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The Impact of Pesticides Exposure on Thyroid Function in Rafsanjan Farmers in 2023-2024: A Case-Control Study

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ABSTRACT

Introduction: Pesticides, particularly highly toxic organophosphates, are widely used in agriculture and can disrupt the endocrine system. This study aims to determine thyroid function indicators in agricultural workers exposed to pesticides in Rafsanjan city, Iran, in 2023.

Material and Methods: This 10-month (from March 2023 to January 2024) case-control study involved 150 participants (men farmers) divided into three groups: pesticide-spraying farmers, rural non-farmers, and urban non-exposed individuals. Socio-demographic information, medical history, and lifestyle factors data were collected by a detailed checklist. Thyroid enzyme levels, including thyroid stimulating hormone (TSH), total triiodothyronine (TT3), total thyroxine (TT4), and free thyroxine (FT4) were tested by ELISA kits. The collected data were analyzed and compared across the groups by one-way analysis of variance (ANOVA), chi-square and Pearson's correlation coefficient.

Results: Significant differences were observed in serum levels of thyroid function indicators such as TSH, Total T4, and Free T4 among the three groups (spraying farmers, rural residents near pistachio orchards, and urban residents), except for Total T3, which showed no significant difference. Moreover, there was a relationship between thyroid function variables in the spraying group and factors such as using personal protective equipment (PPE), days of spraying per year, hours of spraying per day, distance from home to pistachio orchards, and levels of pesticide exposure. However, these relationships were not statistically significant.

Conclusion: The study found no significant effect of pesticide exposure on thyroid function, possibly due to minimal skin contact and equipment maintenance practices among farmers. Further investigations are recommended.

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Introduction

Pesticides are chemical substances used in agriculture to control pests, ensuring crop productivity and quality [1, 2]. They herbicides for weed control, insecticides against pests, fungicides targeting fungal diseases, and rodenticides for rodent management. Each type of pesticide is formulated to address specific agricultural challenges, varying in composition and mode of action [3, 4]. The epidemiology of pesticide use examines patterns, distribution, and health impacts of pesticide application [5]. Factors such as frequency of use, application methods, types of pesticides employed, and demographics of exposed populations are crucial considerations [6].

Epidemiological studies play a pivotal role in identifying health risks associated with pesticide exposure, guiding regulatory measures and protective strategies [7-9]. Pesticides exert their effects through diverse mechanisms. Herbicides disrupt plant metabolic processes essential for growth [10]. Insecticides target nervous system receptors in insects, affecting their neurotransmission. Fungicides inhibit fungal cell wall synthesis or disrupt fungal metabolism. Rodenticides interfere with rodent blood clotting mechanisms, causing fatal hemorrhages [11]. Understanding these mechanisms helps assess their environmental and health impacts, ensuring safer agricultural practices [12, 13].

The thyroid gland, a vital component of the endocrine system, produces hormones such as thyroxine (T4) and triiodothyronine (T3) that regulate metabolism, growth, and energy expenditure throughout the body [14, 15]. These hormones play essential roles in maintaining body temperature, heart rate, and overall physiological balance [16]. Disruption of thyroid function can lead to conditions like hypothyroidism or hyperthyroidism, impacting health and well-being [17]. Certain pesticides, notably organophosphates and pyrethroids, can interfere with thyroid hormone production and regulation [18, 19]. They may disrupt thyroid receptor activity, inhibit hormone synthesis, or alter hormone transport mechanisms [20]. Chronic exposure to pesticides has been linked to thyroid disorders in agricultural workers, highlighting potential occupational health risks [21, 22].

The city of Rafsanjan, a significant agricultural hub, relies heavily on the use of pesticides, exposing agricultural workers to these chemicals regularly [23]. Despite the known risks associated with pesticide exposure, the specific impact on thyroid function among these workers remains underexplored. This study seeks to fill this gap by examining thyroid function indicators among agricultural workers exposed to pesticides in Rafsanjan city.

