Determination and Quantification of Some Organochlorines, Organophosphorus, Carbamates and Pyrethroids Pesticide Residues in Fresh and Frozen Fish in Jordan

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RESEARCH ARTICLE

Abstract

Contamination of aquatic systems with pesticides occurs frequently. This study aimed to detect the levels of some pesticides: organochlorines, organophosphorus, pyrethroids, and carbamates groups in imported frozen and local fresh fish in Jordan. A total of 218 samples were randomly collected (194 imported frozen fish). Samples were analyzed using Liquid Chromatography with Tandem Mass Spectrometry (LC/Ms/Ms). Results showed that 23 different pesticides were found in this study. All 218 fish samples were contaminated with at least 4 compounds and maximally 12 pesticides. In terms of contamination rate, no significant differences between local and imported types were observed. Up to 65.2% of pesticides were detected at concentration higher than the maximum residue limit set by the WHO (10 ppb) in 215 samples (98.6%). These samples concluded heavy contaminations with different pesticides for either imported or locally raised products. Therefore, routine monitoring of pesticides in fish and their environment are officially required.

Keywords: Fresh fish; frozen fish; Jordan; pesticides residues.

INTRODUCTION

Fish is one of the most important source of nutrients as they provide exceptional proteins, vitamins, fat, minerals, and omega fatty acids (Hellberg et al., 2012). However, fish can accumulate pesticides in viscera and flesh from water and via intake of contaminated food. The consumption of pesticides-contaminated fish may pose a crucial threat for human health (Sabra and Mehana, 2015). The capability of fish to metabolize contaminants is moderate, therefore, the contamination in fish is considered as a reflective of the aquatic environment and the state of pollution in surroundings (Stahl et al., 2009). Pesticides are a substance used for the control of insects or pests and other organisms hazardous to economically important plants or animals. Pesticides are delivered to their targets by many methods, such as spraying on the crops and injection into the soil as solids. However, runoff and flash flood transport pesticides into aquatic environments (Willis and McDowell, 1982). The method of bioaccumulation starts once pesticides are applied to the agricultural land which may be eroded into rivers, streams and lately to the oceans where these compounds can remain...
stable for different periods (Poudel et al., 2020). Climate change has a critical impact on the chemical degradation, bioavailability and toxicity of marine life. For instance, increasing water temperatures are shown to extend the toxicity of ordinarily used chemicals (NCOS, 2013). Pesticides may be classified according to their chemical structure as organochlorines, organophosphates, carbamates, and pyrethroids. A few insecticides are extraordinarily poisonous and their toxicity depends on the time that the animals or humans are in direct or indirect exposure (Helfrich et al., 2009). According to International Health Corporation, about 3,000,000 cases of pesticide poisoning and 220,000 deaths occur annually in developing countries (Poudel et al., 2020). Approximately 2.2 million people, especially in developing nations, are at great risk of exposure to insecticides (Hicks, 2013).

Organochlorine pesticides such as aldrin, endrin, dieldrin, heptachlor, and HCB have been banned in most countries. However, organochlorine pesticides are ubiquitous environmental pollutants in the global ecosystem. Such wide occurrence is due to their lipophilicity and persistency as they accumulate along the food chain (Voldner and Li, 1995). Endosulfan has been used on a range of crops and for the management of tsetse fly flies as well as ectoparasites of bovine. Endosulfan exists as two isomers: α- and β-endosulfan, and both are extremely poisonous to fish. Endosulfan sulfate, a breakdown product, is characterized by persistency and poisonous properties more than endosulfan isomers (Nishad, 2006). Endosulfan has been detected in the atmosphere, sediments and water. It was also found in freshwater fish and in marine organisms like shrimp, crabs, and mammals (Navarrete-Rodriguez et al., 2015).

