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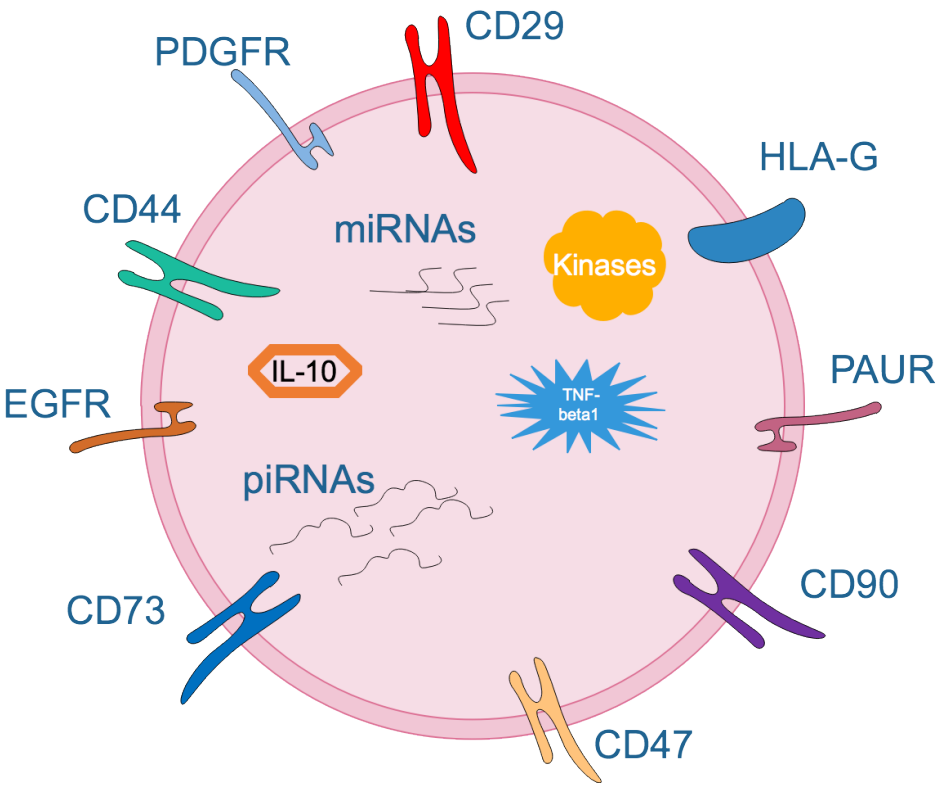

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Letter to Editor

Check for updates

Global strategy and targets to reach end the global tuberculosis epidemic

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Tuberculosis (TB) has the second highest death rate in the world among infectious diseases after HIV/AIDS (Wei et al., 2016). TB epidemic is more important than it was supposed to be (Raviglione and Sulis, 2016). In 2015, 10.4 million new cases were occurred worldwide, among these, 5.9 million (56%) were male, 3.5 million (34%) were female, and 1.0 million (10%) of them was the child. It should be noted that 1.2 million (11%) of all new TB cases were occurred in people that living with HIV (PWLH). Although tuberculosis deaths are declined by about 22% between 2000 to 2015, still is remained among top 10 causes of death in 2015 (Uplekar et al., 2015; WHO, 2016).

The three indicators for the End TB Strategy was conducted by the United Nations in 2015; percent of the reduction in some TB deaths, percent of the reduction in TB incidence rate, and percent of TB-affected families facing catastrophic costs due to TB. Indicators were targeted for the years 2030 and 2035, by milestones in 2020 and 2025, respectively (Lönnroth and Raviglione, 2016; Uplekar et al., 2015; WHO, 2016) **(Table 1)**.

To achieve these targets, the annual TB incidence rates should be reduced to 10% per year by 2025 (from 2% per year in 2015). In second, the case-fatality ratio must be decline to 6.5% by 2025 (from 15% in 2015). As projected to maintain progress after millstone time and achieve the Sustainable Development Goals (SDGs) for 2030 and End TB 2035 targets new tools should be available in 2025 that compromise a new vaccine, conduct a new diagnostic approach, and more effective treatment for TB disease (Taghizade Moghaddam et al., 2016; WHO, 2016) **(Fig. 1)**.

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Table 1. The Sustainable Development Goals (SDGs) for 2030 and End TB 2035 targets

Goal	End the global tuberculosis epidemic			
Indicator	Milestones		Targets	
	2020	2025	SDG 2030	END TB 2035
Reduction in number of TB deaths compared with 2015 (%)	35%	75%	90%	95%
Reduction in TB incidence rate compared with 2015 (%)	20%	50%	80%	90%
TB-affected families facing catastrophic costs due to TB (%)	Zero	Zero	Zero	Zero

It is apparently detectable that failure in achieving to SDGs for 2030 and End TB 2035 targets will take severe concerns in both individual and global public health outcomes (Dirlikov et al., 2015; Lönnroth and Raviglione, 2016). WHO recommended implications for TB preventions such as encouraging the private sector to participate in TB care especially in low-income countries, documenting and evaluating social protection and economic support for TB patients in low- and middle income countries to assess of impact and sustainability, conducts of national surveys to assess of improve services to TB patient, beside these ending TB in world links to ending risk factors of poverty, such as non-communicable disease prevention, food security, and housing (Lönnroth and Raviglione, 2016).

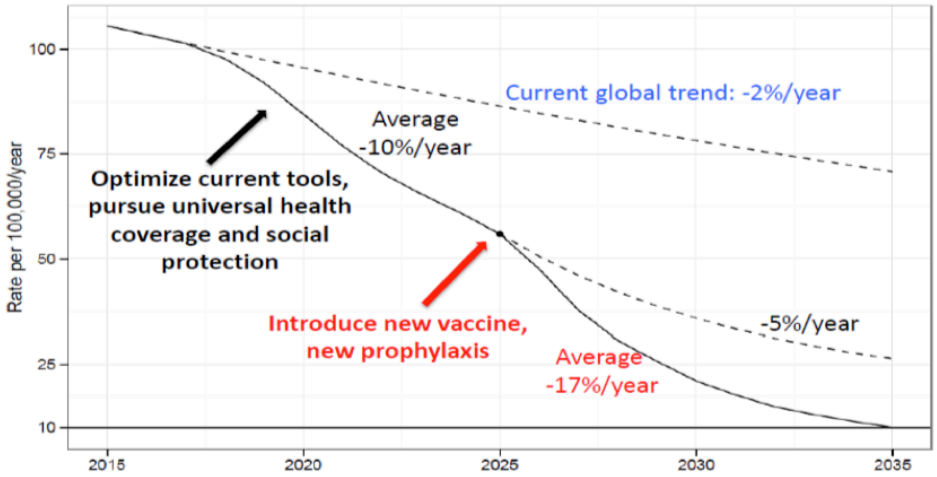

Figure 1. A predicted trend in global TB incidence rates to reach the 2035 targets (Source WHO).

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In finally above prevention strategies must be accompanied by priorities action plan for global progress towards TB and achieve to SDGs for 2030 and End TB 2035. Priorities action plan are following; identify undiagnosed cases (3 million not in TB care), MDR-TB crisis plan to deal with, appropriate and prompt treatment of TB/HIV cases, increase financing support at national level, and support innovation in the field of TB (Cousins, 2016; WHO, 2016).

Abbreviations

Tuberculosis (TB)
Sustainable Development Goals (SDGs)
World Health Organization (WHO)

Author contribution

All authors contributed in manuscript preparation. Veisani,Y and Khazaei,S obtained data and analyzed it. Delpisheh, A interpreted of data analysis. All authors drafted the first version and approve the final draft.

