Lifetime Cancer Risk and Hazard Quotient of BTXs Compounds in Iranian Petrochemical and Petroleum Depot Workers

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ABSTRACT

Exposing to the high-level of BTXs concentration can occur in the oil-dependent industries and may cause considerable adverse impacts on the health of employees. The main objective of the present study firstly is determining the exposure of petrochemical and oil depot workers to benzene, toluene, ortho and methyl xylenes, and p-xylene (BTXs), and secondly is estimating the lifetime cancer risk (LCR) and non-carcinogenic risk using the hazard quotient (HQ). This cross-sectional study is conducted on 85 workers at petrochemical (34 subjects) and oil depot (51 subjects) industries of Iran in 2016. In order to determine the exposure concentrations of BTXs, through inhaling way, individual sampling was carried out according to the NIOSH 1501 method. Then, estimating LCR of benzene, and non-carcinogenic risk of benzene, toluene, ortho and methyl xylenes, and p-xylene, using the HQ, has been conducted regards to criteria recommended by USEPA. The mean exposure concentration of benzene among the oil depot workers was higher than the petrochemical workers, but this difference was not statistically significant (P = 0.162). The mean exposure concentration of benzene in the oil depot workers (2.1 ± 2.53mg/m³) was higher than the occupational exposure limits provided by the American conference of governmental industrial hygienists (ACGIH_TLV = 1.67mg/m³). The mean concentration of toluene in the exposed workers of the oil depot (46.81 ± 22.43mg/m³) was higher than the exposed workers of petrochemical (41.83 ± 30.06mg/m³), but this difference was not statistically significant (P = 0.412). The results of carcinogenic and non-carcinogenic risk assessment revealed high-risk exposure level in the both investigated groups. The mean LCR of benzene in the oil depot workers was higher than the petrochemical workers (1.681E-3 and 1.163E-3, for the oil depot and petrochemical, respectively). The LCR of benzene and HQ of BTXs in the both investigated groups were higher than the reference values. In order to reduce occupational exposure to BTXs in the investigated oil depot and petrochemical, control measures should be taken in the future, as soon as possible.

Key words: Occupational Exposure; Risk Assessment; Workers; benzene; Toluene; Xylenes

