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RESEARCH PAPER

Epidemicity and Genotyping of Pediculus humanus capitis de Geer by Cytochrome Oxidase I gene among Syrian refugees dwelling some camps of Erbil, Kurdistan Region of Iraq

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A B S T R A C T:

The objectives of the current study are to determine risk factors associated with the rampancy and molecular genotyping of the head lice (Pediculus humanus capitis de Geer) infesting Syrian refugees in some temporary camps of Erbil, Kurdistan region of Iraq. To meet the requirements of the study, some primary schools of refugee camps were chosen to collect the relevant data in the afore-mentioned city. The V-Comb trap method was used to obtain specimens of head lice from 202 students of both gender who attend refugee camp schools regularly. The analyzed data revealed that the prevalence rates of pediculosis among 6-10 and 11-15-year-old students were 69% and 64% respectively. The highest infestation rate (79%) was recorded among female students, whilst the lowest outbreak rate (61%) was detected among male students. Regarding the impact of hair lengths on the prevalence of the insect, the highest rate of infection (81.53%) was recorded for medium hair whilst the lowest rate (41.17%) was determined for long hairs. Concerning the hair posture factor, the rates of infestation were 100%, 73% and 55% for spring, straight, and wavy hairs respectively in the 6-10-year-old group. Ultimately, Genomic DNA extraction were performed and Mitochondrion Cytochrome Oxidase I gene was used successfully for PCR amplification and sanger sequencing for genotyping then submission of our query sequences to the NCBI GenBank .

KEY WORDS: Pediculus capitis, Syrian refugees, epidemiology, molecular identification, Erbil
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1.INTRODUCTION :

Pediculosis capitis, colloquially known as head louse, is an obligate external parasite attacks individuals in nurseries, daycare centers, and primary schools particularly in under-developed countries (Willems et al., 2005). The infection of susceptible people with this common and blood-sucking insect is caused by Pediculus humanus (P.h.) capitis de Geer, which belongs to the family Pediculidae (Bonilla et al., 2013, Ashfaq et al., 2015).

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(P.h.) capitis de Geer, which belongs to the family Pediculidae (Bonilla et al., 2013, Ashfaq et al., 2015). Head lice are most rampant during economic crises, natural disasters, wars, starvation and poverty conditions in global communities. These ectoparasites are small arthropods that live on scalps where they get their meals from human blood (Mohammed, 2012). The main route of transmission of head lice is direct contact with a person who is already infested such as head-to-head contact during play at school, camps and at home. On the other hand, less common modes of transmission also occur indirectly via wearing belongings of infested people such as coats, scarves, hats and sport uniforms. Moreover, using

infested towels, combs, brushes or lying on a bed, carpet and pillow of infected individuals are also deemed modes of transmission (Al-Shawa, 2006, Nazari et al., 2006).

Pediculosis capitis can infest anyone and all socioeconomic groups are prone to infection, but children aged 3-11 years are most likely to contract the insect (Nejati et al., 2018). It is noteworthy to mention that people with curly, course hair are less exposed to infestation as compared to those with smoother hair types. Besides, females are more likely to have head lice than males; possibly they tend to have longer hair as compared to boys (Atambay et al., 2007).

The detection of infestation is possible by relying on some salient clinical symptoms such as irritation and scratching of scalp, anemia during heavy infestation, pruritis and visual examination, but exploiting other scientific articles such as molecular techniques are mandatory epidemiologically(Bahamdan et al., 1996, Borges and Mendes, 2002).

Epidemiologically, several factors associated with hosts such as; age, gender, ethnicity, socioeconomic status, and hair characteristics. Furthermore, living in densely populated areas and lice resistance to common pesticides will lead to escalating incidence of infestation (Lesshafft et al., 2013, Chunge, 1986).

Therefore, due to the paucity of studies on the prevalence of P. h. capitis in the area, this research work was carried out to investigate the relationship between rampancy of this insect and the role of some factors such as sex, age, hair posture, and hair lengths of children attending primary schools in the Syrian refugee camps. Additionally, due to the lack of molecular studies on head lice in Kurdistan, this study also aimed to support the morphological identifications and search for the potential of genetic diversities in the lice specimens through utilizing Cytochrome Oxidase I gene and then sequencing the amplified products, this can be considered the novelty of this study

2. MATERIALS AND METHODS

2.1. Study site

This study was conducted in primary schools of some camps in Erbil, the Kurdistan Region of Iraq where Syrian refugees live temporarily. Those displaced people live in a bad situation because their shelters lack simple requirements of life. The camps are suffering from poor sanitation and overcrowding. The climate is moderate in the region. Maximum and minimum temperatures have been recorded as 42C° and 3.5C° in summer and winter, respectively.

