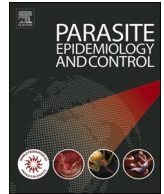




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Pyrethroid-linked resistance allelic mutations by molecular analysis in wild human head louse (Phthiraptera: Pediculidae) populations from schoolgirls of South Iran

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ABSTRACT

Background: Human head louse, *Pediculus humanus capitis* De Geer, 1767 (Anoplura: Pediculidae), is one of the most frequent ectoparasites infesting *Homo sapiens* worldwide. Reduced sensitivity to treatment due to genetic mutations, in particular knockdown resistance (*kdr*) (or target site insensitivity) allele, has led to this infestation prevalence. Molecular characterization of this resistance has a crucial impact on selecting appropriate treatment protocol. The aim of this study was to investigate *kdr* gene mutations on voltage-sensitive sodium channel (VSSC) among wild head lice samples from Fars province, southern Iran.

Methods: Head lice were collected using plastic detection combs on girls enrolled in public schools from 10 counties in Fars province. The specimens were screened in 10 pools (each pool per county containing 35 specimens), with three pools (30%) being positive. Following species identification with valid entomological keys, 350 (68%) out of 514 randomly collected adult head lice were analyzed after their somatic genomic DNA extraction using Sinaclon kit. Samples were investigated by polymerase chain reactions (PCR), and the amplicon was subsequently sequenced.

Results: Sequence analysis showed that the sodium channel genes in the pooled ectoparasites had two intron and three exon regions. Single (L840F), double (I836L, E837K), and triple novel point mutations (V875L, Q876P, S879V); the last involving two concomitant allelic substitutions; were discovered in the second and third exon regions of head louse DNA on chromosome II from three (30%) counties. Other exon or intron regions remained non-mutated from the remaining seven counties.

Conclusions: The detection of six amino acid substitutions from 30% of examined head lice among infested schoolgirls reveal that mutants are minutely developing. These findings provide further incentive to recapitulate the legitimacy of current control measures and resolve dynamics of resistance in human head louse populations.

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1. Background

Human head louse infestation, known as head pediculosis, due to the species of *Pediculus humanus capitis* De Geer, 1767; (Anoplura: Pediculidae) is one of the irritating public health problems in most regions, including high income- and middle- to low-income countries. Although all age groups are susceptible to this infestation, it is primarily present among schoolgirls globally (Candy et al., 2018). This infestation becomes a significant health concern while its frequency rises since interventions to suppress and mitigate these ectoparasites pose even more significant risks to susceptible human populations than the infestation itself (Pollack et al., 2000).

The head lice, *P. h. capitis*, feed exclusively by piercing and sucking on blood from sub-epidermal capillaries of the head with sharp proboscis. They are highly host-specific and spend their entire metabolic life history on humans. Their predominant transmission route is through direct contacts, particularly among 4-13 years old children (Moradiasl et al., 2018). Although this infestation does not categorically lead to specific pathogenic infections, the bite reactions from head lice cause intense pruritus that may lead to secondary microbial wound infections (Veracx and Raoult, 2012). The World Health Organization (WHO) has recently added “scabies and other ectoparasites” (including head lice) to the priority list of neglected tropical diseases (Casulli, 2021).

While the prevalence of head louse infestations varies across regions, a recent meta-analysis-based systematic review has determined that the global rate of these ectoparasites is about 19% (Hatam-Nahavandi et al., 2020). In addition, whereas all age groups are susceptible to infestation, head lice primarily affect children and adolescents, with particular dominance in girls (Veracx and Raoult, 2012; Al-Shahrani et al., 2017; Liao et al., 2019). Its prevalence rate among infested local schoolchildren in Iran is 7.4–10.5% (Nejati et al., 2018; Moosazadeh et al., 2015). These rates express the scope and variability of this menace.