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Letter to Editor

The senility tsunami in Iran

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One of the achievements of the 21st century is the aging population (Angus and Reeve, 2006). According to the World Health Organization (WHO), senility is passing the 60th birthday (Bengtson and Allen, 2009). According to forecasts, by 2050, the population of the world 65-year-old age group will reach over 1.4 billion people from 550 million. It means that the world's aging index, rising from 24 people in 1950 to 33 people in 2000, will increase to 101 people in 2050 (Christensen et al., 2009). Currently, due to lower birth rates, increased life expectancy, health promotion, and disease detection, Iran is also in the age structure transition phase of the population from youth to senility (Noroozian, 2012). Therefore, elderly people are considered as the largest population group in Iran. According to the census conducted in 2016, the ratio of the elderly of Iran reached 6.1% in the past five years from 5.7% (Yearbook, 2017). It is anticipated that by 2050, the Iran's elderly population will reach 31.5% of the total population of the country (Yearbook, 2013, 2017). Due to the WHO, the world's elderly population will reach 21.5% in 2050 and 24% in Asia (Organization, 2009), according to which the population of the elderly people in Iran will be higher than the whole world average and the Asia average till 4 years (Yearbook, 2017). This demographic crisis in Iran can be called the senility tsunami, which can be debatable in various aspects including social, economic, health, medical, and political, in developing countries like Iran.

In terms of care, increasing the population of the elderly leads to an increase in disability and weakness in them, the occurrence of several illnesses simultaneously, exposure to multiple drugs, drug poisoning, and being susceptible to some disasters such as falls which will divest the possibility of independent living from the elderly (Harper, 2014; Rossat et al., 2010); diseases

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that affect not only the individual but care givers, the family, and the whole social and health system of the community and make the care process inevitable (Harper, 2014; Lee and Mason, 2010). On the other hand, studies show that about 80% of the elderly are cared by family members, while in developing countries like Iran, national plans to protect family care givers are inadequate (Noroozian, 2012). Also, changes made in senility including changes in the body's response to drugs, reduced physical ability, the experience of important life events such as retirement; inhabitancy in nursing homes; reducing income, and decreasing social communication opportunities that create loneliness feelings in the elderly (Karel et al., 2012). Obviously, the aging population, double burden of diseases, and the increased risk of disability will lead to development of chronic and untreatable diseases, resulting in increased costs of care and lowering the quality of life (Herrmann et al., 2010). Also, many developing countries have very low incomes and exit/ get out from the labor market before they have enough income to save. In addition, elderly people in developing countries often rely on resources derived from children that those resources do not have reliability, as traditional structures in these countries are changing severely (Bloom et al., 2011).

In developed countries, aging is associated with the improvement of health indicators (especially equity financing), which is facing hesitation in developing countries (Syed et al., 2012). In other words, the emergence of health problems for elderly people in developed countries, unlike developing countries, has a social and private insurance mechanism, and therefore there is no risk of imposing catastrophe expenditure on the elderly and their families in developed countries (Lee and Mason, 2011). Increasing aging population on one hand, and socioeconomic changes and personal and family lifestyles on the other hand, have led to an increase in the number of elderly care institutions (Huber et al., 2009). Admission to the nursing homes has mental consequences including feelings of rejection, mental stress, depression, and loss of chance to be with family and friends (Karel et al., 2012).

Considering the above points, it can be concluded that health of the elderly is one of the health problems in most societies, especially in developing countries including Iran, and coping with these problems requires policy making and accurate planning. Of course, if countries do not plan on this issue, they will face a lot of problems. These problems not only affect the lives of the elderly and cause losing their independence and physical, mental, social, and economic complications, but also have important implications for community health systems and lead to human and social costs and attract resources that could be used to address other community health problems in an inappropriate way. On the other hand, despite the changing population pyramid of Iran towards the senility, it has not yet been focused on the needs of the elderly as a vulnerable group of society. Therefore, considering that care needs of elderly people are different in different societies and they are influenced by several factors including cultural, social, economic and political conditions, consideration and

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attention to the needs of elderly people in different dimensions is felt in Iran and it is recommended that the country's programs for the elderly be organized around the three axes of education for healthy living for the elderly, care for the elderly, and informing the people about the importance of the elderly position and their reverence.

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Review

Efficacy of fluoride varnish for prevention of white spot lesions during orthodontic treatment with fixed appliances: A systematic review study

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Abstract

Background: White spot lesions (WSLs) are a problem commonly found in patients who use orthodontic devices. Fluoride varnish can reduce WSLs during orthodontic treatment with fixed appliances. The aim of this systematic review was to evaluate the efficacy of fluoride varnish compared with other agents for preventing WSLs during orthodontic treatment. **Methods**: Studies were searched from four databases- PubMed, Scopus, Web of Science and Cochrane Library- from January 1980 to May 2017; only studies with English abstracts were included. **Results**: Out of 432 studies searched from the databases, 33 studies were evaluated for eligibility. Of the 33 studies, 19 were excluded with reasons and 14 studies were included in the systematic review. Parameters of WSLs (decalcification score, prevalence, incidence, progression score, ΔQ and ΔZ and *DiagnoDent* (DD) pen score) were compared for the various treatments. **Conclusions**: Although there were some limitations for this systematic review study, the review showed that fluoride varnish combined with *chlorhexidine* (CHX) may be a good treatment for WSLs after orthodontic treatment, especially for a 6-month period, and that resin infiltration might also be effective for preventing WSLs. More studies are needed to further investigate these observations.

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Keywords

Chlorhexidine, Fluoride varnish, Orthodontic treatment, White spot lesions

Introduction

Fixed orthodontic appliances create stagnation areas for plaque and thus difficulties for tooth cleaning. Moreover, the irregular and non-uniform surfaces of brackets, bands and wires limit the naturally occurring self-cleansing mechanism of the oral musculature and saliva (Mount et al., 2016). White spot lesions (WSLs) are a problem commonly found in patients who use orthodontic devices. While it takes around 6 months for caries to progress in a patient not submitted to orthodontic therapy, it takes around 1 month for those who are (Lucchese and Gherlone, 2012). WSLs progress around orthodontic ligatures, brackets and bands because these appliances physically prevent thorough dental cleaning and potentiate bacterial biofilm accumulation on tooth surfaces (Lucchese and Gherlone, 2012; Tufekci et al., 2011). Suitable preventative agents or treatments for WSLs or caries have typically fluoride products (e.g. toothpaste, varnishes, gels and mouth rinse), antimicrobials (e.g. chlorhexidine (CHX)), diet counseling, xylitol gum and casein derivatives (Derks et al., 2004). Topical fluoride varnishes can reduce WSLs during orthodontic treatment and they are assumed to have the same effect following orthodontic therapy (Stecksén-Blicks et al., 2007). The aim of this systematic review herein was to evaluate the efficacy of fluoride varnish, compared to other treatments, for preventing WSLs during orthodontic treatment with fixed appliances.

Materials-Methods

Search strategies and study criteria

The studies were searched from four databases (PubMed, Scopus, Web of Science and Cochrane Library) from January 1980 to May 2017. Only publications with English abstracts were included. The search keywords were "orthodontic treatment" and "white spot" and "fluoride".

Study selection

One author (M.S.) conducted the initial search for articles, with a second author (H.R.M.) blinded to the first author's search. If there was any disagreement between the two authors, the third author (F.R.) resolved the problem. All articles included in the study review were subjected to evaluation for any

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indication of the efficacy of fluoride varnish after orthodontic treatment. Only studies with abstracts written in English were included in the review.