Organochlorine pesticides were determined in seawater, surface water and deep sediment samples from the Gulf of Aqaba, Jordan. Dichlorodiphenyltrichloroethane and its metabolites, HCH, aldrin, heptachlor, and hexachlorobenzene were determined. The samples showed low concentrations of the investigated organochlorine pesticides with a mean of 0.55, 3.77, 4.91, 3.28, 4.67, and 3.27 ppb in seawater, and beach sediments, respectively (Alawi et al., 2017). Therefore, this study aims to determine the level of organochlorines, organophosphorus, carbamates and pyrethroid pesticide residues in nearby freshwater fish and imported frozen fish in Jordan.

MATERIALS AND METHODS

Standards and Reagents

The pesticide standards used in this work were; 10 organochlorines (endosulfan, heptachlor, dieldrin, methoxychlor, lindane, hexachlorobenzene, o’p-DDE, chlordane, heptachlor epoxide, and aldrin), 11 organophosphates (malathion, parathion, methyl parathion, chlorpyrifos, diazenon, dichlorovos, azinphos methyl, azamethiphos, profenofos, fenitrothion, and imidan), 5 carbamates (carbofuran, carbaryl, aldicarb, fenoxycarb, and methomyl), and 4 pyrethroids (deltamethrin, cypermethrin, bifenthrin, and permethrin). These standard materials were purchased from Accu standard (USA), Ref: S-77501-01/S-77501-02, at concentration of 100 µg/ml solvent of Acetonitrile 100%.

MgSO₄ was obtained from Sigma-Aldrich (Steinheim, Germany), NaCl (99%) from fisher scientific, UK. Magnesium sulfate, anhydrous, USP, and n-2 amino ethyl bonded particle, silica type; irregular particle 40-63 micron, 60 angstrom pores, lot number;716111-FXCUPSA were obtained from UCT, (USA), (ECMAG00D), lot number: 717111-BC. Acetic acid, ACS grade, Ethyl acetate, HPLC grade, Methanol, HPLC grade, ammonium acetate, and formic acid, ACS grade were purchased from Fisher Scientific, UK.

Sample collection

Studies on local fishes on Jordan or neighboring areas showed almost 50% positive of residues incidences (Alawi et al., 1995). Thus, to calculate the samples number to be tested, a prevalence rate of 50% was assumed and according the following statistical formula:

\[
N = \frac{z^2pq}{d^2}
\]

(Lwanga and Lemeshow, 1991)

where, N is the sample size, p is the probability (0.5), q is the expected contamination (1-p), z is the standard deviation (1.96) at 95% CI, and d is the accuracy given at 0.05 significance (0.07). Therefore, N = 195 samples.

A total of 218 samples representing different 26 fish species were collected randomly from different regions according to harvest areas (Atlantic, Pacific, Indian oceans) that represented eight geographical FAO zones (Supplementary file, table 1). Local fish samples (N = 24) were collected from Gulf of Aqaba and Alaghoar regions (Jordan Valley). Imported frozen 194 fish samples were collected and kept frozen and to be examined within the recommended shelf life whereas the local fish samples were kept under cool condition and examined within five days to one week of capturing time.

Sample Preparation

Samples preparations were done according to the protocol “Screening for Pesticides by LC/MS/MS” as per
A weight of 200–2000 gm. of muscle tissues were minced with ice by a food blender. The resulting sample homogenate was kept in a closed sample cups and kept at -70 °C.

Sample Extraction
Sample extraction was carried out according to the method of United States Department of Agriculture Food Safety and Inspection Service, (OPHS, 2018) with some modifications. Briefly, twenty grams of already prepared samples were transferred to 50 ml polypropylene tubes containing 100 µL (0.1 ml) of internal standard in addition to 36 ml of ethyl acetate. The mixture was vortexted for 3 minutes to remove any possible precipitation, then followed by 10 minutes of centrifugation at 3500 rpm. A volume of 20 ml of supernatant were filtered by 0.2 µm filter then transferred into 50 ml polypropylene centrifuge tube stored at -10 °C for 30 minutes. After removal of frozen lipids by filtration, 2 gm of NaCl with 8 g MgSO₄, as a drying agent, were added and shook for 1 minute. The mixture was transferred to glass tubes and vortexted for 5 minutes. Then 1 ml of Methanol into each tube with 50 mg of PSA (primary secondary amine) as a sorbent for reducing matrices (pigments and sterols) and with 8 g MgSO₄ were evaporated at 45 °C up to elute the suspended remaining fat. One ml of the mixture was placed into an Eppendorf centrifuge tube and centrifugation for 3 minutes (11000 rpm). A 200 µl are then placed into vial and this sample will be ready to use for LC/MS / MS.