A range of interventions is available to control head lice as they are not invulnerable to physical or chemical practices. The mainstay of therapy has explicitly been insecticides (Moosazadeh et al., 2015). *P. h. capitis* control depends primarily on the topical application of pharmaceutically based products. These are usually available as over-the-counter (OTC) medicines that may have different classes of insecticides, in particular pyrethroids. Large-scale application of these OTC products is inevitable since the infestation is widely spread worldwide. Consequently, increasing therapeutic resistance in different head lice populations is widely reported (Downs et al., 2002; Hunter and Barker, 2003; Kristensen et al., 2006; Moemenbellah-Fard et al., 2016; Kalari et al., 2019).

Treatment with the pyrethrins- and pyrethroid-based products has led to a high degree of resistance even though organochlorine insensitivities were effectively operative (Mohammadi et al., 2021). Subsequently, it is essential to use efficient drugs with different active ingredients to treat this infestation. Pyrethroids, such as permethrin, attack the site of voltage-sensitive sodium channels (VSSC) on the nodes of Ranvier along the nerve axon. This action causes long opening of the gates and extensive influx of sodium ions which results in uninterrupted depolarization (reduction or reversal of the potential difference across the nerve cell membrane at rest) and muscle seizure, and finally, a reversible sudden “death-like effect” of head lice (Hemingway et al., 1999). Excessive use of permethrin induces through direct epigenetic mechanism single-base point mutations in the VSSC coding gene subunits, which leads to reduced neuronal sensitivity in the head louse (Firoozian et al., 2017).

Table 1

The allelic and amino acid substitutions tabulated for each head lice pool geographical sampling area alongside their abundance in each case. The mutated alleles are in color.

County	Coordinates	Number of head lice collected	Adult male head lice	Adult female head lice	Number of homogenized samples	Sample weight (mg)	Mutations	Amino acid substitution	Nucleotide substitutions
Shiraz	29°36'36"N, 52°32'33"E.	62	25	37	35	20	L840F	Leucine-Phenylalanine	CTT/TTT
Marvdasht	29°87'87"N, 52°82'06"E.	89	51	38	35	22	V875L, Q876P, S879V.	Valine-Leucine, Glutamate-Proline, Serine-Valine	GTC/CTC CAG/CCG TCG/GTG
Fasa	28°94'40"N, 53°63'39"E.	41	24	17	35	18			
Larestan	27°67'41"N, 54°33'58"E.	47	16	31	35	24			
Kazeroun	29°62'71"N, 51°65'18"E.	46	13	33	35	19			
Abadeh	31°15'95"N, 52°64'40"E.	40	16	24	35	22			
Firoozabad	28°50'38"N, 52°34'15"E.	60	31	29	35	23			
Zarindasht	28°20'00"N, 54°20'00"E.	39	18	21	35	20			
Noorabad	34°06'65"N, 47°97'63"E.	37	17	20	35	21	I836L, E837K.	Isoleucine-Leucine, Glutamic acid-Lysine	ATT/CTT GAA/AAA
Sepidan	30°10'00"N, 52°00'00"E.	53	23	30	35	22			

Knockdown resistance (*kdr*) gene mutation is the most typical resistance mechanism to insecticides through target site insensitivity of the VSSC α -subunit gene (Mohammad Rezaei et al., 2019), where single-base mutations are used as biomarkers. Since *kdr* mutation could be closely associated with the lack of proper response to pyrethroids, its existence as a mechanism in the field has grave consequences for sustained use of these chemicals in head lice control. There is an essential need to monitor these mutations through geographically-specific genetic biomarkers due to increasing failures to first-line treatment (Fox et al., 2020). This failure took place since allele mutations related to pyrethroid resistance differ between regions. This study aimed to explore permethrin-associated *kdr* gene mutations through molecular analysis of human head lice in schoolgirls in south Iran.