List of abbreviations

VOCs Volatile Organic Compounds
BTEX Benzene, Toluene, Ethyl benzene and Xylenes
IARC International Agency for Research on Cancer
WHO World Health Organization
US EPA United States Environmental Protection Agency
LCR Lifetime Cancer Risk
HQ Hazard Quotient
NIOSH National Institute for Occupational Safety and Health
CDI Chronic Daily Intake
SF Slope Factor
IR Inhalation Rate
ED Exposure Duration
EF Exposure Frequency
BW Body Weight
INTRODUCTION
Many chemical compounds such as heavy-chain hydrocarbons, light-chain volatile compounds, and short-chain organic compounds are mined, processed, produced and distributed through the oil-dependent industries. Some of these compounds have toxic properties and exposure to them can have adverse effects on human health. Toxic petroleum products are mainly light-chain volatile aromatic compounds [1]. The most important materials that are included in these products are Volatile Organic Compounds (VOCs) that are defined as organic compounds having a boiling point between 50°C and 260°C, and amongst VOCs, the most commonly found are benzene, toluene, ethyl benzene and xylenes (so-called BTEX) [2].
Adverse health effects caused by chronic exposure to VOCs are varied and can consider into two groups, carcinogenic and non-carcinogenic effects. Allergy, sensitization, and liver, kidney, nervous and respiratory disorders are viewed as non-carcinogenic effects [3-6]. Furthermore, lung, liver, kidney and biliary cancers and leukemia are some cases of carcinogenic effects [4, 7, 8]. Amongst BTEX, benzene is the most toxic compound, and prolonged exposure to a low concentration of it may lead to increase the incidence of leukemia and aplastic anemia in individuals who are exposed. The international agency for research on cancer (IARC) has classified benzene as a definite carcinogenic compound (Group I) [8]. Also, world health organization (WHO) has reported that prolonged exposure to 1.7μg/m³ of benzene causes leukemia in 10 people per million [9].
Oil-dependent chemical industries (e.g. Oil terminals, oil and gas transmission lines, petrochemicals, oil depots, refineries and etc.) generally are major industries that have a crucial role in mining, processing and distributing petroleum products. Petrochemicals and oil depots are two essential parts of oil-dependent chemical industries and much of the workforce is employed in these industries. Recently, several studies have been carried out for determining VOCs concentrations in the air of oil-dependent industries, especially BTEX concentrations, in order for determining environmental emissions and occupational exposures. The results of these studies have suggested high emissions of these compounds into the environment. Also, occupational exposure to the VOCs has been reported in high values [10-14].
Recently, several studies carried out in Iran to survey the concentrations of BTEX in the air of petrochemicals, fuel stations (gasoline and gas), oil depots and urban air [15-21]. Although many international organizations such as the WHO, the united states environment protection agency (US EPA) and the united states food and drug administration are using quantitative risk assessment for legislating on chemical compounds problems, but studies in Iran are mainly aimed to determine only exposure levels of BTEX and only a few studies are conducted on exposed workers of Iran’s process industries for assessing carcinogenic quantitative risk and these studies have not been conducted in any oil depots [15, 18, 20, 22-24]. Furthermore, some conducted studies mainly have used semi-quantitative methods to evaluate the risk level of exposure to chemical substances; however, the validity of these methods is questionable [25-28]. Zare Jeddii et al. conducted a study to quantify the carcinogenic risk assessment of chemical substances in fuel stations. They found that the lifetime cancer risk was 3 out of 1,000 in occupied workers [29]. Also, Rahimnejad et al. determined the lifetime cancer risk in the petrochemical industry employees 3 out of 10,000, and the estimated health hazard of xylenes was higher than the reference value [24]. Therefore, due to limit studies, determining the concentration levels of BTXs in the oil-dependent industries is important, and carcinogenic quantitative risk estimation and non-carcinogenic assessment of exposed workers to BTXs in these industries are helpful for planning and taking work control measures. Since there are no published data on carcinogenic and non-carcinogenic quantitative risk assessment in the oil depot workers and these data are rare for petrochemical workers, this study was aimed to determine occupational exposure to BTXs and estimating the LCR and non-carcinogenic assessment of these chemical compounds using the HQ, in some petrochemical and oil depot workers in Iran.

MATERIALS AND METHODS
Study design
This cross-sectional study is conducted on 85 workers at petrochemical (34 subjects) and oil depot (51 subjects) industries of Iran in 2016. The sample size was calculated based on the concentration of benzene exposure among Iranian petrochemical workers and according to a study conducted by Rahimpoor and colleagues (1.192±0.639) using equation 1 (α=0.05, δ=0.14 and σ= 0.639). According to the mentioned study, sample size estimated 80 subjects (30).

Equation 1
\[ n = \frac{(Z_{1-α/2})^2 \times (σ^2)}{(d^2)} \]

The participant workers in our study were asked to disclose their demographic and occupational characteristics and smoking habits in a questionnaire form. Also, they were selected randomly by workers who have work experience of two years or more and without any history of smoking and employment in the second job with exposure to BTXs compounds. This study was approved by the Ethical Committee of Shiraz University of Medical Sciences, Shiraz, Iran. All the participants filled out the informed consent and signed it.

Personal air sampling
In order to determine the concentrations of benzene, toluene, and xylene isomers (ortho-, meta- and para-xylene) in the breathing zone of workers, individual sampling was performed according to the national institute for occupational safety and health (NIOSH) 1501 method, using the charcoal tube (bipartite derived from coconut 50/100 Manufactured by SKC Co) and a SKC sampling pump [31]. Before sampling, charcoal tube ends were broken and connected to a calibrated pump with a flow rate of 200 ml/min. Then charcoal tubes were attached to the collar of workers using holders, and sampling was carried out from the breathing zone air. After completion of the sampling period, tubes were detached and the two ends of the absorber tubes were closed by plastic caps and moved to the laboratory for preparation and analysis.