Material and Methods

Study Population

In this case-control study, health centers in Rafsanjan city handled participant recruitment, while the Faculty of Medicine of Rafsanjan University of Medical Sciences conducted thyroid enzyme levels assessment. This 10-month (from March 2023 to January 2024) study investigated 150 participants (men farmers) divided into three groups, including spraying farmers (n = 50), villagers near pistachio orchards (n = 50), and urban residents without toxin exposure (n = 50) in Rafsanjan. Ethical approval (IR.RUMS.REC.1399.124) was obtained, with inclusion criteria focusing on living conditions and specific exposures and exclusion criteria including subjects with thyroid problems, history of surgery, or exposure to harmful chemicals other than pesticides. Purposive sampling included spraying farmers, villagers near pistachio orchards, and urban residents without toxin exposure, who were matched in terms of age and education and did not work at the Sarcheshme copper complex. Informed consent was obtained, and participants completed a detailed checklist encompassing demographic information, medical history, and lifestyle factors. Blood samples (8 cc) were collected using standard venous methods, centrifuged to separate plasma, and serum was then stored at -70 °C. The thyroid enzyme levels (TSH, Free T4, Total T4, Total T3) were analyzed using ELISA kits from Ideal Diagnostic Company.

Data collection

The data collection tool included a checklist of demographic characteristics, medical history

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(including medications and supplements), history of smoking, alcohol, tea, and coffee consumption, exposure to toxins, and details related to thyroid diseases, with the activity of thyroid health indicator enzymes measured using blood samples. Exposure to pesticides was examined based on various factors, such as transportation, mixing, application, and equipment maintenance, with influencing factors including the type of activity (e.g., application of poisons, mixing), method of application (e.g., backpack, hand sprayer, speed sprayer), use of personal protective equipment (PPE) (e.g., gloves, masks, boots, or clothing), and work habits and personal hygiene (e.g., changing clothes or bathing after work). The intensity of exposure to pesticides was calculated as [24]:

$$SE = MM + MU + ER) \times PPE \quad (1)$$

Where, SE is the severity of exposure, MM is mixing mode which had three levels (never mixed = 0, mixed less than 50% of the time = 3, and mixed more than 50% of the time = 9), MU is the method of use which had seven levels (no spraying = 0, aerial use = 1, tablet distribution = 1, pit application = 2, tractor use = 3, backpack use = 8, and hand sprayer use = 9), and ER is equipment repair or washing status which had two levels (no repair or washing = 0 and equipment repair or washing = 2). PPE is the use of PPE which had four groups including PPE-0 (0% protection, no PPE used), PPE-1 (20% protection, including face shields or glasses, leather or fabric gloves, and boots), PPE-2 (30% protection, including special breathing masks and disposable clothes), and PPE-3 (40% protection, special rubber gloves resistant to chemicals). PPE use was categorized into eight levels, including (PPE-0 = 1, PPE-1 = 0.8, PPE-2 = 0.7, PPE-3 = 0.6, PPE-2 and PPE-1 = 0.5, PPE-3 and PPE-1 = 0.4, PPE-3 and PPE-2 = 0.3, PPE-4 and PPE-3 and PPE-2 = 0.1).

$$PEI = (YS \times NDSY) / 30 \text{ days} \quad (2)$$

Where, PEI is the pesticide exposure index per month, YS is years of spraying, NDSY is number of days of spraying per years.

$$CEI = PEI \times YS \times NDSY$$

Where, CEI is the cumulative exposure index [25].

Statistical Analysis

Data analysis was conducted using SPSS version 22 software. Quantitative data were presented as mean ± standard deviation, while qualitative data were reported as numbers (percentage). The normality of quantitative variables was assessed initially. The homogeneity of the three groups in terms of education level was examined using one-way analysis of variance (ANOVA) and either chi-square or Fisher's exact test. Quantitative variables were compared among the three groups using two-sample t-tests or ANOVA, controlling for qualitative variables. Pearson's correlation coefficient was used to explore relationships between two quantitative variables under parametric conditions. The significance level was set at 0.05.