2.2. Collection of samples and visual identification of lice

After a short interview, each participant was inspected for head lice infestation by direct ectoparasitological visualizations. The head, the nape of the neck and behind the ears were inspected carefully with the naked eyes. Hairs that had at least one of the developing stages of P.h. capitis, including nit residues, were considered infested. In this study, 202 specimens including adult lice and their nits were collected using the V-Comb traps. The obtained specimens were kept in ethanol 95% until used for genotyping.

2.3. Questionnaire application

A questionnaire form was filled out for each student. The form included age, gender, school grade, family size, hair lengths, hair types, sharing combs and sharing clothes or bed.

2.4. DNA Extraction

Genomic DNA was isolated from five adults of human head lice. Each specimen was extracted by ZYMO Quick-DNA Tissue/Insect kit (Microprep Kit, USA- No. D6015). Then isolated DNA was electrophorized in 1% Agarose.

2.5. PCR amplification of Cytochrome Oxidase 1 (CO1)

According to the manufacturer's instructions, DNA samples, extracted from five head lice. PCR amplification for COI partial gene was done in 50 µl of reaction mixture containing; 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S Stenhuggervej 22), 10 picomol of forward primer LCO1490 (GGTCAACAAATCATAAAGATATTGG), 10 pmol reverse primer HCO2198(TAAACTTCAGGGTGACCAAAAAATC

A), DNase free water and template DNA by Bioresearch PTC-200 Gradient thermocycler.

The thermal cycle included an initial denaturation at 95 C for 5 min, followed by 35 cycles of denaturation at 95C for 35 seconds, a primer annealing at 58C for 35 seconds, an extension at 72C for 1 min and final step is an extra extension at 72C for 10 min.

2.6. Sequencing of DNA

The five samples of PCR product COI partial gene have been sequenced by ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Microgene Center in Korea . Chromatograms of COI gene were edited and base calls checked using Finch TV program software.

2.7. Sequence alignment and submission

The COI gene sequence were applied to Basic Local Alignment Search Tool (BLAST) is a searching tool that applies the sequence alignment method (https://blast.ncbi.nlm.nih.gov/Blast.cgi) , and is available at the National Center for Biotechnology Information (NCBI) website to comparing and alignment laboratory or query sequence with other biological sequence to find out more similarity with other targets. The sequences were aligned and trimmed using BioEdit sequence alignment editor software. The phylogenetic tree was constructed using Mega 11 Molecular Evolutionary Genetics analysis software (https://doi.org/10.1093/molbev/msab120)

2.8. Statistical analysis

The data were collected and analyzed using the statistical program; SPSS version 21 and one-way ANOVA was applied .

3. RESULTS AND DISCUSSION

3.1. Epidemiological studies

3.1.1. Relationship of age groups with infestation

The study revealed that morbidity rates as result of infection with P. h. capitis in the 6-10-year group and 11-15-year group were 69 and 64, respectively. Accordingly, the 6-10-year group was more susceptible to infestation as compared to the 11-15-year group in both sexes (Table 1).

3.1.2. Gender susceptibility to infection

The results exhibited that female student were more prone to infestation than male students in both age groups. The morbidity rates among the 6-10-year group were 79 and 45 in females and males, respectively, whilst among the 11-15-year group, the morbidity rates were 65 and 61 in females and males, respectively. There was a statistically significant difference ($P<0.05$) between female and male students for their susceptibility to contract this ectoparasite. The calculated P value was 0.048 (Table 2).

3.1.3. Association of hair lengths with severity of infestation

The impact of hair length on the intensity of infestation in both sexes is demonstrated in Table 3.

The highest rate of infestation (81.53%) was recorded for medium hair of the 6-10-year group, whilst the lowest rate (41.17%) was determined for long hair of the same group. There was a significant difference ($P<0.05$) between both groups. The calculated P value was 0.0040. Having said that no significant difference (($P>0.05$) was observed within the 10-15-year group.