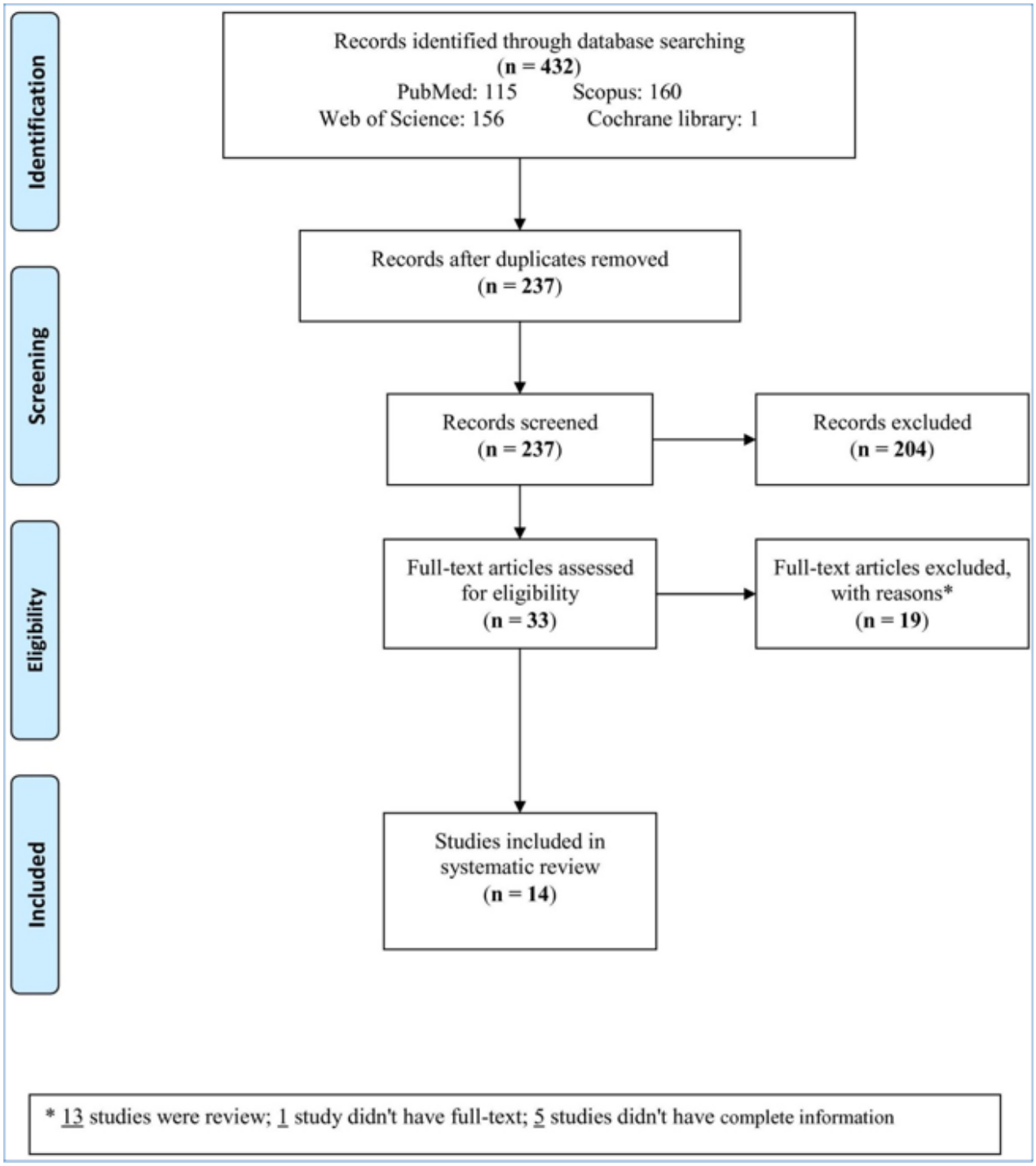

Figure 1. Flowchart of the study.

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Data extraction

The name of the author, year of publication, country, parameters of WSLs, the comparison of groups, and *P*-values were the relevant data extracted from each study. A *P*-value<0.05 was considered to be statistically significant.

Results

Study characteristics

The flowchart of the study selection is shown in **Fig. 1**. Of the 432 studies searched among the databases, 33 studies were evaluated for eligibility. Of the 33 studies, 19 were excluded for several reasons (e.g. articles were reviews and not original studies, they did not have available full-text, or they contained incomplete information) (**Fig. 1**). Thus, the remaining 14 studies were included in the systematic review (**Table 1**). The studies were reported from 1990 to 2016. Of these reported studies, 1 was from Belgium, 1 from Sweden, 4 from Brazil, 1 from Denmark, 1 from Egypt, 2 from China, 1 from Romania, 1 from the USA, 1 from India, and 1 from Poland.

Table 1. Characteristics of the studies included in the systematic review (n=14)

Study, year	Country	Parameters of WSLs	Comparison of groups	P-value
Adriaens et al., 1990	Belgium	Decalcification score at each time, in vitro study	Fluoride varnish less than Control	<0.05
		Decalcification score after 2 years, in vivo study	Fluoride varnish less than Control (4/52 molars vs. 19/52 molars)	<0.001
Ogaard et al., 2001*	Sweden	Prevalence (debonding) of WSLs on maxillary incisors	CHX varnish in combination with a fluoride varnish vs. Control (1.07 ± 0.15 vs. 1.23 ± 0.38)	<0.05
			Fluoride varnish alone vs. Control (1.13 ± 0.28 vs. 1.23 ± 0.38)	<0.05
			CHX varnish in combination with a fluoride varnish vs. Fluoride varnish alone (1.07 ± 0.15 vs. 1.13 ± 0.28)	<0.05
		Increments of WSLs during treatment on maxillary incisors	CHX varnish in combination with a fluoride varnish vs. Fluoride varnish alone (0.04 ± 0.20 vs. 0.08 ± 0.30)	>0.05
		Developed WSLs during treatment	CHX varnish in combination with a Fluoride varnish (58%)	-
			Fluoride varnish alone (61%)	-
Demito et al., 2004*	Brazil	Decalcification depths	Fluoride varnish vs. Control (108.3108± 64.79095 vs. 173.2384± 77.90322)	0.003
		Maximum decalcification depths	Fluoride varnish vs. Control (150.2912± 73.8283 vs. 209.9584± 92.0585)	0.005

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Vivaldi-Rodrigues et al., 2006*	Brazil	Change in enamel decalcification index, after 12 months vs. baseline	Fluoride varnish vs. Control (0.34 ± 0.64 vs. 0.61 ± 1.15)	0.035
		Demineralization	Fluoride varnish had 44.3% less than Control	<0.05
Stecksén-Blicks et al., 2007	Denmark	Prevalence (debonding) of WSLs, %	Fluoride varnish vs. Placebo varnish (11.7 vs. 29.7)	<0.001
		Incidence of WSL, %	Fluoride varnish vs. Placebo varnish (7.4 vs. 25.7)	<0.001
		Progression score*	Fluoride varnish vs. Placebo varnish (0.8±2.0 vs. 2.6±2.8)	<0.001
Shinaishin et al., 2011*	Egypt	Roughness height	Fluoride varnish vs. Control (370.54±2.19 vs. 569.7±2.3)	<0.001
			Unfilled sealant vs. Control (330.28±1.62 vs. 569.7±2.3	<0.001
			Filled sealant (pro seal varnish) vs. Control (307.24±2.58 vs. 569.7±2.3	<0.001
		Total surface area	Fluoride varnish vs. Control (2577.2±5.3 vs. 2886.6±9.20)	<0.001
			Unfilled sealant vs. Control (2561.2±8.07 vs. 2886.6±9.20	<0.001
			Filled sealant (pro seal varnish) vs. Control (2507.2±7.08 vs. 2886.6±9.20)	<0.001
Du et al., 2012*	China	DD scores, baseline vs. 3 months	Fluoride varnish (17.66±5.36 vs. 11.88±4.27)	9.402×10−7
			Placebo (16.19±5.70 vs. 13.75±4.76)	0.024
		DD scores, baseline vs. 6 months	Fluoride varnish (17.66±5.36 vs. 10.10±4.86)	3.794×10−10
			Placebo (13.75±4.76 vs. 13.10±5.19)	0.006
		DD scores, 3 months vs. 6 months	Fluoride varnish	0.0513
			Placebo	0.536
		DD scores, in 3 months	Fluoride varnish vs. Placebo	0.046
		DD scores, in 6 months	Fluoride varnish vs. Placebo	0.004
Jumanca et al., 2012*	Romania	DD scores immediately after take-off	Fluoride varnish vs. not receiving any special treatment; were instructed about the correct dental brushing which must be done 2 times per day (19.76±4.89 vs. 18.40±5.30)	0.197
		DD scores immediately at 3 months	Fluoride varnish vs. not receiving any special treatment; were instructed about the correct dental brushing which must be done 2 times per day (14.15±4.14 vs. 15.98±4.50)	0.045
		DD scores immediately at 6 months	Fluoride varnish vs. not receiving any special treatment; were instructed about the correct dental brushing which must be done 2 times per day (12.35±4.75 vs. 14.75±5.14)	0.004
			MI Paste Plus vs. Normal home care	>0.05