Results

The analysis of the results among the three studied groups showed that, out of 150 male participants, 71 (47.3%) subjects lived in the city, and 79 (52.7%) subjects lived in the village. Each group included 50 participants. The control group comprised urban residents whose jobs were not related to agriculture. A statistically significant difference was observed in terms of disease history (P-value = 0.006), with rural residents having a higher prevalence of diseases. The most common conditions among the groups were diabetes, high blood pressure, and high blood cholesterol. No statistically significant difference was observed between the groups regarding the history of using certain drugs (P-value = 0.058), indicating homogeneity in this area. Similarly, there was no significant difference in the history of herbal medicine and alcohol use ($p > 0.05$), which also indicates homogeneity. However, a significant difference was observed in the history of smoking, coffee, and tea consumption ($p < 0.05$), indicating non-homogeneity among the groups in these behaviors. Clinical variables, except for signs and symptoms of hypothyroidism, did not show statistically significant differences ($p > 0.05$),

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indicating homogeneity except for these specific symptoms. Regarding signs and symptoms of hypothyroidism, a significant difference was observed between the groups ($p<0.05$) with hair loss (25 cases) and weakness and fatigue (19 cases) being the most common complaints. For hyperthyroidism (thyrotoxicosis), irritability (20 cases) and heat intolerance (15 cases) were the most commonly reported symptoms. Surgical history revealed 3 cases of appendectomy and 1 case of hernia among the participants. Thyroid examinations, including size, consistency, palpation, and erythema, were normal in all participants (100 %) (Table 1).

Table 1: Frequency and percentage of illness history, drug use, herbal medicine use, smoking, alcohol, coffee, tea, and clinical variables related to thyroid across three studied groups

Variable		Sprayers (%) frequently	Living in rural areas (%) frequently	Control (%) frequently	P-Value
Disease	Yes	(64) 32	(52) 26	(82) 41	0.006
	No	(36) 18	(48) 24	(18) 9	
Consumption certain drugs	Yes	(84) 42	(72) 36	(90) 45	0.058
	No	(16) 8	(28) 14	(10) 5	
Consumption herbal medicine	Yes	(98) 49	(100) 50	(100) 50	1
	No	(2) 1	(0) 0	(0) 0	
Smoking	Yes	(72) 36	(86) 43	(94) 47	0.01
	No	(28) 14	(14) 7	(6) 3	
Alcohol consumption	Yes	(78) 39	(86) 43	(86) 43	0.49
	No	(22) 11	(14) 7	(14) 7	
Coffee and tea consumption	Yes	(12) 6	(26) 13	(50) 25	0.00
	No	(88) 44	(74) 37	(50) 25	
Thyroid examinations	Yes	(0) 0	(0) 0	(0) 0	-*
	No	(100) 50	(100) 50	(100) 50	
History of thyroid disease or thyroid cancer in the family	Yes	(90) 45	(78) 39	(90) 45	0.13
	No	(10) 5	(22) 11	(10) 5	
History of recent surgery or hospitalization, tumor, radiotherapy	Yes	(94) 47	(98) 49	(100) 50	0.32
	No	(6) 3	(2) 1	(0) 0	
Hypothyroid	Yes	(50) 25	(58) 29	(80) 40	0.006
	No	(50) 25	(42) 21	(20) 10	
Thyrotoxicosis (Hyperthyroidism)	Yes	(66) 33	(60) 30	(76) 38	0.22
	No	(34) 17	(40) 20	(24) 12	

* No P-value test

Table 2 shows that chlorpyrifos, an organophosphorus compound, was the most commonly used pesticide among the 50 men in the spray group. A significant difference in total T3 levels was observed among the three studied groups ($p < 0.05$), while no statistically significant difference was observed in other laboratory variables ($p > 0.05$). Post hoc analysis showed a significant difference in total T3 levels between the spray group and the control group and between rural residents and the control group (Table 3).

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Table 2: Frequency and percentage of pesticide used in the spraying group of farmers in Rafsanjan, Iran

Poisons	Frequency (Percent)
Chlorpyrifos	32 (64)
Chlorpyrifos / Abamectin	1 (2)
Chlorpyrifos / Amitraz	2 (4)
Chlorpyrifos / Euphoria	2 (4)
Chlorpyrifos / Diazinon	2 (4)
Chlorpyrifos / Roland	1 (2)
Chlorpyrifos / Rolan / Endosulfan	1 (2)
Chlorpyrifos / Zolon	1 (2)
Chlorpyrifos / Fenvalerit	2 (4)
Chlorpyrifos / Kaolin	1 (2)
Chlorpyrifos / Kaolin / Amitraz	1 (2)
Chlorpyrifos / sulfur	1 (2)
Chlorpyrifos/acetamipride	1 (2)
Chlorpyrifos / Endosulfan	1 (2)
Chlorpyrifos / Diazinon	1 (2)