Pesticides Column
Pesticides column from Phenomenex (Torrance, CA, USA) - E-816240

Mobile Phase
LC/MS/MS Mobile Phase A:
5 ml (1M CHCONH) + 1 liter distilled water + 1 ml formic acid.

LC/MS/MS Mobile Phase B:
1 ml of formic acid / 1 liter Methanol.

Pesticides quantitation
Pesticides identification and concentration were analyzed using Liquid chromatography with tandem mass spectrometry (LC/MS/MS) as per United States Department of Agriculture Food Safety and Inspection Service (OPHS, 2018). The measurements include homogenization of the muscles followed by extraction and clean up and finally measurement techniques.

Control preparation
Positive and negative controls were prepared by weighing 20 g of fish homogenate and then entrenched with 100 µl screening levels of the mixed standard solution. Young and pesticides-free Comet goldfish (Carassius auratus auratus) were purchase from the market to make blank and spiked controls by known concentrations of stock solutions (5 ml), and then it was dissolved in 10 ml ethyl acetate. Positive and negative controls were prepared by weighing 20 g of fish homogenate and then entrenched with 100 µL screening levels of the mixed standard solution. The same extraction technique of the analyte procedure was applied to prepare the calibration curve. A 200 µL of stock solution were added to 25 ml of methanol as a solvent. Serial solutions were done with 12 points 1:1 to be used for spiking. The prepared samples were injected for LC/MS/MS (See supplementary file). Screening for pesticides by LC/MS/MS as per United States Department of Agriculture Food Safety and Inspection Service (OPHS, 2018).

Validation
The performance of the analysis was fixed by retrieval of an Internal Standard, 50 µl of Amitraz belongs to triazines herbicides was added in a constant amount to unknown samples, that were added to correct analyte losses and avoid of fluctuation at the beginning of the sample preparation is accompanied that must be show similar behavior to the analyte.

Cleaning of Equipment
All tableware, liquidizer and pestles, centrifuge tubes, pipettes, and beakers were brushed with soap and rinsed with running water. An extra rinse was done with water and followed by final rinse with solvent. Equipment were
then place within the kitchen appliance to dry at 45°C. Frozen samples were allowed to thaw before removal of scales and dissection to obtain samples (Grice et al., 1999).

**Statistical Analysis**

The statistical package for social sciences (SPSS, version 20, Chicago, Inc.) was used for data processing and analysis. Chi square was used to examine the different prevalence of pesticide between local and imported samples.

**RESULTS**

**Organochlorines prevalence**

Eight pesticides out of 10 Organochlorine residues were found in examined fish samples, whereas two of them (Heptachlor epoxide and Lindane) were undetected. A total of 198 samples (91%) out of the collected 218 samples were contaminated with organochlorines. Endosulfan was the most commonly found pesticide in 91% of samples. Only hexachlorobenzene and o,p′-DDE were below the maximum permissible limit (10 ppb). The detailed distribution of detected pesticides, as well as their concentrations, are summarized in table 1. The percentage of samples that exceeded the MRL for these pesticides were 22.2%, 74.3%, and 37.5% for endosulfan, dieldrin, and methoxychlor, respectively. The highest residual concentration (276.51 ppb) was detected in the Red Snapper species, (the Arabian Sea and the Indian Ocean). The overall prevalence rate of endosulfan for all geographical locations was 89.6%, of which 19.26% were more than the MRL. Zone 27, representing the Northeast Atlantic Ocean, was the highest contaminated site (46.8%).