Sample preparation and analysis
In order for preparing air samples, at first, the front section of the charcoal tube was broken, then activated carbon absorbent containing absorbed contaminants was transferred into 4 ml vials. For extracting BTXs compounds, 1 ml of GC-grade carbon disulfide (CS2) (Merck Co) was added to the vial of the activated charcoal absorbent. In order to transfer BTXs from activated carbon absorbent to carbon disulfide solvents, the vial door was firmly closed and was enclosed in an ultrasonic bath (Soltect 2200 MH model) for 30 min. After preparing the solution, 1 μL of it was injected into the port of gas chromatography (GC) [31]. The analysis was performed using GC (SCION-455GC) equipped with an FID detector using a capillary column (RTX, 624 with 25m 0.22mm 0.25mm). Helium (99.999%) was used as the carrier gas at a flow rate of 1.5 ml/min. The injector was set in the splitless mode and the temperature of it was set at 180°C. The column temperature program was set at 30°C initially, then increased to 180°C at 20°C/min and held for 30 Sec. Each chromatography’s run was completed in 8 min.

Sampling and analytical quality control
In order to control breakthrough of the Sorbent tubes, front and back sections of the tubes were analyzed separately, and samples which the concentration of BTXs in the back section was more than the front section, 10%, were recognized as invalid samples, and for the samples of less than 10%, corrections were performed according to the NIOSH 1501 method formulas. In order to remove errors in sampling and analysis, blank samples were considered as constant as the obtained samples and all sampling and preparation steps were conducted similarly. The amount of analytical method recovery was tested by injection 2, 4, 6, 8 and 10 μg of standard solutions to 5 charcoal tubes. The average recovery was given in 94% (91-101 percent). The calibration curves were plotted using injection different concentrations of 6 standard samples of each compound into the GC device [31].

Lifetime Cancer Risk (LCR) and Hazard Quotient (HQ) Estimation
Carcinogenic risk (LCR) and non-carcinogenic risk (HQ) were calculated using the method provided by the US EPA (32, 33).

LCR was calculated by multiplication of the chronic daily intake (CDI) by the slope factor for Benzene (Equation 2).

Equation 2
\[ LCR = CDI \times SF \]

The slope factor is an upper level, with a 95% confidence interval, on the increased cancer risk from a lifetime exposure to a chemical through ingestion or inhalation (mg/kg-day). The slope factor of benzene through inhalation was obtained from the California Environmental Protection Agency (0.1 mg/kg-day) [34].

CDI is the amount of absorbed chemical through the human body for the duration of working life. CDI be identified using Equation 3.

Equation 3
\[ CDI = \frac{C_{adj} \times 24h \times IR \times ED \times EF}{(BW) \times (AT) \times (NY)} \]
Where $C_{adj-24h}$ is the pollutant concentration in inhalation for 24 hours in day (mg/m$^3$), IR is the rate of inhalation (m$^3$/day), ED is the exposure duration (years), EF is the exposure frequency (days per year), BW is the body weight (kg), AT is the average time and NY is the number of days per year. In this study, the inhalation rate (IR) and body weight (BW) were considered 20 m$^3$/day and 70 kg respectively. The average time (AT) was reckoned 70 years for carcinogens and 30 years for non-carcinogens. The exposure duration (ED) and exposure frequency (EF) were considered 30 years and 240 days, respectively [32].

For calculating non-carcinogenic risk, the hazardousness through inhalation was measured by calculating the HQ using Eq. (4).

$$\text{HQ} = \frac{\text{CDI}}{\text{RFD}}$$  \hspace{1cm} \text{Equation 4}

Where CDI is the chronic daily intake of non-carcinogenic substances (mg/kg-day) and RFD is the reference dose factor (mg/kg-day). In general, the RFD is a value to estimate a daily exposure for individuals who are liable to be without risks of injurious effects for their lifetime (including sensitive subgroups). In the other words, the RFD is the proportion of the No-Observable Averse Effect Level (NOAEL) over the Uncertainty Factor (UF) [33].

RFD for each compound was calculated using Eq. (5)

$$\text{RFD} = \frac{\text{RFC} \times \text{IR}}{\text{BW} \times \text{a}}$$  \hspace{1cm} \text{Equation 5}

Where RFC is the reference concentration factor (mg/m$^3$), IR is the rate of inhalation (set at 20 [m$^3$/day]), BW is the body weight (set at 70 [kg]). The RFC of benzene, toluene, and xylenes (m, o, p-isomers) obtained from the California Environmental Protection Agency (0.003, 0.3 and 0.7 mg/m$^3$, respectively) [34].