3.1.4. Association of family sizes with severity of infestation

Regarding 6-10-year group, the highest morbidity rate (70%) was recorded among families had >7 members, while the lowest morbidity rate (65.38%) was recorded among households had 1-3 members. For 11-15-year group, the highest morbidity rate (71.42%) was recorded among families had >7 members, while the lowest morbidity rate (54.16%) was recorded among households had 4-5 members. A significant difference ($P<0.05$) was observed in the 6-10-year group, whilst no significant difference ($P>0.05$) was observed in the 11-15-year group. Other details are shown in Table 4.

3.1.5. Relation of hair types with severity of infestation

The highest morbidity rate (100%) was recorded among spring hair students in the 6-10-year group; however a very small number of students had spring hairs. There was a significant difference ($P <0.05$) among all hair types of students regarding

severity of infestation. Details are shown in Table 5.

Table 1. Relationship between age and human lice among some primary school in Erbil city

Age Group	Total number	Infected	Percentage %	Non- Infected	Percentage %
6-10	144	99	69	45	31
11-15	58	37	64	21	36
Total	202	136	67.32	66	32.67

Table 2. Prevalence of head lice pediculus humans capitis infestation in relation to gender among some primary schoolin Erbil city

Age	Male				Female				P value
	Infected	Percentage %	Non-Infected	Percentage %	Infected	Percentage %	Non-Infected	Percentage %	
6-10	20	45	24	55	79	79	21	21	0.048
11-15	11	61	7	39	26	65	14	35	

Table 3. Association of hair lengths and ages of Syrian refugee students with severity of Pediculus humanus capitis infestations in Erbil

Type of hair	Short hair			Medium hair			Long hair		
Age(Years)	Total	Infested	%	Total	Infested	%	Total	Infested	%
6--10	62	39	62.90%	65	53	81.53	17	7	41.17%
11--15	18	11	61.11%	26	17	65.38	14	9	64.28%

Table 4 Association of family members of Syrian refugee students with severity of Pediculus humanus capitis infestations in Erbil

Type of hair	≥7 members			4-6 Members			1-3 members		
Age(Years)	Total	Infested	%	Total	Infested	%	Total	Infested	%
6--10	40	28	70%	78	54	69.23	26	17	65.38%
11--15	21	15	71.42%	24	13	54.16	13	9	69.23%

Table 5. Association of hair types of Syrian refugee students with severity of Pediculus humanus capitis infestations in Erbil

Type of hair	Spring hair			Wavey hair			Straight hair1		
Age (Years)	Total	Infested	%	Total	Infested	%	Total	Infested	%
6--10	2	2	100%	36	20	55.55	106	77	72.64%
11--15	4	3	75%	16	11	68.75	38	23	60.52%

3.2. Molecular studies

3.2.I. Isolation of genomic DNA

Electrophoresis of genomic DNA, isolated from five adults of Pediculus humanus capitis, is demonstrated in Figure 1.

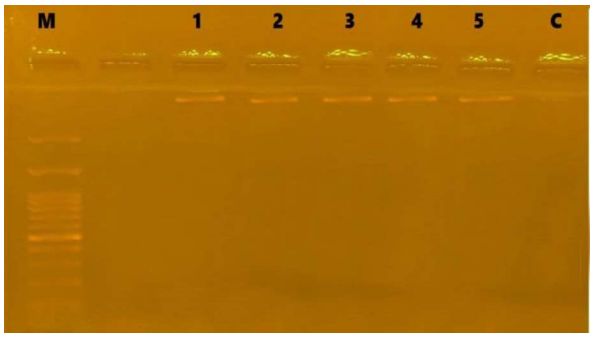

Figure 1. Agarose gel electrophoresis 1% showing genomic DNA isolated from five adults of Pediculus humanus capitis de Geer

3.2.2. PCR amplification of partial COI gene

Mitochondrion gene specific primers were designed for using the sequences of cytochrome c oxidase subunit I synthesized by Micro-gene Company (South Korea). The primers could yield a band ~700bp. The PCR product was electrophoresed and visualized by 1.5% Agarose gel (Figure 2).

3.2.3. Sequencing and phylogenetic analysis

The PCR products of the five samples, used as a source of DNA template for sequencing specific PCR amplification, are shown in Figure 3.