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Huang et al., 2013	USA	Mean improvement of WSLs over 8-week period	PreviDent fluoride varnish vs. Normal home care	>0.05
			MI Paste Plus vs. PreviDent fluoride varnish	>0.05
Restrepo et al., 2015*	Brazil	ΔZ (%vol/μm) in 3 months after the last application	Fluoride varnish vs Control (7459±960.1 vs. 7608±7608)	>0.05
			CHX gel vs. Control (7670±7699.6 vs. 7680±7680)	>0.05
		Lesion depth (μm) 3 months after the last application	Fluoride varnish vs Control (224.43±76.3 vs. 208.9±92.8)	>0.05
			CHX gel vs. Control (266.7±87.2 vs. 208.9±92.8)	>0.05
He et al., 2016	China	ΔQ value [estimate; 95% CI]	Fluoride varnish vs. Control [-11.83; 95% CI, -15.39 to -8.26]	<0.0001
			Fluoride film vs. Control [-7.72; 95% CI, -11.34 to -4.10]	<0.0001
			Fluoride Film vs. Fluoride varnish [4.11; 95% CI, 0.48 to 7.73]	0.0266
Restrepo et al., 2016*	Brazil	Fluorescence values, the end of the intervention (3 months) vs. Baseline	Fluoride varnish (7.2±1.6 vs. 17.2±2.3)	<0.05
			CHX gel (9.2±1.6 vs. 16.8±1.8)	<0.05
			Control (10.5±2 vs. 17±1.7)	<0.05
		Number of WSLs, the end of the intervention (3 months) vs. Baseline	Fluoride varnish (2 active caries vs 17 active caries)	<0.001
			CHX gel (7 active caries vs. 17 active caries)	<0.001
			Control (6 active caries vs. 17 active caries)	<0.001
Singh et al., 2016	India	Description of DD scores at various time intervals of observation (T0 to T4)	Fluoride toothpaste	0.744
			Fluoride toothpaste plus fluoride varnish	0.378
			Fluoride toothpaste plus CPP-ACP	0.614
		Between the group comparisons of visual scores at various time intervals of observation	Fluoride toothpaste	>0.05
			Fluoride toothpaste plus fluoride varnish	>0.05
			Fluoride toothpaste plus CPP-ACP	>0.05
		Between the group comparisons of DD scores at various time intervals of observation	Fluoride toothpaste	>0.05
			Fluoride toothpaste plus fluoride varnish	>0.05
			Fluoride toothpaste plus CPP-ACP	>0.05
Turska-Szybka et al., 2016	Poland	Progression of the treated lesions after 1 year, %	Resin infiltration and fluoride varnish vs. Fluoride varnish (7.9 vs. 29.4)	<0.001

*CHX: Chlorhexidine; ΔQ: Product of ΔF and area, and indicates the volume of the lesion; ΔF: the percentage of fluorescence loss comparing sound enamel with an identified lesion; ΔZ: the integrated mineral loss; T0: prior to application of remineralizing agents; T1: 1 month after the use of remineralizing agents; T2: 3 months after the use of remineralizing agents; T3: 6 months after the use of remineralizing agents; DD: DiagnoDent pen; CPP: casein phosphopeptide; and ACP: amorphous calcium phosphate. *The values were mean±SD. #The WSL scores of baseline between groups in each study were similar (P>0.05).*

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Parameters of WSLs

Decalcification Score

This score was significantly less for fluoride varnish than for control in an *in vitro* study and after 2 years of an *in vivo* study (Adriaens et al., 1990). Another study reported that decalcification depths and maximum decalcification depths were significantly lower in fluoride varnish compared to control (Øgaard et al., 2001). The change in the enamel decalcification index after 12 months vs. baseline was less for fluoride varnish vs. control (P=0.035) (Vivaldi-Rodrigues et al., 2006).

Prevalence and Incidence

Prevalence (debonding) of WSLs on maxillary incisors was significantly less for CHX varnish combined with fluoride varnish, for fluoride varnish compared to control, and also for CHX varnish combined with fluoride varnish compared to fluoride varnish alone (Demito et al., 2004). This prevalence was 11.7% for fluoride varnish versus 29.7% for placebo varnish (P<001) (Stecksén-Blicks et al., 2007). The incidence of WSLs was 7.4% for fluoride varnish vs. 25.7% for placebo varnish (P<0.001) (Stecksén-Blicks et al., 2007). The number of WSLs at the end of the intervention (3 months) versus baseline was less for fluoride varnish compared to control, or for control compared to CHX gel (Restrepo et al., 2016).

Progression score

Increments of WSLs during treatment on maxillary incisors for CHX varnish combined with fluoride varnish was less than the increments for fluoride varnish alone (P<0.05); progression of WSLs was 58% vs. 61% (Øgaard et al., 2001), whereas was it was 0.8% for fluoride varnish vs. 2.6% for placebo varnish (P<0.001) (Stecksén-Blicks et al., 2007). The mean improvement of WSLs over the 8-week period for MI Paste Plus and fluoride varnish were less than that for normal home care; the mean improvement of WSLs for MI Paste Plus was less than for fluoride varnish (P<0.05) (Huang et al., 2013). Progression of the treated lesions after one year was 7.9% in resin infiltration combined with fluoride varnish compared to 29.4% for fluoride varnish alone (P<0.001) (Turska-Szybka et al., 2016).

ΔQ and ΔZ

ΔQ value was significantly less for fluoride varnish and fluoride film compared to control, and also less for fluoride varnish compared to fluoride film (He et al., 2016). Indeed, ΔZ in 3 months after the last application of fluoride varnish and CHX gel was less than for control (P>0.05), ΔZ for fluoride varnish was less than that of CHX gel (P>0.05). However, lesion depth in 3 months after the last application of control was less than that for fluoride varnish and CHX gel (P>0.05); lesion depth for CHX was less than that for fluoride varnish (P>0.05)

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(Restrepo et al., 2015). Demineralization in fluoride varnish was 44.3% less than that for control (Vivaldi-Rodrigues et al., 2006). Fluorescence value at the end of the intervention (3 months) vs. baseline was (7.2±1.6 vs. 17.2±2.3), (9.2±1.6 vs. 16.8±1.8), and (10.5±2 vs. 17±1.7) for fluoride varnish, CHX gel, and control, respectively ($P<0.05$). These values were less than fluoride varnish compared to CHX gel after 3 months, and compared to baseline (Restrepo et al., 2016).

DD Scores

DD scores in 3 months and 6 months (versus baseline) were less for fluoride varnish and placebo, and also less for 6 months compared to 3 months ($P<0.05$) (Restrepo et al., 2015). At these two time points, the DD score of fluoride varnish was less than that of placebo ($P<0.05$) (Restrepo et al., 2015). Additionally, DD scores for 3 months and 6 months (versus baseline) were less for fluoride varnish compared to placebo ($P<0.05$), and less for 6 months compared to 3 months (Jumanca et al., 2012). DD and visual scores at various time intervals of observation (prior to application, 1 month, 3 months and 6 months) for fluoride toothpaste, fluoride toothpaste + fluoride varnish, fluoride toothpaste + *DiagnoDent* pen, casein phosphopeptide (CPP), and amorphous calcium phosphate (CPP-ACP) were not different ($P>0.05$). Moreover, DD scores between treatment groups at various time intervals of observation were not different ($P>0.05$) (Singh et al., 2016).

Other

Roughness height and total surface area of WSLs after fluoride varnish, unfilled sealants and filled sealants (pro-seal varnish) were all less than control ($P<0.001$). Also, roughness height for filled sealant (pro-seal varnish) was less than that for unfilled sealant, while roughness height for unfilled sealant was less than that for fluoride varnish. The total surface area for unfilled sealant was less than that for fluoride varnish; and total surface area for fluoride varnish was less than filled sealant (pro-seal varnish) (Shinaishin et al., 2011).

Discussion

This systematic review evaluated the efficacy of fluoride varnish for preventing WSLs during orthodontic treatment and compared its efficacy to other treatments. The results indicated that fluoride varnish was an effective treatment for orthodontic patients, compared to patients without treatment (control or placebo). By increasing treatment time (of fluoride varnish) to 6 months, one can reduce WSLs; thus, 6 months of fluoride varnish was more effective than a shorter treatment time. Importantly, fluoride varnish combined with CHX was more effective than fluoride varnish alone.