Table 3: Thyroid hormones in three groups of pesticide-spraying farmers, rural non-farmers, and urban non-exposed individuals in Rafsanjan, Iran

Thyroid hormones	Sprayers (n = 50)	Living in rural Areas (n = 50)	Control (n = 50)	P-value*
TSH	1.73 ± 1.63	1.81 ± 2.09	1.84 ± 2.49	0.966
Total T3	1.22 ± 0.33	1.25 ± 0.34	1.61 ± 1.06	0.006
Total T4	9.35 ± 1.48	9.15 ± 1.24	9.04 ± 1.09	0.630
Free T4	1.47 ± 0.60	1.39 ± 0.46	1.32 ± 0.41	0.306

*ANOVA

Table 4 indicates that mean values of laboratory variables in the spraying group, except for the storage of pesticides until use, did not exhibit statistically significant differences ($p > 0.05$). However, the mean level of Total T4 in the spraying group showed a statistically significant difference ($p < 0.05$) based on the variable of pesticide storage until use.

Table 4: Comparison of variables related to exposure to pesticides in the spraying group of farmers in Rafsanjan, Iran

Variables		TSH	Total T3	Total T4	Free T4
PPE	Yes	1.0 ± 52.92	1.0 ± 29.38	9.1 ± 59.58	1.0 ± 31.48
	No	1.1 ± 89.99	1.0 ± 17.29	9.1 ± 18.41	1.0 ± 59.66
P-value*		0.435	0.225	0.343	0.114
Mixing pesticides	Yes	1.1 ± 82.72	1.0 ± 24.34	9.1 ± 40.51	1.0 ± 46.56
	No	1.0 ± 11.24	1.0 ± 30.18	9.1 ± 29.0	1.0 ± 55.89
P-value*		0.325	0.146	0.539	0.759
Cleaning or spraying equipment	Yes	1.1 ± 80.91	1.0 ± 25.35	9.1 ± 60.49	1.0 ± 42.60
	No	1.0 ± 58.74	1.0 ± 14.30	8/1 ± 81.35	1.0 ± 59.60
P-value*		0.659	0.264	0.076	0.358
Smoking while spraying	Yes	3.5 ± 75.16	1.0 ± 22.27	10.1 ± 35.25	1.0 ± 40.84
	No	1.0 ± 56.85	1.0 ± 22.34	9.1 ± 27.48	1.0 ± 48.59
P-value*		0.460	0.985	0.175	0.791
Bathing after spraying	Yes	1.1 ± 79.74	1.0 ± 24.34	9.1 ± 36.50	1.0 ± 53.61
	No	1.0 ± 41.41	1.0 ± 08.21	9.1 ± 27.45	1.0 ± 15.47
P-value*		0.576	0.253	0.876	0.131
Eating and drinking while spraying	Yes	2.2 ± 48.08	1.0 ± 23.33	9.1 ± 58.30	1.0 ± 47.63
	No	1.0 ± 52.70	1.0 ± 21.34	9.1 ± 21.58	1.0 ± 47.59
P-value*		0.344	0.877	0.385	0.993

* Independent Samples t-test - PPE= Personal Protective Equipment

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Table 5 shows that in the spraying group, there were non-significant ($p > 0.05$) positive relationships between using PPE and the intensity of pesticide exposure with the TSH variable. There were also non-significant negative relationships between the number of spraying days per year, hours of spraying per day, distance to the pistachio garden, monthly exposure index, and cumulative exposure index with TSH. Moreover, non-significant positive relationships existed between spraying hours per day, intensity of toxin exposure, and monthly exposure index with Total T3, while non-significant negative relationships were found between PPE use, spraying days per year, distance to the garden, and cumulative exposure index with Total T3. Similar non-significant relationships were observed for Total T4 and Free T4, with both positive and negative relationships across various exposure variables.