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Prevalence rate</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>91%</td>
<td>8.68±6.60</td>
<td>1.66 – 36.54</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>5.5%</td>
<td>98.4±76.8</td>
<td>8.37 – 242.17</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>0.45%</td>
<td>1.50±0.24</td>
<td>1.33 – 1.68</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.45%</td>
<td>87.94±10.07</td>
<td>80.82 – 95.06</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>3.7%</td>
<td>17.98±6.02</td>
<td>2.98 – 77.8</td>
</tr>
<tr>
<td>o,p′-DDE</td>
<td>0.45%</td>
<td>3.47±1.07</td>
<td>2.715 – 4.22</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>17.88%</td>
<td>50.3±13.01</td>
<td>2.694 – 276.51</td>
</tr>
<tr>
<td>Y - Chlordane</td>
<td>0.45%</td>
<td>39.7±5.65</td>
<td>35.7 – 43.7</td>
</tr>
</tbody>
</table>

**Organophosphates prevalence**

Eight out of 11 (72.72%) organophosphates compounds were detected in various concentrations. Only fenitrothion, methyl parathion, and dichlorovos were not found in analyzed samples (Table 2). Malathion, diazinon, chlorpyrifos, and parathion exceeded the maximum permissible limit (10 ppb). The percentage of samples exceeding the MRL were 100%, 22.9%, 35%, and 82.3% of total contaminated samples for malathion, diazinon, chlorpyrifos and parathion, respectively. Parathion has the highest residual concentration (126.34 ppb) found in Basa, whereas Diazinon has the lowest concentration in Tilapia (1.29 ppb).

<table>
<thead>
<tr>
<th>Pesticide</th>
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<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>98.6%</td>
<td>53.06±18.14</td>
<td>22.8 – 99.1</td>
</tr>
<tr>
<td>Diazinon</td>
<td>88.1%</td>
<td>8.46±2.11</td>
<td>1.29 – 23.8</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>93.1%</td>
<td>10.8±3.02</td>
<td>1.87 – 79.9</td>
</tr>
<tr>
<td>Profenofos</td>
<td>1.8%</td>
<td>3±0.77</td>
<td>2.31 – 4.13</td>
</tr>
<tr>
<td>Azinphos methyl</td>
<td>8.7%</td>
<td>2.49±0.72</td>
<td>1.68 – 4.48</td>
</tr>
<tr>
<td>Imidan</td>
<td>3.2%</td>
<td>2.53±0.57</td>
<td>1.73 – 3.36</td>
</tr>
<tr>
<td>Azamethiphos</td>
<td>1.3%</td>
<td>2.76±1.33</td>
<td>1.38 – 4.04</td>
</tr>
<tr>
<td>Parathion</td>
<td>23.3%</td>
<td>36.5±30.63</td>
<td>2.84 – 126.34</td>
</tr>
</tbody>
</table>

**Pyrethroid pesticides prevalence**

All the studied pyrethroid pesticide residues were recovered from 9, 65, 4, and 214 examined samples for cypermethrin, deltamethrin, permethrin, and bifenthrin respectively. The range mean concentrations of these pesticides are summarized in table 3. The percentage of fish samples that exceeded the MRL for each pesticide were
88.9%, 41.5%, 25%, and 79% for cypermethrin, deltamethrin, permethrin, and bifenthrin, respectively. The general occurrence of pyrethroids in this study was 33.45% in all tested samples of the target species, where 58.6% of them were contaminated at levels more than the MRL. While the prevalence for residual concentration in all geographical sites was 25% including the Gulf of Aqaba and local freshwater fish. Indeed, most contaminated samples were those originated from Gulf of Aqaba (37%), while the lowest contaminated area was the zone of Al Aghoar (14.2%). Regarding the carbamates, three pesticides were detected in 207 fish samples (94.95%) (Table 3). Only fenoxycarb and carbofuran were detected, but only the former was found to exceed the MRL set by WHO. Aldicarb and methomyl were not found in any sample.

