software, and wrote the initial draft of the manuscript. All authors reviewed and approved the final versions for submission.

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