2. Materials and methods

2.1. Sample collection

P. h. capitis specimens were collected from primary schools in ten different counties which included Shiraz, Zarindasht, Sepidan, Firoozabad, Fasa, Kazeroon, Larestan, Noorabad, Marvdasht, and Abadeh (Table 1, Fig. 1). The specimens were screened in 10 pools with three pools (30%) being positive. As previously reported, standard plastic detection combs (PDC) were used (Kalari et al., 2019). *P. h. capitis* specimens were examined for morphological characteristics using a stereomicroscope according to a valid diagnostic key for the adult stage (Control CfD, Prevention, 2003). Specimens were collected in accord with their accessibility. The Directorate of Shiraz University of Medical Sciences (SUMS), School of Health, Ethics Committees, confirmed the head louse collection protocol under the IR license.SUMS.SCHEANUT.REC.1400.011. The parent or guardian of each louse-provider gave written informed consent. The collected *P. h. capitis* specimens were taken to the laboratory and preserved in 70% ethanol preceding identification and molecular analysis.

2.2. Primer design

The partial genome of *kdr* gene in *P. h. capitis*, sequenced by other researchers, was retrieved from the National Center for Biotechnology Information (NCBI) database. First, the DNA sequences of VSSC genes, including AY191157.1, KX302005.1, KX301992.1, KX301991.1, and KX301988.1, were aligned using the MEGA6.0 software (Version 6.0). Following analysis, two regions



Fig. 1. Map of Fars province within Iran shows the counties (enlarged map on the left) where samples were collected.

were chosen to design gene-specific primers (GSPs) (Alipour et al., 2017). Two forward and reverse primers, including HLF (GAATTTGTGGCCTTACTTGTATTCCGA) and HLR (TAAATTACCCAAAGCTCCAACAGTTC), were allocated as start and end primers, respectively, to identify the partial sequence of the target gene. The expected size amplicon was 582 bp. This study implemented Gene Runner 0.04, Oligo 0.7, and BLAST (online tool) to design primers on the exon regions (Ebrahimi et al., 2021a).

2.3. DNA extraction

Randomly selected head lice were arranged into 10 pools, each pool of 35 specimens per county, transferred to sterile micro-tubes, and subjected to DNA extraction and molecular analysis. Each sample was homogenized with a tissue homogenizer (Thomas Scientific, United States) at 12000 g for 600 s. DNA extraction was conducted using the commercial kit (Sinaclon Co., Sina Gene, Tehran, Iran) following the manufacturer’s instructions and stored at –20 °C.

2.4. Polymerase Chain Reaction (PCR) assay

The PCR assay was conducted on 350 out of 514 (68.1%) head lice specimens obtained from the surveyed counties. The total volume of PCR mixtures was 20 µl and included 10 µl of Master Mix Red, 7 µl double distilled water (2H₂O), 1 µl of 10 pmol/µl of each primer, and 1 µl of DNA template (100–200 ng/µl). All PCR components were purchased from Sinaclon Company, Iran. Negative control was run in each trial. PCR program was as follows: 5 min at 94 °C as initial denaturation, and 35 cycles of 30 s each at 95 °C, 60 °C, and 72 °C, with a terminal extension for 10 min at 72 °C (Alipour et al., 2017). All PCR constituents were exposed to agarose gel electrophoresis in 0.5× Tris-Acetate EDTA buffer. The amplified fragments were seen after staining using the DNA Safe stain (Sinaclon Co.) with UV light Gel Documentation systems (Bio-Equip, UK).

2.5. DNA Extraction from agarose gel

According to the manufacturer’s protocols, the expected size bands were recovered from the agarose gel using the GF1 gel extraction kit (Vivantis, Malaysia). Then, sequencing in both directions was performed according to the forward and reverse GSP primers obtained from Pishgam Company in Iran.

2.6. Sequencing

One PCR production sample from each population (#10) with sizes close to the predicted range (582 bp) was sequenced using the GSPs forward (HLF) and reverse (HLR) primers. Then, sequencing in both directions was performed according to the forward and reverse GSP primers obtained from Pishgam Company in Iran. EditMan and SeqMan (Version 7.10, 2006) then performed their analyses by assembling. The resulting nucleic acid sequence was aligned with ClustalW (MEGA 6) then the sequences were BLAST in NCBI. Finally, the samples were characterized in accord with the saved sequences in GenBank. Using Sanger sequencing, the desired region was sequenced by Pishgam company/ Iran in the present study.