Table 5: Correlation between exposure-related variables and laboratory variables in spraying group of farmers in Rafsanjan, Iran

Variables	TSH	Total T3	Total T4	Free T4
	Correlation coefficient (significance level)			
PPE	0.122 (0.339)	- 0.177 (0.220)	- 0.124 (0.390)	0.140 (0.320)
The number of spraying days per year	- 0.073 (0.615)	- 0.041 (0.776)	0.168 (0.243)	0.104 (0.472)
Spraying hours in one day	- 0.207 (0.148)	0.149 (0.302)	0.163 (0.257)	- 0.072 (0.622)
The distance from the house to the pistachio garden	- 0.045 (0.757)	- 0.070 (0.627)	- 0.158 (0.275)	0.113 (0.433)
The intensity of pesticide exposure	0.177 (0.218)	0.020 (0.890)	0.056 (0.700)	0.005 (0.972)
Pesticide exposure index per month	- 0.109 (0.451)	0.025 (0.864)	0.137 (0.342)	- 0.119 (0.410)
Cumulative exposure index	- 0.058 (0.687)	- 0.016 (0.910)	0.143 (0.321)	- 0.061 (0.672)

PPE= Personal Protective Equipment

Discussion

This observational study was conducted in Rafsanjan city in March 2023 to January 2024, which included 150 male participants divided into three groups, including farmers who were directly exposed to pesticides, rural residents with indirect poisons, and urban residents without exposure to pesticides. The mean age of the farmers was about 43 years, and 68% had graduated from high school. All participants were male, which was probably due to the use of tractors for spraying. Similar trends were observed among farmers in Laos, Cambodia, and Vietnam who were of similar age (mean age of 45 years for Laos and Cambodia and 50 years for Vietnam), but their education level was lower than high school (29%). In the present study, 28% of farmers smoked and 22% consumed alcohol, similar to the rates observed in Brazilian farmers; however, the current cohort study had relatively lower alcohol consumption [26].

One of the aims of this study was to measure mean levels of TSH, Total T3, Total T4, and Free T4 hormones in three distinct groups (agricultural workers directly exposed to pesticides, rural residents with indirect pesticide exposure, and urban residents with no pesticide exposure). The findings revealed a statistically significant difference in mean level of Total T3 hormone between the spraying group and the control group, as well as between rural residents and the control group ($p<0.05$). Specifically, the control group had higher Total T3 levels compared to the other two groups. However, mean levels of other hormones (TSH, Total T4, and Free T4) did not show

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statistically significant differences and were consistent across all three groups ($p > 0.05$). In a study conducted by Kongtip et al. (2021), results indicated that levels of chlorpyrifos metabolites in urine significantly increased the day after spraying, while free triiodothyronine (FT3) and total triiodothyronine (TT3) levels decreased. Additionally, an increase in total thyroxine (TT4) was observed, while cypermethrin metabolites also showed a rise in urine post-spraying. An increase in thyroxine (T4) was correlated with higher glyphosate levels in urine, and higher paraquat levels were associated with a significant decrease in FT3 and TT3 hormones [27]. This study generally indicated that exposure to pesticides like chlorpyrifos and paraquat could reduce T3 hormone levels (both total and free). The researchers also noted that chronic exposure to pesticides over an average of 25 years among farmers might obscure acute effects [27]. The study showed higher total T3 levels in the control group compared to the spraying and rural resident groups, indicating that chronic pesticide exposure, particularly to chlorpyrifos, likely contributes to lower T3 hormone levels in the exposed groups. This suggests that direct pesticide spraying or proximity to pesticide-treated areas, such as pistachio orchards, may be responsible for these hormonal changes. A study on 122 Danish greenhouse workers reported that pesticide exposure in autumn led to decreased TSH levels and increased Total T3, Total T4, and Free T4 levels. Researchers noted limitations such as seasonal dietary changes and challenges in accurately measuring pesticide exposure due to skin absorption and contact with sprayed plants [28]. These findings contrast with the current study, which found that pesticide exposure decreased Total T3 levels.