3.2.4 Molecular identification of genus and species of the insect

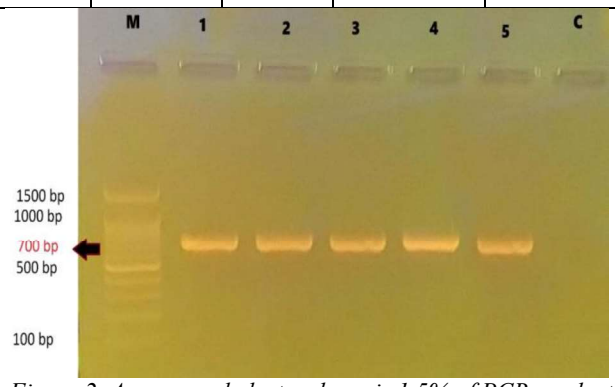

Figure 2. Agarose gel electrophoresis 1.5% of PCR product of the COI gene region of pediculus humanus capitis de Geer (Lane M: DNA Marker of 1500bp, Lane 1 to 5 are Samples, Lane C: negative control).

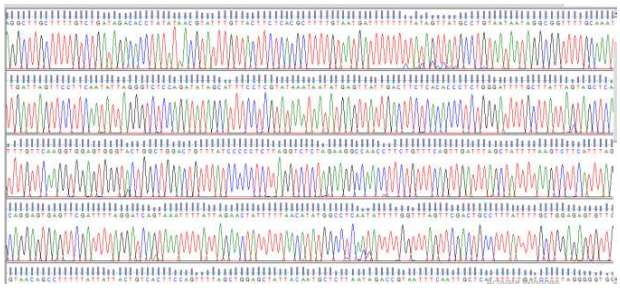

Figure 3. The partial chromatogram sequence result of cytochrome oxidase I sequence of Pediculus humanus capitis de Geer

The five COI sequence samples with sizes 690-700 were alimented by BLAST program. The GenBank (http://blast.ncbi.nlm.nih.gov/) was used to compare our amplified sequences with other stored species of lice sequences. The results obtained from the BLAST indicated that the highest identity number query sequence was 100%, whilst the lowest identity number query sequence was 99.84%. Accordingly, our insect samples were identified as P. h. capitis (Figure 4). The query sequences submitted to the NCBI GenBank and the following accession numbers

were given: MW518864, MW518865, MW518866, MW518867, and MW518868.

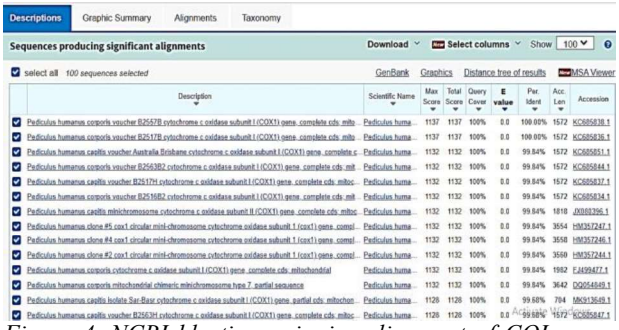

Figure 4. NCBI blasting pairwise alignment of COI query sequence of Pediculus humanus capitis de Geer

The results of the phylogenetic analysis in this study showed that the samples clustered with sequences of P. h. capities identified from other countries such as Australia/KC685851, Egypt/KJ840219, Pakistan/KJ840267 and one similar study from Basra-Iraq/MK913649). However the sequences showed divergence with other two sequences of the parasite carried out in Canada/ MG376067 and Iran/ MK908949 respectively (Figure 5).

4. Discussion

Head lice attack people of under-developed nations, particularly school-age children in Africa, South America, Asia and other continents. Depending on many associated risk factors, the global rampancy range of this ectoparasite is very wide (from 0 to 70%) (Falagas et al., 2008). The collected data from the present study revealed that the 6-10-year group is slightly more exposed to infestation with pediculus capitis as compared to the 11-15-year group. This variation in the morbidity rates may be due to unawareness of such an age about the transmission risks of the insect and less attention to personal hygiene.