**Statistical analysis**

Data were analyzed using SPSS (Chicago, Illinois, USA). The distribution of parameters was examined with Shapiro wilk test. Independent two-sample t-test was conducted for comparing mean concentrations of BTXs compounds between petrochemical and petroleum depot workers. In all tests, the level of significance was set at $P< 0.05$.

**RESULTS**

The demographic and job characteristics of petrochemical and oil depot workers are presented in Table 1. The mean (Std. Deviation) of age and Work experience of subjects were 32.41 (4.97) and 8.14 (4.38) respectively.

<table>
<thead>
<tr>
<th>Industry</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Petrochemical (n=34)</strong></td>
<td>Age (year)</td>
<td>32.5</td>
<td>5.06</td>
<td>24.00</td>
</tr>
<tr>
<td></td>
<td>Work experience (year)</td>
<td>7.29</td>
<td>5.24</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>175.47</td>
<td>4.65</td>
<td>169.00</td>
</tr>
<tr>
<td></td>
<td>Weight (kg)</td>
<td>80.38</td>
<td>8.52</td>
<td>66.00</td>
</tr>
<tr>
<td><strong>Petroleum depot (n=51)</strong></td>
<td>Age (year)</td>
<td>31.35</td>
<td>4.95</td>
<td>22.00</td>
</tr>
<tr>
<td></td>
<td>Work experience (year)</td>
<td>8.70</td>
<td>4.50</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>178.17</td>
<td>4.93</td>
<td>165.00</td>
</tr>
<tr>
<td></td>
<td>Weight (kg)</td>
<td>81.27</td>
<td>9.05</td>
<td>65.00</td>
</tr>
<tr>
<td><strong>Total (n=85)</strong></td>
<td>Age (year)</td>
<td>32.41</td>
<td>4.97</td>
<td>22.00</td>
</tr>
<tr>
<td></td>
<td>Work experience (year)</td>
<td>8.14</td>
<td>4.83</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>177.09</td>
<td>4.97</td>
<td>165.00</td>
</tr>
<tr>
<td></td>
<td>Weight (kg)</td>
<td>80.91</td>
<td>8.80</td>
<td>65.00</td>
</tr>
</tbody>
</table>

Exposure concentrations of the BTXs compounds in both petrochemical and oil depot workers’ groups, and also statistical differences between them are shown in Table 2. The mean concentration of benzene in the oil depot workers group was higher than the petrochemical workers, but this difference was not statistically significant ($P = 0.162$). Also, the mean concentration of benzene in the oil depot workers group (2.1 ± 2.53 mg/m$^3$) was higher than the occupational exposure limits provided by the American conference of governmental industrial hygienists (ACGIH_TLV = 1.67 mg/m$^3$). The measurement results of workers’ exposure to toluene and xylenes (ortho-xylene, methyl-xylene and p-xylene) in the petrochemical and oil depot, showed that the mean concentrations of toluene, ortho-
 Unlike the benzene and toluene, the mean concentration of xylenes in the exposed workers of the petrochemical (122.98 ± 116.87 mg/m³ and 121.76 ± 95.96 mg/m³ for o, m-xylene and p-xylene, respectively) was higher than the exposed workers of oil depot (88.71 ± 77.99 mg/m³ and 95.18 ± 76.08 mg/m³ for o,m-xylene and p-xylene, respectively), but the differences were not statistically significant (P = 0.139 and P = 0.159 for o,m-xylene, and p-xylene, respectively).

The results of carcinogenic and non-carcinogenic risk assessment of BTXs compounds for the petrochemical and oil depot workers are given in Table 3. These results indicated a high risk in both of the study groups. The mean lifetime cancer risk of benzene in the oil depot workers was higher than the petrochemical workers (1.681E-3 and 1.163E-3, for the oil depot and petrochemical, respectively). The mean lifetime cancer risk of benzene in both groups was higher than the reference value (Reference value: LCR < 1*10⁻⁶). The results of average non-carcinogenic risk of BTXs showed that the HQ value of these compounds in both groups was higher than the reference value and the highest value was for the xylene (Reference value: HQ ≤ 1). HQ values of benzene and toluene in the petrochemical workers were higher than the oil depot workers, while the HQ values of o, m-xylene, and p-xylene in the petrochemical workers were higher than the oil depot workers.