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The prevalence of WSLs in patients that seek orthodontic treatment is in the range of 50% to 96% (Geiger et al., 1992; Øgaard et al., 1996; Vivaldi-Rodrigues et al., 2006). Lucchese and Gherlone (2013) showed that the first 6 months are of particular importance in the development of WSLs because the majority of adolescent patients need to adapt their hygienic practices to the requirements of orthodontic therapy. It is assumed that calcium, fluoride and phosphate will penetrate the deepest areas of lesions (Llena-Puy, 2013).

Fluoride varnishes have been suggested to be safe and feasible for topical application. They include high concentrations of fluoride (not the fluoride used daily in toothpaste and mouth rinse). The varnish may also remain on tooth surfaces for several hours after application, and may be capable of releasing sufficiently high concentrations of fluoride ions to maintain surface fluoridation (Seppä, 1983). One study (Farhadian et al., 2008) revealed a 40% reduction in depth of demineralization around brackets after application of high concentration fluoride varnish. Many clinicians have applied topical fluoride to WLSs as the first step in treatment.

When the pH of oral fluids increases to normal levels, the calcium and phosphate ions of saliva are transmitted through the pellicle into the enamel; according to the laws of chemical equilibrium, this leads to remineralization (de Leeuw, 2004). Certainly, this process is greatly enhanced by fluoride in saliva and plaque. Another study (McNeill et al., 2001) reported that fluoride inhibits mineral loss during the acid dissolution process and enhances remineralization in a similar manner to that which occurs in dental enamel.

The benefits of using antimicrobial agents for the control of gingivitis have been widely discussed in the literature, and at present, CHX is considered the most effective agent for this purpose (Sari and Birinci, 2007). CHX has the capacity to prevent biofilm formation, which is one of the main etiological factors of caries disease. However, CHX affects the prevention and control of WSLs (Twetman, 2004). In a short-term *in vivo* study, a combination of daily mouth rinsing with fluoride and CHX was more effective at decreasing mineral loss and lesion development than fluoride mouth rinsing alone (Ullsfoss et al., 1994). Huizinga et al. (1991) calculated that CHX would be need to be released from the varnish for at least 6 months after a topical application (Huizinga et al., 1991). Some studies have shown the efficacy of CHX varnishes in decreasing the prevalence of caries during orthodontic treatment, yet other studies have not shown the efficacy of a varnish of 40% CHX (Kronenberg et al., 2009; Øgaard et al., 2001). Moreover, the main benefits of CPP-ACP are their ability to localize at the tooth's surface and penetrate into the supragingival plaque to provide bioavailable calcium and phosphate ions where they are most needed (Reynolds et al., 2003).

Turska-Szybka et al. (2016) concluded that resin infiltration in conjunction with fluoride varnish treatment of early facial smooth-surface caries lesions in deciduous teeth is superior to fluoride varnish treatment alone for reducing

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lesion progression (Turska-Szybka et al., 2016). The studies (Hammad et al., 2012; Mueller et al., 2006; Paris and Meyer-Lueckel, 2010) showed a good effect of resin infiltration on WSLs after debonding orthodontic brackets. The studies concluded that treatment with resin infiltration in conjunction with fluoride varnish is promising for controlling proximal lesions (Ekstrand et al., 2010) and in fact, teeth treated with resin infiltration showed higher Vickers hardness values than untreated teeth (Palamara, 2010). Based on our knowledge, there were no study on the efficacy of resin infiltration versus fluoride varnish+ CHX for WSL treatment; therefore, more studies in the future are necessary to follow up on this comparison.

Limitations of this systematic review included: (1) heterogeneity between the studies; (2) difference in age and sex between the studies; (3) few studies reported the efficacy of a type of treatment compared with other treatments; (4) many parameters of WSLs in the studies; and (5) different types of study environment (*in vitro* and *in vivo*). The studies concluded that treatment with resin infiltration in conjunction with fluoride varnish is promising for controlling proximal lesions

Conclusion

While this systematic review has several limitations, it also demonstrates that fluoride varnish combined with CHX could be an effective treatment for WSLs after orthodontic procedure. It is best that fluoride varnish be available for 6-month period of treatment, at least. The study review also demonstrated or concluded that treatment with resin infiltration in conjunction with fluoride varnish is a promising combination for controlling proximal lesions (e.g. WSLs). More studies in the future are needed to explore that.

Abbreviations

WSL: White spot lesion
CHX: Chlorhexidine
ΔQ: Product of ΔF and area
ΔF: The percentage of fluorescence loss comparing sound enamel with an identified lesion
ΔZ: The integrated mineral loss
T: Time
DD: DiagnoDent
CPP: Casein phosphopeptide

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ACP: Amorphous calcium phosphate
SD: Standard deviation

Author Contribution

All authors drafted the first version and approve the final draft.

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Review

Concise review: Extracellular vesicles from mesenchymal stem cells as cellular therapy

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Abstract

Extracellular vesicles (EVs) from mesenchymal stem cells (MSCs) are microvesicles produced from cells throughout their life. From research over recent years, there has been greater understanding about EVs, including their physiological characteristics and the role they play in cell targets. Indeed, EVs carry information (in the form of RNA, DNA and protein) to cell targets. Some of their main biological properties include angiogenesis and immune-modulation. Therefore, these properties can be exploited to treat various diseases, including bone disorders, spinal cord injury and diabetes mellitus. Recently, new methods have been developed to isolate and enrich EVs with high performance and low-toxicity. Thus, EVs have emerged as the new generation of stem cell therapy. This concise review aims to highlight some recent achievements of EVs in preclinical and clinical applications.

Keywords

Acellular therapy, Exosome, Extracellular microvesicle, Mesenchymal stem cell, Microvesicle, Stem cell

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Introduction

Stem cells are unspecialized cells with long-lasting self-renewal potential. After differentiation they can become specialized cells with new physiological functions (Bongso and Lee, 2005). In recent years, stem cells have been discovered to exhibit other useful functions, including secretion of cytokines

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(Kilroy et al., 2007), i.e. growth factors which help stimulate tissue regeneration (Boomsma and Geenen, 2012), and notably immune modulation of mesenchymal stem cells (Abdi et al., 2008; da Silva Meirelles et al., 2009; Prockop and Oh, 2012; Yanez et al., 2006). To date, stem cells (particularly MSCs) have been discovered to have at least 3 functionst: homing and differentiation into tissue specialized cells, production of cytokines and growth factors, and immune modulation.

Given these functions, stem cells have been tested for various diseases; in fact over the last 50 years they have been evaluated in more than 50 different diseases (Ginn et al., 2013; Squillaro et al., 2016; Van Pham, 2016). Hematopoietic stem cell transplantation has been widely used to treat hematopoietic malignancies, and MSC therapy has already been approved as routine treatment for a number of diseases in Canada, Japan, Korea, China, India and Vietnam. Compared to HSCs, MSCs play many more functions *in vivo* and possess unique characteristics, such as immune modulation and tissue healing via secreted factors (Chase and Vemuri, 2012).

One of the most well-known secreted factors is extracellular vesicles (EVs). EVs have distinct physiological characteristics and have been studied for disease applications for the past 5 years. This review aims to summarize the characteristics of EVs and their applications in the clinic.

What are extracellular vesicles?

Extracellular vesicles are nano-sized particles produced from the live cells during their lifespan. EVs can be classified into two main kinds based on their size: exosomes and microvesicles. Exosomes are about 40-150 nm in diameter whereas microvesicles are about 50nm-2000 nm in diameter. They differ in the way they are produced and, thus, exosomes and microvesicles exhibit different properties.

Generally, exosomes are produced from secretory mechanisms and are regulated by endosomal sorting complex mechanisms, which are associated with transport proteins (e.g. ESCRT), Rab proteins, tumor protein p53 pathway, tumor suppressor-activated pathway 6, and ceramide/neutral sphingomyelinase (Lespagnol et al., 2008; Ostrowski et al., 2010; Rak, 2013). Moreover, exosomes are rich in tetraspanins (CD63, CD81, CD9, etc.), gangliosides, sphingomyelin, and saturated lipids. Exosomes generally have a more rigid membrane than that of microvesicles which allow them to be more resistant to degradation and thus more stable (Pols and Klumperman, 2009; Raposo and Stoorvogel, 2013; Stoorvogel et al., 2002).