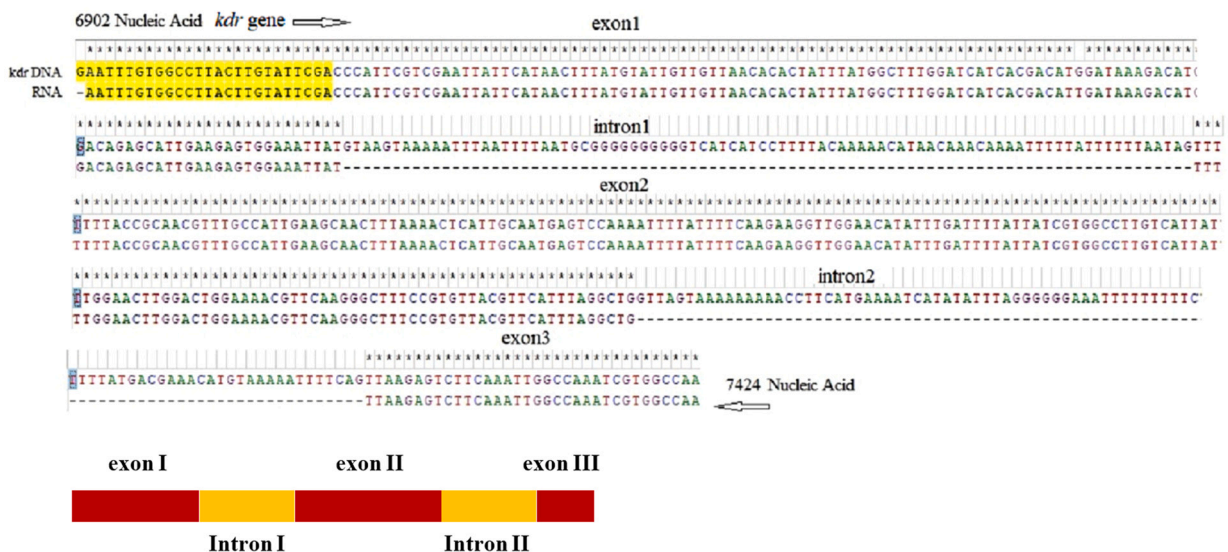


Fig. 2. The NCBI-derived *kdr* allelic DNA and RNA coding sequences of 582 bp amplicon (above) contain two introns and three exons (below).

and recently in northwest Iran (Firoozian et al., 2017). In the last report, researchers likewise identified six novel mutations in the head louse's VSSC α -subunit gene of the nerve axon. These findings revealed that resistance-related alleles operated through *kdr* gene mechanisms with most recorded haplotypes of RS heterozygotes (Firoozian et al., 2017).

Head pediculosis is indeed a neglected disease both at the national and individual family level in Iran, where several other infectious diseases could concomitantly occur in high-risk groups of people (Fakoorziba et al., 2006; Azizi et al., 2016; Neghab et al., 2014; Neghab et al., 2006). Its resistance patterns to pyrethroid-based medications differ geographically [25] since its developmental process has many individual steps, at each of which interaction may occur between the environment and the genotype or between various elements of the louse genotype. In a recent systematic review and meta-analysis, the *kdr* gene frequencies of only about a third of human head louse populations were susceptible to the pyrethroid-based OTC products in different countries of the world (Mohammadi et al., 2021). The remaining two-thirds were, however, resistant to the treatments. Most insensitivity to pyrethrin- and pyrethroid-based pediculicides (and to a lesser extent to the organochlorine DDT) was due to the *kdr*-type allelic mutations in the VSSC α -subunit target receptor-coding gene of head lice. Moreover, this resistance phenomenon is a reversible event following recessive allele mutations at one or more gene loci due to persistent and uncontrolled exposure to DDT, a pyrethroid, or both, at some point in their humanoid-associated co-evolutionary history.