### Table 2: Concentrations of BTXs among the petrochemical and oil depot workers and statistical differences between both groups (mg/m³)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean (mg/m³)</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1.49</td>
<td>1.31</td>
<td>0.03</td>
<td>4.45</td>
<td>0.162</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.1</td>
<td>2.53</td>
<td>0.008</td>
<td>14.23</td>
<td>0.412</td>
</tr>
<tr>
<td>O, m-Xylene</td>
<td>46.81</td>
<td>23.43</td>
<td>0.2</td>
<td>78.90</td>
<td>0.02</td>
</tr>
<tr>
<td>P- Xylene</td>
<td>122.98</td>
<td>116.87</td>
<td>14.40</td>
<td>412.50</td>
<td>0.139</td>
</tr>
<tr>
<td>Petrochemical</td>
<td>88.71</td>
<td>77.99</td>
<td>1.90</td>
<td>321.50</td>
<td>0.159</td>
</tr>
<tr>
<td>Petroleum depot workers</td>
<td>121.76</td>
<td>95.96</td>
<td>4.12</td>
<td>459.87</td>
<td>0.519</td>
</tr>
<tr>
<td>Petroleum depot workers</td>
<td>95.18</td>
<td>76.08</td>
<td>2.70</td>
<td>341.80</td>
<td>0.312</td>
</tr>
</tbody>
</table>

### DISCUSSION

According to our knowledge, there is no survey on the carcinogenic and non-carcinogenic risk assessment of BTXs that has conducted on exposed workers of both petrochemical and oil depot at the same time. The results showed that oil depot workers have higher levels of exposure to benzene and toluene, in comparing with the petrochemical. Also, the average occupational exposure to benzene for both groups of workers was higher than the occupational exposure limits recommended by the Iranian Ministry of Health and Medical Education and ACGIH organization. Also, the results of the carcinogenic and non-carcinogenic risk assessment of BTXs indicated a high-risk level in both groups. The mean lifetime carcinogenic risk of benzene in both groups was greater than the reference value. Furthermore, despite the lower average occupational exposure to toluene and xylenes, risk assessment results indicated much higher value than the reference value.

In this study, the mean exposure level of benzene in most of the workers was higher than the permissible limits, however, the mean exposure levels of toluene and xylenes were lower than the occupational exposure limits. There are some studies that have surveyed occupational exposure levels to BTXs in the petrochemicals and oil depots industries.
values in these studies are different together and most of these values are lower than the obtained values of the present study. Chan et al. have determined mean exposure to benzene and toluene in Taiwan petrochemical industries, 0.0466mg/m³, and 0.177mg/m³, respectively [35]. Lerner et al. have determined the concentrations of benzene, toluene, p,m-xylene and ortho-xylene in the ambient air of Argentina petrochemicals, 0.016mg/m³, 0.021mg/m³, 0.012 mg/m³ and 0.0027mg/m³, respectively [36]. The difference of BTXs concentrations in this study and the cited studies may be explained by the dissimilarity of sampling methodology and probably more properly controlling measures in Taiwan and Argentina petrochemical industries than Iranian petrochemicals. In the studies carried out by Chan et al. and Lerner et al., determining the compound concentrations was performed utilizing passive sampling and continuous monitoring system, respectively. However, in this study, determining the amounts of samples was performed using the NIOSH 1501 method. Thus, the difference of sampling methods used for evaluating occupational exposure to compounds may cause different results.