Microvesicles are directly produced from the plasma cellular membrane and therefore they contain some cytoplasmic components. Unlike exosomes,

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microvesicles contain markers of the original cells, such as common proteins of the cellular membrane like integrins, glycoprotein Ib (GPIb), and P-selectin (Kastelowitz and Yin, 2014; Raposo and Stoorvogel, 2013).

Besides exosomes and microvesicles, apoptotic bodies and oncosomes can also be found in EVs (Crescitelli et al., 2013; Meehan et al., 2016). Apoptotic bodies are products of the apoptotic process while oncosomes are larger vesicles produced from cancer cells.

Physiological functions of EVs

EVs are comprised of exosomes, microvesicles, apoptotic bodies and oncosomes, and play important physiological roles, especially in cellular communication. They are important not only in the normal physiological processes but also in pathological conditions. The roles that EVs play are dependent on the content they cargo. It was initially discovered that EVs contain siRNA molecules (Eirin et al., 2014; Kumar et al., 2015; Lai et al., 2015; Vallabhaneni et al., 2015). Nowadays, it is known that they carry many more forms of "information". In fact, exosomes have been described as "information cargos" for their transport of siRNA, DNA, peptides, and proteins (Baglio et al., 2012; Biancone et al., 2012; Camussi et al., 2010; Lai et al., 2015; Rani et al., 2015; Yu et al., 2014). All of these aforementioned molecules help regulate cell targeting by modulating gene expression and gene regulation in target cells at the level of post-transcription and translation (Camussi et al., 2011; Collino et al., 2010; Mokarizadeh et al., 2012; Zhang et al., 2015b).

EVs from mesenchymal stem cells (MSCs)

It has been known for over a decade that MSCs can produce EVs. MSC-derived EVs (MSC-EVs) contain at least 2 components: exosomes and microvesicles. Both exosomes and microvesicles express tetraspanin molecules and MSC markers on their surface; these include CD9, CD63, CD81 and CD107, and CD29, CD73, CD44 and CD105, respectively (Lai et al., 2015; Yu et al., 2014).

The components inside EVs have been the focus of many research studies (Baglio et al., 2012; Lo Sicco et al., 2017; Wang et al., 2017; Yuan et al., 2017). The main components found inside MSC-EVs are miRNAs (De Luca et al., 2016; Fafian-Labora et al., 2017; Livingston and Wei). Notably, MSC-EVs have been found to contain miR-223, miR-564, and miR-451 (De Luca et al., 2016; Nawaz et al., 2016). These miRNAs play the important roles in cell survival, cell differentiation, and immune regulation (Yanez-Mo et al., 2015)). Besides miRNAs, other RNAs can be found in MSC-EVs (Borger et al., 2017). Such RNAs include transcription factor CP2/clock homolog (which regulates transcription),

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retinoblastoma-like 1 (which also regulates transcription), small ubiquitin-related modifier 1 (which regulates cell proliferation), and interleukin-1 receptor antagonist (which regulates immune responses) (Tomasoni et al., 2013).

MSC-EVs are generally comprised of 3 main groups of proteins/molecules, including surface receptors, signaling molecules, and cell adhesion molecules. These proteins were demonstrated to regulate cell self-renewal and differentiation. Surface receptors and cell adhesion molecules are present on the surface of EVs, and likely originated from cell membrane. Conversely, signaling molecules are usually found within EVs, and likely originated from secretory processes. Some common surface receptors found in MSC-EVs include platelet-derived growth factor receptor, epidermal growth factor receptor, and plasminogen activator urokinase receptor. Some common cell adhesion proteins include fibronectin, ezrin, IQ motif containing GTPase activating protein 1, CD47, integrins, lectin galactose binding soluble 1 (LGALS1), and lectin galactose binding soluble 3 (LGALS3) (**Fig. 1**).

Other important molecules to be found within MSC-EVs include RAS-related protein/neuroblastoma RAS, mitogen-activated protein kinase 1 (MAPK1), guanine nucleotide-binding protein subunit 13/G protein subunit 12 (GNA13/GNG12), cell division control protein 42 homolog, Vav guanine nucleotide exchange factor 2, transforming growth factor beta, mitogen-activated protein kinase, and peroxisome proliferator-activated receptor (Baglio et al., 2012). Given these important aforementioned components, MSC-EVs represent a cellular therapeutic approach with great potential in regenerative medicine (**Fig. 1**).

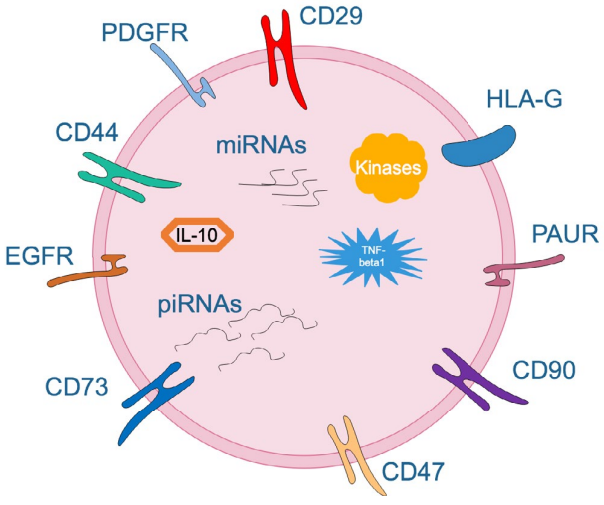

Figure 1. The components of EVs from MSCs. EVs from MSCs contain some markers included CD29, CD44, CD73, CD90, CD47; some receptors in the their surfaces, and some miRNAs, piRNAs, IL-10, TNF-beta1, some kinds of kinases… inside the EVs.

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Applications of EVs

Critical size bone defects

MSCs-EVs have been evaluated for treatment of certain bone defects. Recently, MSC-EVs were shown to stimulate bone regeneration in a bone defect model (Qin et al., 2016). MSC-EVs promoted cartilage restoration and subchondral bone regeneration in osteochondral defects (Zhang et al., 2016). They were also capable of preventing bone loss and enhancing neo-angiogenesis in a femoral head necrosis model (Liu et al., 2017). Qin et al. (2016) isolated MSC-EVs by gradient ultracentrifugation and ultrafiltration. These EVs were used to treat osteogenesis both *in vitro* and *in vivo*. The authors showed that MSC-EVs could induce bone formation in Sprague Dawley rats with calvarial defects (Qin et al., 2016). Qin et al. showed evidence that miR-196a in MSC-EVs may play an essential role in the regulation of osteoblast differentiation (Qin et al., 2016).

Zhang et al. (2016) also tested the intra-articular injection of 100 ug EVs per rat bearing osteochondral defects (n=12 adult rats); the EVs were derived from human embryonic MSCs. After 12 weeks of injection, the EV-treated group showed an histological score greater than that of PBS. Moreover, cartilage and subchondral bone were restored (Zhang et al., 2016).

EVs derived from MSCs can differentiate from induced pluripotent stem cells, according to a study by Liu et al. (2017) (Liu et al., 2017). Indeed, EVs are sometimes referred to as induced pluripotent stem cell-/differentiated mesenchymal stem cell-derived exosomes (iPS-MSC-Exos). These exosomes can stimulate endothelial cells to proliferate and migrate, and stimulate tube forming via expression of PI3K/Akt signaling pathway (Liu et al., 2017). By this mechanism, the iPS-MSC-Exos can prevent the bone loss and increase microvessel density in the femoral head compared to the placebo group in the rat model.

Epidermolysis bullosa (EB)

Epidermolysis bullosa (EB) is a rare genetic disorder of which dystrophic epidermolysis bullosa (DEB), which causes skin fragility, is one of the major forms. In this disorder, patients lack collagen type 7 (C7) and have defective anchoring fibrils at the dermal-epidermal junctions (Fine et al., 2014). Recently, this disease was treated by infusion of the MSC-EVs. EVs derived from human embryonic stem cell differentiation, from human biblical cord, and from adipose tissue were used to treat this disease in animal models. The first pre-clinical trial was conducted in a murine model; the authors injected MSC-EVs (from human ESCs) and evaluated allogenic skin grafts in the mouse model. The results showed that the infusion induced the M2 phenotype in monocytes *in vitro* and regulatory T cell polarization *in vivo*, as well as enhanced the survival of skin grafts (Zhang et al., 2014).