These recessive resistance alleles appear to be rare in intervention-free populations of the head louse. It is, therefore, conceivable that increased abundance of diagnosis and treatment would precede more severe selection pressure on pediculicide reduced sensitivity, causing heterozygotes to propagate first through gene flow, while reversion of head louse target receptor coding genes to fully-sensitive allele status against a particular pediculicide medication could originate from its disuse during the long evolutionary history (Mohammadi et al., 2021).

Six new gene mutations located in the IIS5 (I927F, L928A, R929V, L930M, and L932M) and IIS1–2 (H813P) of the α -subunit of VSSC of the nerve axon have been reported from northwest Iran. These findings revealed resistance-linked alleles performed through *kdr* gene mutation mechanisms, with most haplotypes recorded as RS heterozygotes (Firoozian et al., 2017). Another recent study in Turkey reported three amino acid mutation sites at L920F, M815I, and T917I, all identified using real-time PCR (Karakuş et al., 2020). Of the studied specimens, resistance allele frequency (RAF) was 0.99 for L920F, 1.00 for M815I, and 0.98 for T917I. The presence of *kdr*-related new gene mutations in the sodium channel was possibly due to frequent treatment failures against head lice based on the uncontrolled use of permethrin insecticides (Firoozian et al., 2017). It suggests that failures commonly occurring in these countries may be due to the permethrin resistance observed in these populations (Ghahvechi Khaligh et al., 2021). However, one cannot state that a specific gene mutation determines a particular character or *vice versa*.

The strength of this study was that a sufficient sample size (350) was collected from 10 climatically and topographically diverse localities in Fars province, leading to the attainment of three pools with possibly permethrin-linked mutant head lice. The salient outcome of getting triple mutations from wild head louse specimens collected in the county of Marvdasht points unprecedentedly to the likely severity of this disease in this corner of the world.

5. Limitations

One of the fundamental limitations in this research study was that this work was not set to discriminate overall genotype as outlined in other studies since homozygous or heterozygous states could be identified using RFLP-PCR or ARMS-PCR, which were not implemented due to a predefined proposal. Using pools of 35 specimens per county was another hurdle in this work which was imposed due to the minimum sample collection size of 37 head lice from one county. The existence of SARS-CoV-2 (Covid-19) pandemic added another hurdle to our research progress.

6. Conclusions

Since the triple mutations exist *en bloc* as a permethrin-resistant haplotype, the two adjacent novel mutations (V875L and Q876P) may function to compensate for any fitness disadvantage associated with the validated T917I or T929I substitutions in permethrin-resistant human head lice from other reports. In contrast to the other seven counties in the Fars province of Iran, the detection of six amino acid mutations in human head lice in Shiraz, Noorabad, and Marvdasht could point to the region-specific treatment failures from the constant use of permethrin in recent years. Regional health executives should thus be warned. They are advised to monitor the increasing frequency of allele mutations before any resistance trend changes into the fixation state, which will be very hard to eradicate in vulnerable hot spot regions.

Declaration of Competing Interest

Authors declare that they have no conflict of interests.