Also, some studies have conducted in Iran in order for evaluating occupational exposure to BTXs in petrochemical and oil depot industries. Azari et al. have determined occupational exposure to benzene in Tehran’s oil depots 0.491 to 5.2mg/m³ and the mean exposure to toluene 0.76 to 10.25mg/m³ [15]. Also, they have determined the mean exposure to benzene higher than the occupational exposure limit, and the mean exposure to toluene lower than the occupational exposure limit, that these findings were consistent with the present study. Furthermore, they concluded that the concentration of xylene was lower than the detection limit, which this finding is in contradiction to our study, which the amounts of xylene were not lower than the detection limit in any sample. Rahimnejad et al. has surveyed levels of exposure to BTXs in southern Iranian oil fields which the mean levels of occupational exposure in their study were mostly lower than the present study. In their study, the exposure levels of benzene, toluene, ortho and meta-xylene, and p-xylene were 0.236 to 1.323mg/m³, 0.09 to 0.877mg/m³, 0.144 to 1.49mg/m³, 0.047 to 1.185mg/m³, respectively [24]. Also in other studies conducted in Iran’s petrochemicals, the resulted occupational exposure levels were less than the present study [22, 23]. The causes of the differences are not clear but may be rooted in the taken controlling measures in the present study in comparison with the other, differences in equipment maintenance in the present study with other studies which in turn determines a number of compounds leakage to air. Also differences between the present study and Rahimnejad et al. study may be due to the amounts and produced compounds, and the differences between our study and the Azari et al. study may be due to rates of loading petroleum products in the investigated oil depots.

The results of evaluating the carcinogenic and non-carcinogenic risk of BTXs in both investigated groups showed high exposure risk. Mean LCR values were higher than the reference value in both groups. Furthermore, mean HQ value of BTXs in both groups was higher than the reference value and the most HQ value was related to xylenes.

The carcinogenic effect of benzene, and non-carcinogenic effects of BTXs, such memory, behavior and balance disorders, renal effects, liver effects, and central nervous system injury, have been proven through many studies. Chan et al. and Lerner et al. have determined the lifetime cancer risk in separate studies in petrochemical workers exposed to benzene, 1.4×10⁻⁵ and 5×10⁻⁵, respectively [35, 36]. Also, Rahimnejad et al. have determined this value of 2.83×10⁻⁴, which in all of the mentioned studies LCR value was lower than the present study [24].

There are several studies dealt with the hazards, and the prevalence of some diseases in the oil depot workers, while there is no study surveying carcinogenic and non-carcinogenic risk of exposing to BTXs in oil depot workers. Anthony et al. in two separate studies has reported Liver and cardiovascular diseases among the oil depot workers [37, 38]. Also, LI Li-Chun et al. have examined the occupational hazards in oil depots, which one of the most important hazards was exposure to high-level benzene that is consistent with the findings of the present study [39]. According to Anthony et al. study, the prevalence of liver and cardiovascular diseases in the oil depot workers can be related to the non-carcinogenic risk of exposing chemical compounds, which are consistent with high-level non-carcinogenic risk in the present study. In the other study conducted by LI Zheng-hong et al. in some oil depots of China, the non-carcinogenic risk of exposure to toluene and benzene was in the acceptable limit, which is in contrast to the present study [40]. The most significant cause of this difference may be explained by the different investigated exposure way. LI Zheng-hong et al., have surveyed pollution of groundwater with BTXs, and they have estimated the non-carcinogenic risk through the oral way, while in the present study it estimated through inhalation way.

Generally, the findings of the present study imply high-risk of carcinogenic and non-carcinogenic effects of occupational exposure to BTXs in petrochemical and oil depot workers, and control measures in order for reducing occupational
exposures, can be taken. It is recommended for further studies to consider exposure to the other chemical compounds simultaneously and also determining the carcinogetic and non-carcinogetic effects and the prevalence of related disease can be surveyed in order to achieve more realistic information.

CONCLUSION
The LCR of benzene, and HQ of benzene, toluene, o,m-xylene, and p-xylene in the both investigated groups, petrochemical and oil depot workers, were higher than the reference values, and in some cases, these values were higher in the oil depot workers than the petrochemical workers. In order to reduce the occupational exposure of workers to BTXs in the investigated oil depot and petrochemical, control measures should be taken in future, as soon as possible.

ETHICAL ISSUES
This study was approved by Ethical Committee of Shiraz University of Medical Sciences, Shiraz, Iran

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest.

AUTHORS’ CONTRIBUTION
All authors worked equally. All authors have contributed to the review and finalization of this manuscript. All authors read and approved the manuscript.

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