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EVs from umbilical cord-MSCs have demonstrated to activate the WNT4 signaling pathway in deep second degree burn injury in rats. Therefore, these EVs can accelerate skin regeneration (Zhang et al., 2015a). EVs from ADSCs can recruit fibroblasts to the wound areas, increase collagen type I and III, and reduce scar formation (Hu et al., 2016).

Spinal cord injury (SCI)

Spinal cord injury is a condition related to a disconnection of axons which direct signals from the brain to peripheral organs. Injection of EVs was shown to be an effective treatment for SCI in animals. Both EVs from MSCs and embryonic neurons successfully reduced inflammation and promoted neuro-regeneration in rats after SCI (Doeppner et al., 2015; Han et al., 2015; Rivero Vaccari et al., 2016). The mechanisms of action are likely a weakening of TLR4 mediated signaling and reduction of the IL-1beta and TNF-alpha axes (Teixeira et al., 2015).

GVHD in hematopoietic stem cell transplantation

Graft versus host disease (GVHD) is a common condition that arises in almost all cases of hematopoietic stem cell transplantation (HSCT), especially allo-graft transplantation. GVHD arises when the new immune system that comes from the HSCs attack to the owner cells. Recently, EVs from MSCs can contribute to improving the allo-HSCT allograft. Indeed, the MSC-EVs can modulate the immune system (Blazquez et al., 2014; Budoni et al., 2013; Chen et al., 2016; Conforti et al., 2014), therefore, they can be used to prevent or reduce the immunoreactions as GVHD. In the clinical study, Kordelas et at. showed that bone marrow MSC-EVs could alleviate the GVHD symptoms in grade IV GVHD patients with no side effects (Kordelas et al., 2014). This study also showed that MSC-EVs contained some anti-inflammatory factors included IL-10, TGF-beta and HLA-G.

In another study in animal model, Wang et al. also showed that umbilical cord blood derived MSCs-EVs can prevent the acute GVHD in mouse model of allo-HSCT (Wang et al., 2016).

Acute renal injury

Acute renal failure (ARF) is characterized by the loss of renal function with concurrent accumulation of creatinine and nitrogen metabolism products (e.g. urea). This condition is associated with ischemia, reperfusion injury, and/or exposure to nephrotoxic agents. The effects of EVs in ARF have been investigated in some models of ARF, including models of kidney injury induced by glycerol, cisplatin, and gentamicin. In these models, high inflammatory reactions were observed, with an increase of interstitial infiltrate, apoptosis and tubular necrosis. MSC-EVs have been evaluated as treatment in these models. In almost all cases, injection of EVs decreased inflammation and inhibited

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apoptosis. To date, there are 3 clinical trials using MSCs to evaluate the efficacy and safety of ARF: NCT01275612, NCT00733876 and NCT01602328. However, there has not been any clinical trial using EVs for the treatment of ARF.

Diabetes mellitus

The first documented study showing the application of MSC-EVs for treatment of diabetes type 1 (T1D) was reported this year; Shigemoto-Kuroda et al. (2017) demonstrated that MSC-EVs effectively prevented the onset of disease in T1D. In this study, the authors showed that the effects MSC-EVs were similar to that of MSCs in terms of immune modulation potential. EVs have been shown to be capable of inhibiting antigen presenting cells, and Th1 and Th17 cells (Shigemoto-Kuroda et al.).

Conclusion

EVs from MSCs contain some biological components such as DNA, RNA and proteins. These molecules help EVs exhibit particular physiological activities and functions, similar to those of MSCs, such as stimulation of tissue regeneration and immune modulation. Therefore, EVs from MSCs have become increasingly popular to study in recent years. Importantly, primary investigations have indicated the promise of EVs in applications of regenerative medicine.

Abbreviations

ARF: Acute renal failure
EB: Epidermolysis bullosa
EVs: Extracellular vesicles
GVHD: Graft versus host disease
HSCT: Hematopoietic stem cell transplantation
MSCs: Mesenchymal stem cells
SCI: Spinal cord injury
T1D: Diabetes type 1

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Original Research

Evaluation of Quetiapine Fumarate and its Solid Lipid Nanoparticles as antipsychotic drug in rat model of schizophrenia

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Abstract

Background: The present study compares the efficacy of quetiapine fumarate (QF) and QF-loaded solid lipid nanoparticles (QFSLN) as antipsychotic drugs for schizophrenia. **Methods**: To induce schizophrenia-like symptoms, a group of rats was injected intraperitoneally (i.p.) with ketamine (25mg/kg b.w.) for 1 week to establish a rat model of schizophrenia. The incidence of schizophrenic symptoms was estimated to be equivalent to the control group. To estimate the pronounced antipsychotic effect of QF, a low dose (LD) of 10 mg/kg b.w. and a high dose (HD) of 30 mg /kg b.w. were orally administrated to two groups of rats (designated L.QF and H.QF) for 3 weeks (2 weeks without ketamine injection; the last week with ketamine). To achieve the optimal therapeutic response of QF drug, 2 other groups of rats were administered orally the equivalent low and high doses of QF in its solid lipid nanoparticle form (L.QFSLN) and (H.QFSLN) for 3 weeks in the same manner. The treatments were given after 1 h of ketamine injection. To assess the effect of different doses of treatment on hyperlocomotion and cognitive impairment induced by ketamine, an open field test and passive avoidance test were conducted. In addition, excitatory and inhibitory amino acids, as well as catecholamines, were estimated in brain regions (cortex and hippocampus). The study was extended to estimate the side effects of different treatments on hepatorenal functions and lipid profile. Additionally, samples were subjected to immunohistochemical analysis. **Results**: QFSLN treatment showed enhanced effect over QF in a dose-dependent manner with minimal side effects in schizophrenic rats. In addition, immunohistochemical examinations of brain tissues confirmed the biochemical data.

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Antipsychotic drugs, Catecholamines, Excitatory and inhibitory amino acids, Nanoparticles, Schizophrenia

Introduction

Schizophrenia is a complex neuropsychiatric disease that consists of positive symptoms (delusions and hallucinations), negative symptoms (blunted affect and social withdrawal), and cognitive impairments (Pickard, 2015). Previous investigations have shown an imbalance of several neurotransmitter systems in the pathophysiology of schizophrenia (Arion et al., 2007; De Oliveira et al., 2011). The dopaminergic pathway was the first to be studied in schizophrenia. Dopaminergic hyper-function manifests in the aforementioned positive symptoms. In addition to dopamine (DA), glutamate is one of the main neurotransmitters involved in schizophrenia pathophysiology (De Oliveira et al., 2011). The scientific evidence of the involvement of glutamate is from the fact that phencyclidine (PCP) and ketamine are two N-methyl-D-aspartate (NMDA) glutamatergic receptor antagonist drugs which cause severe symptoms similar to those observed in schizophrenia (Arion et al., 2007; De Oliveira et al., 2011).

Ketamine has been widely used to induce characteristics of cognitive impairment seen in schizophrenia (e.g. difficulty in functions related to attention, memory and behavior). Indeed, ketamine induces schizophrenic-like psychotic symptoms in normal adult subjects (Krystal et al., 1994). Moreover, Malhotra et al., (1996) estimated that sub-anesthetic doses of NMDA receptor antagonists (e.g. ketamine) induces a spectrum of behavioral responses in healthy human volunteers that resemble positive, negative and cognitive schizophrenic-like symptoms (Malhotra et al., 1996).

Antipsychotic drugs are classified as typical or atypical antipsychotics. Typical antipsychotic such as chlorpromazine and haloperidol ameliorate only the positive signs. Atypical antipsychotics such as clozapine, quetiapine and risperidone are useful in treating the positive, negative and cognitive signs. Both typical and atypical antipsychotics can block DA receptors (Bellino et al., 2006). The blockade of DA receptor D2 in the mesolimbic area is considered to be responsible for the reverse of positive symptoms by antipsychotics. Atypical antipsychotics can also bind to serotonin (5-HT) receptors. QF is an atypical psychotropic agent that belongs to the thienobenzodiazepine class of schizophrenia drugs (Barch et al., 2001). In 1985 scientists at AstraZeneca Pharmaceuticals developed QF, which was later approved by the US FDA in 1997 (Riedel et al., 2007). Since then, QF has been used in the treatment of

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several mental disorders in more than 70 countries, such as Canada, Europe and Japan. Nevertheless, QF has poor oral bioavailability (about 9%) with a plasma half-life of 6 hours (Mehnert and Mäder, 2001). Narala and Veerabrahma (2013) reported that the poor bioavailability of QF was solved by using the SLN formulation of QF (Narala and Veerabrahma, 2013). The SLN form was optimized based on nanoparticles and drug release features (Kreuter, 2001; MuÈller et al., 2000).