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References

- Alipour, H., Raz, A., Zakeri, S., Djadid, N.D., 2017. Molecular characterization of matrix metalloproteinase-1 (MMP-1) in *Lucilia sericata* larvae for potential therapeutic applications. *Electron. J. Biotechnol.* 29, 47–56.
- Al-Shahrani, S.A., Alajmi, R.A., Ayaad, T.H., Al-Shahrani, M.A., Shaurub, E.-S.H., 2017. Genetic diversity of the human head lice, *Pediculus humanus capitis*, among primary school girls in Saudi Arabia, with reference to their prevalence. *Parasitol. Res.* 116 (10), 2637–2643.
- Azizi, K., Shahidi-Hakak, F., Asgari, Q., Hatam, G.R., Fakoorziba, M.R., Miri, R., et al., 2016. In vitro efficacy of ethanolic extract of *Artemisia absinthium* (Asteraceae) against *Leishmania major* L. using cell sensitivity and flow cytometry assays. *J. Parasit. Dis.* 40 (3), 735–740.
- Candy, K., Nicolas, P., Andriantsoanirina, V., Izri, A., Durand, R., 2018. In vitro efficacy of five essential oils against *Pediculus humanus capitis*. *Parasitol. Res.* 117 (2), 603–609.
- Casulli, A., 2021. New global targets for NTDs in the WHO roadmap 2021–2030. Public Library of Science San Francisco, CA USA.
- Control CfD, PPrevention, 2003. Pictorial keys to arthropods, reptiles, birds, and mammals of public health significance: Department of Health & human services. Centers for Disease Control and ..., 192 p.
- Downs, A., Stafford, K., Stewart, G., Coles, G., 2000. Factors that may be influencing the prevalence of head lice in British school children. *Pediatr. Dermatol.* 17 (1), 72–74.
- Downs, A., Stafford, K., Hunt, L., Ravenscroft, J., Coles, G., 2002. Widespread insecticide resistance in head lice to the over-the-counter pediculocides in England, and the emergence of carbaryl resistance: therapeutics. *Br. J. Dermatol.* 146 (1), 88–93.
- Ebrahimi, S., Alipour, H., Azizi, K., Kalantari, M., 2021a. Construction of PX-LmGP63 using CRISPR-Cas9 as primary goal for GP63 gene knockout in *Leishmania major* and *Leishmanization*. *Jundishapur Journal of Microbiology.* 14 (1), 1–7.
- Ebrahimi, S., Shahriari-Namadi, M., Shahabi, S., Moemenbellah-Fard, M.D., Alipour, H., 2021b. Permethrin-associated kdr mutations through molecular analysis of human head lice (Phthiraptera: Pediculidae) populations in school children in the south of Iran. *Journal of Health Sciences & Surveillance System.* 9 (4), 257–264.
- Fakoorziba, M., Neghab, M., Alipour, H., Moemenbellah-Fard, M., 2006. Tick-borne Crimean-Congo haemorrhagic fever in Fars province, southern Iran: epidemiological characteristics and vector surveillance. *Pak. J. Biol. Sci.* 9 (14), 2681–2684.
- Firoozian, S., Sadaghianifar, A., Taghilou, B., Galavani, H., Ghaffari, E., Gholizadeh, S., 2017. Identification of novel voltage-gated sodium channel mutations in human head and body lice (Phthiraptera: Pediculidae). *J. Med. Entomol.* 54 (5), 1337–1343.
- Fox, K., Larkin, K., Sanchez, A., 2020. Global trends in genetic markers of *Pediculus humanus capitis* resistance mechanisms. *Current Tropical Medicine Reports.* 7, 65–73.
- Ghahvechi Khaligh, F., Djadid, N.D., Farmani, M., Asadi Saatlou, Z., Firoozian, S., Abedi Astaneh, F., et al., 2021. Molecular monitoring of knockdown resistance in head louse (Phthiraptera: Pediculidae) populations in Iran. *J. Med. Entomol.* 58 (6), 2321–2329.
- Hatam-Nahavandi, K., Ahmadpour, E., Pashazadeh, F., Dezhkam, A., Zarean, M., Rafiei-Sefiddashti, R., et al., 2020. Pediculosis capitis among school-age students worldwide as an emerging public health concern: a systematic review and meta-analysis of past five decades. *Parasitol. Res.* 119 (10), 3125–3143.
- Hemingway, J., Miller, J., Mumcuoglu, K., 1999. Pyrethroid resistance mechanisms in the head louse *Pediculus capitis* from Israel: implications for control. *Med. Vet. Entomol.* 13 (1), 89–96.