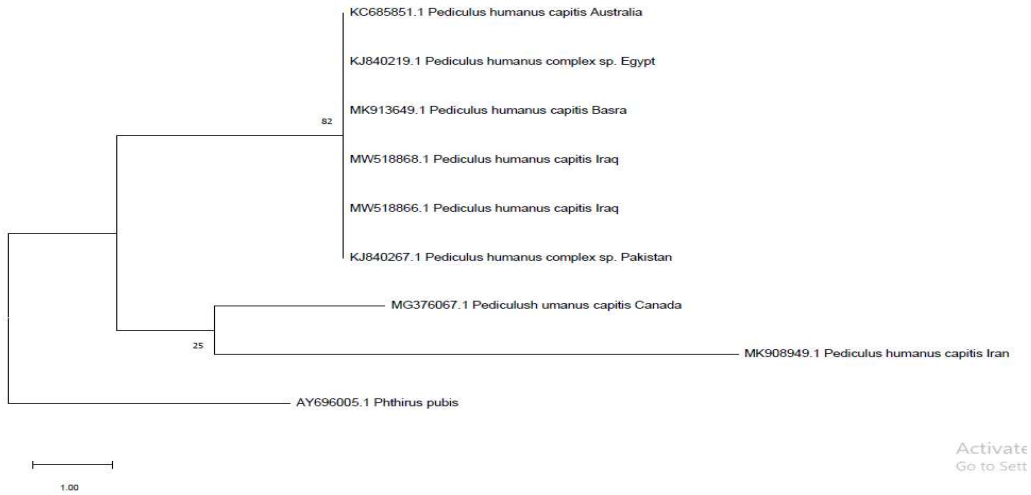

Figure 5. Phylogenetic trees for primate head lice

Moreover, children at this age sleep and play together in these eastern societies (Ziaoddini et al., 2019). Regarding the susceptibility of both sexes, it has been found that girls in both age groups are more prone to infestation as compared to boys. In this regard, (Tohit et al., 2017) have mentioned that sharing hair accessories by females may help in transmission of head lice. Furthermore, sharing personal things such as combs, towels and hairbrushes are also deemed possible routes in passive transmission of head lice from an infested girl to non-infested one (Amirkhani et al., 2011).

The present survey embraced the factor of hair lengths which may enhance the intensity of infestation among students as well. The results exhibited that the highest rate of infestation (81.53%) was recorded in the 6-10-year group with medium hair students, whilst the lowest infestation rate (41.17%) was recorded with long hair students in the same group. But pertaining to the 11-15-year group, the infestation rates of short, medium and long hair students were very close to each other, so no distinct variation observed (Table 1). Nevertheless, most studies conducted in the field of epidemiology of

pediculus capitis vary in their results. For instance, the studies of (Vahabi et al., 2012) revealed that the hair lengths do not play any role in intensity of infestaion among susceptible people. In contrast (Tohit et al., 2017) confirmed that there is a tie between hair lengths and severity of infestations.

The impact of family size was also elaborated in the current study because usually Syrian families reproduce many offspring. In the light of the results of this study, it was found that the family size plays a role in the severity of infestation. For instance, the morbidity rate of households with >7 members were 70%, while in families with 1-3 members, the rate was 65.38%, both rates recorded in the 6-10-year group. Having said, this difference was not detected in the 11-15-year group. The disparity in the severity of infestation between the two aforementioned groups may be due to the habit of sleeping kids on one bed and sharing clothes in Middle East communities (Tohit et al., 2017).

The last factor investigated in this study is the influence of hair types of students on the prevalence of pediculus capitis. The study revealed that spring hair students in both groups are more subjected to infestation as compared to wavy and straight hair students. However, the morbidity rate of spring hair students in the 6-10-year group was 100%, whilst the rate in the 11-15-year group was 75%. It is noteworthy to mention that the numbers of attending spring hair students in the camp schools are very low as compared to wavy and straight hair students (Table 3).

Our study also comprised molecular data on the head lice infesting Syrian refugees who reside in some camps in Erbil. Sequencing of COI gene confirmed that the lice identified in this study are P.h. capitis. Phylogenetic analysis showed 100% homology with lice COI genes of other studies from GenBank which have been carried out in different countries such as Australia, Egypt, Iraq, Pakistan and Canada. This could be explained as geographical factors and natural selections have not had much impact on genetic diversity of the parasite and hence made it cosmopolitan with a highly conservative COI gene. To the best of our knowledge, this is the first study of its kind in our region, which investigates the mitochondrion CO1 gene for genotyping P. h. capitis.

5. CONCLUSIONS

In the light of the collected data and their analysis, we have concluded that pediculosis capitis is highly prevalent among Syrian refugee students who attend the camp schools in Erbil. Young children were more susceptible to head lice as compared to adolescents and girls were more exposed to infestation than boys. The influence of hair lengths on infestation was insignificant, whilst the effect of family size was detected. The impact of hair posture was significant in both young children and adolescents. Regarding genotyping of the ectoparasite, the mitochondrion CO1 gene was utilized successfully.

Acknowledgements

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Conflict of Interest (1)

The author attests that there is no competing interest concerning the contents of this research article.

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