In this study, only Total T3 levels showed significant differences, while other hormone levels remained consistent across groups. Both studies reported no changes in TSH levels. A study conducted by Mardhiyah et al. (2021) found no significant differences in thyroid hormone levels between farmers and non-farmers, except for FT3 and TT4. It revealed that 32% of farmers had clinically low iodine levels, whereas 49% of non-farmers had high iodine levels, suggesting that iodine intake, rather than pesticide exposure, might explain these findings [29]. Changes in FT3 and TT4 among farmers were attributed to iodine deficiency, and blood sampling during the rainy season might have affected the results [29].

Organophosphate pesticides like diazinon and chlorpyrifos are metabolized in the body to their more toxic forms by cytochrome P450 enzymes. These metabolites can be detoxified by esterases such as PON1 [30]. Lacasaña et al. found that individuals with higher PON1 activity can detoxify organophosphate metabolites more effectively [31]. Despite the prevalent use of organophosphorus pesticides among farmers, no significant differences were detected in thyroid hormone levels. This outcome may be influenced by genetic variations affecting PON1 activity among farmers in this region and possibly among the broader population. These genetic factors contribute to the efficient removal of pesticide metabolites from the body. However, further research is necessary to establish more precise and reliable conclusions in this area.

Smoking is associated with many health problems. Although we found no significant differences in thyroid hormone concentrations based on smoking status, previous studies have reported an association between smoking and thyroid function. These studies noted that smoking is associated with decreased thyroid peroxidase antibody (TPOAb) levels, potentially impairing thyroid hormone synthesis [32]. However, the exact mechanism remains unclear and requires further research. Smoking during pesticide spraying is common in agricultural environments, but in the present study, only 8% of farmers smoked while spraying and 38% ate and drank while spraying. Oral exposure to pesticides through smoking or eating and drinking is a significant risk factor if contaminated hands are not properly washed beforehand [33, 34]. The study examined the cumulative exposure index, monthly pesticide exposure, and intensity of pesticide exposure. Results showed that although there were positive or

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negative relationships between laboratory variables and these indicators in the spraying group, they were not significant. In contrast, a study by Nankongnab et al. in 2021 found that increased cumulative days of insecticide spraying significantly increased TSH and FT3 levels while decreasing FT4 levels. Differences in pesticides used, sample size, race or ethnicity of workers, genetic factors in pesticide metabolism, or different spraying methods may explain the discrepancies between the present study results and those of previous studies [35].

The study limitations included the lack of assessment of acute toxin exposure immediately after spraying, the difficulty in isolating the effects of individual pesticides due to simultaneous exposure to multiple agricultural and household pesticides, the absence of data on daily food consumption despite the possibility of pesticide exposure through contaminated food and drinks, and the omission of dietary factors that can affect iodine levels, which are crucial for thyroid biosynthesis.

Conclusion

Significant differences were observed in serum levels of thyroid function indices, including TSH, total T4, and free T4 (but not total T3), among the studied groups. Moreover, positive or negative correlations were found between thyroid function and variables such as the use of PPE, number of spraying days per year, daily spraying hours, distance from home to pistachio orchards, intensity of contact, monthly pesticide exposure index, and cumulative exposure index in the spraying group, although these correlations were not statistically significant. These results may be affected by indirect methods of mixing pesticides using tractors, inadequate cleaning and maintenance of equipment, and the use of PPE by more than half of the sprayers, all of which may reduce direct pesticide exposure and its effect on thyroid function. Furthermore, residents of Rafsanjan may have a genetic sensitivity to PON1, an enzyme that detoxifies pesticide metabolites, thereby reducing the effects of these toxins on various organs. Further studies with improved methodology and sensitivity in measuring pesticide-induced thyroid function changes are required for more accurate and reliable results.

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Competing Interests

The authors declare no competing interest.

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Code of Ethics

This study was approved by the Ethics Committee of Rafsanjan University of Medical Sciences, Rafsanjan, Iran, with ethical code of IR.RUMS.REC.1399.124.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Sadegh Salehipour, Alireza Saedi, Zahra Assadollahi, Mohammad Amin Lotfi, Mohammadreza Rahmani, Feezeh Nosratabadi, and Mahdieh Azin. The first draft of the manuscript was written by Sadegh Salehipour, Mahdieh Azin, Soudeh Khanamani Falahati-pour, and Hadi Eslami, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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