- Hunter, J., Barker, S., 2003. Susceptibility of head lice (*Pediculus humanus capitis*) to pediculicides in Australia. *Parasitol. Res.* 90 (6), 476–478.
- Kalari, H., Soltani, A., Azizi, K., Faramarzi, H., Moemenbellah-Fard, M.D., 2019. Comparative efficacy of three pediculicides to treat head lice infestation in primary school girls: a randomised controlled assessor blind trial in rural Iran. *BMC Dermatol.* 19 (1), 13.
- Karakuş, M., Atıcı, T., Karabela, Ş.N., Baylan, O., Limoncu, M.E., Balcıoğlu, İ.C., 2020. Detection of permethrin resistance and phylogenetic clustering of Turkish head lice (*Pediculus humanus capitis*); De Geer, 1767 populations. *Acta Trop.* 204, 105362.
- Kristensen, M., Knorr, M., Rasmussen, A.-M., Jespersen, J.B., 2006. Survey of permethrin and malathion resistance in human head lice populations from Denmark. *J. Med. Entomol.* 43 (3), 533–538.
- Liao, C.-W., Cheng, P.-C., Chuang, T.-W., Chiu, K.-C., Chiang, I.-C., Kuo, J.-H., et al., 2019. Prevalence of *Pediculus capitis* in schoolchildren in Battambang, Cambodia. *J. Microbiol. Immunol. Infect.* 52 (4), 585–591.
- Moemenbellah-Fard, M.D., Nasiri, Z., Azizi, K., Fakoorziba, M.R., 2016. Head lice treatment with two interventions: pediculosis capitis profile in female schoolchildren of a rural setting in the south of Iran. *Annals of Tropical Medicine and Public Health.* 9 (4), 245.
- Mohammad Rezaei, F., Hashemzadeh, S., Ravanbakhsh Gavgani, R., Hosseinpour Feizi, M., Pouladi, N., Samadi Kafil, H., et al., 2019. Dysregulated KDR and FLT1 gene expression in colorectal cancer patients. *Reports of Biochemistry and Molecular Biology.* 8 (3), 244–252.
- Mohammadi, J., Azizi, K., Alipour, H., Kalantari, M., Bagheri, M., Shahriari-Namadi, M., et al., 2021. Frequency of pyrethroid resistance in human head louse treatment: systematic review and meta-analysis. *Parasite.* 28, 86.
- Moosazadeh, M., Afshari, M., Keianian, H., Nezammahalleh, A., Enayati, A.A., 2015. Prevalence of head lice infestation and its associated factors among primary school students in Iran: a systematic review and meta-analysis. *Osong public health and research perspectives.* 6 (6), 346–356.
- Moradiasl, E., Habibzadeh, S., Rafinejad, J., Abazari, M., Ahari, S.S., Saghafipour, A., et al., 2018. Risk factors associated with head lice (pediculosis) infestation among elementary school students in Meshkinshahr county, north west of Iran. *Int. J. Pediatr.* 6 (3), 7383–7392.
- Neghab, M., Moosavi, S., Moemenbellah-Fard, M., 2006. Prevalence of intestinal parasitic infections among catering staff of students canteens at Shiraz, southern Iran. *Pak. J. Biol. Sci.* 9 (14), 2699–2703.
- Neghab, M., Moemenbellah-Fard, M.D., Naziaghdam, R., Salahshour, N., Kazemi, M., Alipour, H., 2014. The effects of exposure to pesticides on the fecundity status of farm workers resident in a rural region of Fars province, southern Iran. *Asian Pac. J. Trop. Biomed.* 4 (4), 324–328.
- Nejati, J., Keyhani, A., Kareshk, A.T., Mahmoudvand, H., Saghafipour, A., Khoraminasab, M., et al., 2018. Prevalence and risk factors of pediculosis in primary school children in south west of Iran. *Iran. J. Public Health* 47 (12), 1923.
- Pollack, R.J., Kiszewski, A.E., Spielman, A., 2000. Overdiagnosis and consequent mismanagement of head louse infestations in North America. *Pediatr. Infect. Dis. J.* 19 (8), 689–694.
- Veracx, A., Raoult, D., 2012. Biology and genetics of human head and body lice. *Trends Parasitol.* 28 (12), 563–571.
- Yoon, K.S., Previte, D.J., Hodgdon, H.E., Poole, B.C., Ho Kwon, D., Abo El-Ghar, G.E., et al., 2014. Knockdown resistance allele frequencies in north American head louse (*Anoplura: Pediculidae*) populations. *J. Med. Entomol.* 51 (2), 450–457.