### Table 3. The concentration (ppb) of detected pyrethroid and carbamates pesticides

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Prevalence rate</th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyrethroids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>4.1%</td>
<td>14.59±3.46</td>
<td>8.34 – 21.18</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>29.8%</td>
<td>17.40±6.22</td>
<td>1.52 – 65.35</td>
</tr>
<tr>
<td>Permethrin</td>
<td>1.8%</td>
<td>5.41±4.20</td>
<td>1.71 – 10.50</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>98.1%</td>
<td>61.05±37.22</td>
<td>2.17 – 376.61</td>
</tr>
<tr>
<td><strong>Carbamates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>94.95%</td>
<td>11.25±4.63</td>
<td>1.42 – 95.37</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>0.917%</td>
<td>1.8±0.43</td>
<td>1.5 – 2.12</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>10.6%</td>
<td>1.92±0.50</td>
<td>1.22 – 3.18</td>
</tr>
</tbody>
</table>

This study showed that local and imported fish in Jordan are heavily contaminated with 23 compounds. All 218 analyzed fish samples (24 local fresh and 194 imported frozen) were contaminated with at least four different compounds simultaneously (Figure 1). The results of overall contamination rates for pesticides showed that 26%, 43%, 25%, and 6.18% of samples contained organochlorines, organophosphorus, pyrethroids, and carbamates, respectively.

![Figure 1](image.png)  
**Figure 1.** Distribution of pesticides along with their levels exceeding the MRL in fish samples.

The general contamination rates for all seawater fish species in all locations were 25%, 41.1%, 26.2%, and 6.86% for organochlorines, organophosphorus, pyrethroids, and carbamates pesticide residues, respectively. Based on FAO classification, zone–27 (Northeast Atlantic Ocean), zone–51 (Indian and Arabian gulfs, and Gulf of Aqaba), and zone–71 (western central Pacific) were the highest contaminated areas of 36.3%, 57.1%, 37.5%, and 14.2% for organochlorine, organophosphorus, pyrethroid, and carbamate residues, respectively. On the other hand, contamination in freshwater fish species showed a contamination prevalence of 28.6%, 57.1%, and 14.2% for organochlorine, organophosphorus, and pyrethroid pesticides, respectively. Nonetheless, no carbamate residues were detected.

The total contamination rates in all freshwater fish samples were 42.57%, 21.8%, 58.6% and 4.62% for organochlorine, organophosphorus, pyrethroid, and carbamate insecticide residues, respectively. Almost samples derived from all FAO zones showed pesticides levels that violate the acceptable residue level (Figure 2). The prevalence of contamination in levels higher than MRL among different fish species revealed that Argentinian Hake fish have the highest contamination level with a prevalence rate of 40% whereas, Mackerel species from Iceland have the lowest contamination with a prevalence rate of 10%.
Figure 2. Distribution of pesticides concentrations (>MRL) in local Jordanian fish and imported frozen fish from different geographical world locations (FAO zones).

DISCUSSION

Despite the paramount benefits of pesticides in controlling diseases and improving productivity, various abuses and negative effects were reported around the globe (Poudel et al., 2020). Contamination of food and water is the most commonly encountered issue. For instance, DDT organochlorine pesticide was banned from use in agriculture because of its serious health effects on animals (Sharma et al., 2020). Marine organisms concentrate DDT and its degradation product, DDE, by factors of millions (Darko et al., 2008). The observation of o,p′-DDE level detected in the current study was only in Grouper fish (*Epinephelus polylepis*) with a mean concentration of 3.47±1.07 ppb. This is in parallel with a study in Ghana that showed DDE as the predominant pesticide (58%) in tilapia fish (*Tilapia zilli*) at concentrations of 5.232±1.30 ppb, which is less than the MRL (Darko et al., 2008).

Endosulfan is also a banned organochlorine pesticide that is a genotoxic and endocrine disruptor (Cone, 2010). In the current study, endosulfan, dieldrin, and methoxychlor were detected in all tested samples at the prevalence of 91%, 17.88%, and 3.7%, respectively. This is in agreement with a study carried on Libyan fish (Enbaia et al., 2014), that showed endosulfan, dieldrin and methoxychlor concentrations to be higher than the MRL. Moreover, higher prevalence rates of organochlorine pesticides in fish samples have been reported in Densub River Basin in Ghana where β-HCH, δ-HCH, lindane, heptachlor, aldrin, dieldrin, endrin, α-endosulfan, β-endosulfan, endosulfan sulfate, methoxychlor, fenvalerate, γ-chlordane, p, p′-DDE, p, p′-DDD and p, p′-DDT were detected (Kuranchie-Mensah et al., 2018).

The overall prevalence of pyrethroids ranged from 1.8% to 98.1%. Pyrethroids have been reported in fish samples in various concentrations. For instance, the low levels found in this work are in agreement with a study from Jorhat district of Assam markets in India (Choudhury et al., 2013), where contamination by pyrethroids such as fenpropathrin, λ-cyhalothrin and α-cypermethrin ranged from 1 to 2.1 ppb. It is known that certain areas/zones that are near industrial or agricultural activities tend to be more contaminated with pyrethroids (Deng et al., 2020). An Australian study investigated the impacts of pyrethroid and neonicotinoid pesticides on shrimp cultivation in Northeast Australia have shown that crustaceans, together with commercially vital species, are extraordinarily sensitive to these pesticides (Hook et al., 2018).

Organophosphates are employed in both urban and agricultural environment. Chlorpyrifos is a widely-used insecticide that poses risks to aquatic organisms and ecosystems (Giddings et al., 2014). Exposure to this pesticide is wide via direct uptake from water. Although dietary exposure to organophosphate may result from residues absorbable to food items like algae, macrophytes, and invertebrates, or from eaten sediment particles (Li et al., 2019).

The observation of high levels of parathion in the present work is not compatible with a study of
organophosphorus residues in fish from the Tono, Gold Coast, Ghana, that showed mean concentrations of organophosphorus pesticides (methamidophos, entomophos, phorate, dimethoate, diazinon, fonofos, pirimiphos-methyl, fenitrothion, malathion, chlorpyrifos, parathion, chlorfenvinphos and profenofos) in ranges of 0.080 – 0.90 ppm, 0.080 – 0.09 ppm and 0.05 – 0.06 ppm in C. anguillaris, S. intermedius and M. senegalensis, respectively (Akoto et al., 2016). Another study was done to determine organophosphorus insecticides (malathion, and chlorpyrifos,) residues in two fish samples collected from Kolleru Lake, India. The maximum concentrations of pesticide residues were 2.5 ppm for malathion and 88.6 ppm for chlorpyrifos (Rao and Pillala, 2001) which are in parallel with this study. Additionally, these observations are also in agreement with another study in Nigeria that showed the residual concentrations of organophosphates (dichlorvos, diazinon, chlorpyrifos and fenitrothion) in fish to be above the MRL as set by EU (Akan et al., 2013).

The results revealed that the overall prevalence and contamination rates for both Jordanian regions Aqaba and Al Aghoar) were quite similar. Comparison of seawater fish (from Aqaba gulf) with fish from Al Aghoar Lake shown no statistical differences between means of pesticides residues. This observation reflects the amount of accumulation within fish types and species under study from these two regions. Such uniformity of results could indicate that factors such as; location nearby fruit and vegetable farms or from seawater and presence of the port, contamination of the fish farms by water treatment, feed and feed supplements does not reveal clear significant affect under this study condition. Lastly, no significant differences between local and imported fish were observed in terms of total contamination.

CONCLUSIONS

The main conclusion of this study is that examined fish are heavily contaminated with different pesticides. Fish samples were contaminated with at least four compounds and up to 12 compounds and the majority of samples showed concentrations above MRL. The local authorities should be aware of the high pesticides’ contamination levels of both imported and local fish and routine periodical surveillance should be run on marketed fish. Further regulations of pesticides usage should be implemented and monitored.

Author Contributions: A.R.A. and B.S.J. Conceptualized the study; A.R.A. and D.A.A. Mentored the study; B.S.J. Carried out the laboratory analysis; D.A.A. Wrote the manuscript. All authors analyzed the data, reviewed and revised the final manuscript.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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