Bcl-2, one of the key proteins that suppress cell apoptosis, was examined to discern the neuroprotective effects of the antipsychotic drugs. Up-regulation of Bcl-2 expression is a critical mechanism for cell survival (Adams and Cory, 2001).

The aim of this study was to evaluate and compare QF and QFSLN as antipsychotic drugs to treat ketamine-induced schizophrenia-like symptoms in albino rats.

Materials-Methods

Materials

Drugs

Quetiapine and ketamine were purchased from the local Egyptian market. Ketamine was dissolved in normal saline and injected intraperitoneally (i.p.) at a dose of 25 mg/kg b.w. (Malhotra et al., 1997). QF was orally administered to rats in two doses, 10 mg/kg b.w. (equivalent to therapeutic human daily dose) and 30mg/kg b.w., for 3 weeks.

All other chemicals were of high-performance liquid chromatograph (HPLC) analytical grade and commercially available.

Chemical preparation of QF loaded solid lipid nanoparticles (QFSLN): QFSLN were prepared by hot homogenization followed by ultrasonication. QF, solid lipid and lecithin were then dissolved in 10 ml of a mixture of methanol and chloroform (1:1). Organic solvents were completely removed using a rotary evaporator. The embedded lipid layer was melted by heating to 5°C above the melting point of the lipid. An aqueous phase was prepared by adding the stabilizer Tween 80 in distilled water (1.5% w/v) and heating to the same temperature of the oil phase. The hot aqueous phase was added to the oil phase and homogenization was performed at 12,000 rpm using a homogenizer (DIAX 900 Heidolph, Germany) for 5min. The coarse oil in water emulsion was obtained by sonication using a probe (12T) sonicator (Vibracell Sonics, USA) for 20 min. QF-loaded SLN were finally obtained by allowing the hot nano-emulsion to cool at room temperature (Blasi et al., 2013).

Animals

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Thirty-six male albino rats weighing about 170 g (±10 g) were used in this study. Animals were obtained from the animal house of NODCAR, Egypt. They were kept under strict hygienic conditions and allowed free access to diet and tap water. They were acclimated to the environment for two weeks prior to the start of the experiment. The experimental animal protocol was approved by the ethical committee of NODCAR overseeing animal care and usage.

Experimental design

Rats were randomly divided into six groups (six rats each). Group 1 was the control (C) group wherein rats were fed a basal diet and injected i.p. with 0.2 ml of saline per rat; Group 2 was the ketamine (Ket) group wherein rats were injected i.p. with ketamine (25 mg/kg b.w.) daily for 1 week; Group 3 was the Low Dose QF (L.QF) treated group wherein rats were orally administered with QF (10 mg/kg b.w.) for 3 weeks; and Group 4 was the high dose QF (H.QF) treated group wherein rats were orally administered QF (30 mg/kg b.w.) for 3 weeks (2 weeks without ketamine and the last week with ketamine). Additionally, Group 5 was the low dose QF-loaded solid lipid nanoparticle (L.QFSLN) treated group wherein rats were orally administered with a daily dose of QFSLN (10 mg/kg b.w.) for 3 weeks, and Group 6 was the high dose QF-loaded solid lipid nanoparticle (H.QFSLN) treated group wherein rats were orally administered with a daily dose of QFSLN (30 mg/kg. b.w.) for 3 weeks. At the third week, ketamine was injected i.p. (25 mg/kg b.w.) in groups 2-6.

At the end of the experiment, blood samples were collected from the retro-orbital plexus veins, rats were sacrificed, and serum samples were separated at 3000 rpm for collection and storage at -20°C until analysis. Brain tissues were quickly removed from each animal and washed in ice-cold saline. Two brain tissue samples from each cortex and hippocampus area of each rat were used. One part was immersed in formalin for immunohistochemical evaluation and the other part was homogenized separately in 70% iced methanol to yield a 10% homogenate (w/v) solution, which was stored at -20°C until further analysis.

Methods

Animal behavior

Open field test: To estimate locomotor activity, rats were placed in an open field installation (El-Sisi, 2015), i.e. a square wooden field measuring 90x90x25 cm. The wood of the equipment was covered with a plastic laminate (Formica, Cincinnati, OH), which prevents absorption of fluids (e.g. urine of rats). The platform was divided by black lines into 36 small squares (15x15 cm). The open field maze was cleaned between each rat test using 70% ethyl alcohol to bypass odor cues. The rats were transferred to the test room in their home cages and examined one at a time for 5 min each. Rats were handled by the base of their tails at all times. The rats were brought from their home cages and placed randomly into one of the four corners of the open field facing the center. The

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behavioral scores were estimated, in this analysis, by the total number of line crossings.

Passive avoidance test: Passive avoidance test was used to assess the impact of the different treatments on memory. The apparatus was comprised of identical illuminated and non-illuminated boxes. The illuminated compartment (20x20x20 cm) connected with the non-illuminated compartment (20x20x20 cm) by a guillotine door (5x5 cm). The floor of the non-illuminated compartment was composed of 2 mm stainless steel rods spaced 1cm apart. Each rat were gently placed into the illuminated compartment for an acquisition trial and the door between the two compartments was opened after 10 sec. The rat was placed again in the illuminated compartment for a retention trial. The time taken for a rat to enter the dark part after opening of the door was recorded as the latency time for both acquisition and retention trials. Latency for entering the dark compartment was recorded up to 150 sec. If a rat did not enter the dark chamber within 30 sec, the rat was removed and assigned a latency score of 150 sec. QF was given 1 h after the acquisition trial (McLamb et al., 1990).

Biochemical analysis

Assessment of brain areas (cortex& hippocampus) amino acids: In the brain tissues, excitatory (glutamate) and inhibitory (gamma-Amniobutyric acid; GABA) amino acids were determined using HPLC methods (Heinrikson and Meredith, 1984). Catecholamines norepinephrine (NE), serotonin (5-HT) and DA were estimated by HPLC methods according to Pagel et al., (2000) (Pagel et al., 2000).

Assessment of liver function: The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and creatine kinase (CK) were measured by commercial kits according to the method of IFCC (2010) (Schumann et al., 2010). Alkaline phosphatase (ALP) was measured by a commercial kit according to the method of Tietz (1994) (Tietz, 1994). Albumin concentration was measured by a commercial kit according to the method of Tietz (1995) (Tietz, 1995).

Evaluation of kidney function: Determination of serum urea and creatinine were carried out by commercial kits according to the methods of Tietz (2005) and Levey et al., (2007), respectively (Levey et al., 2007; Tietz, 2005).

Determination of lipid profile: Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were measured by commercial kits following the methods described by NCEP (2001) (Panel, 2001).

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) for Bcl2 was performed. Sections were fixed in formalin and then embedded in paraffin. The sections were pre-treated using

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pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH 6) and then incubated at 1/1000 dilution for 15 min at room temperature. A goat anti-chicken biotinylated secondary antibody was used to detect the primary antibody and visualized using an HRP conjugated ABC system. The sections were counterstained with hematoxylin and mounted with DPX mounting medium.

Statistical analysis

Data values were expressed as mean ± S.E of 6 rats. One-way ANOVA tests (from SPSS version 23) were used to study the relationship between the different variables. $P<0.05$ was considered statistically significant (Armitage, 2008).

Results

The QF-loaded nanoparticles were generated are shown in **Figure 1**. The surface morphology was analyzed using scanning electronic microscope, which confirmed that nanoparticles were mostly spherical in shape (**Fig. 1A, B**). The nanoparticles had an approximate particle size of 134-162 nm (**Fig. 1C**).

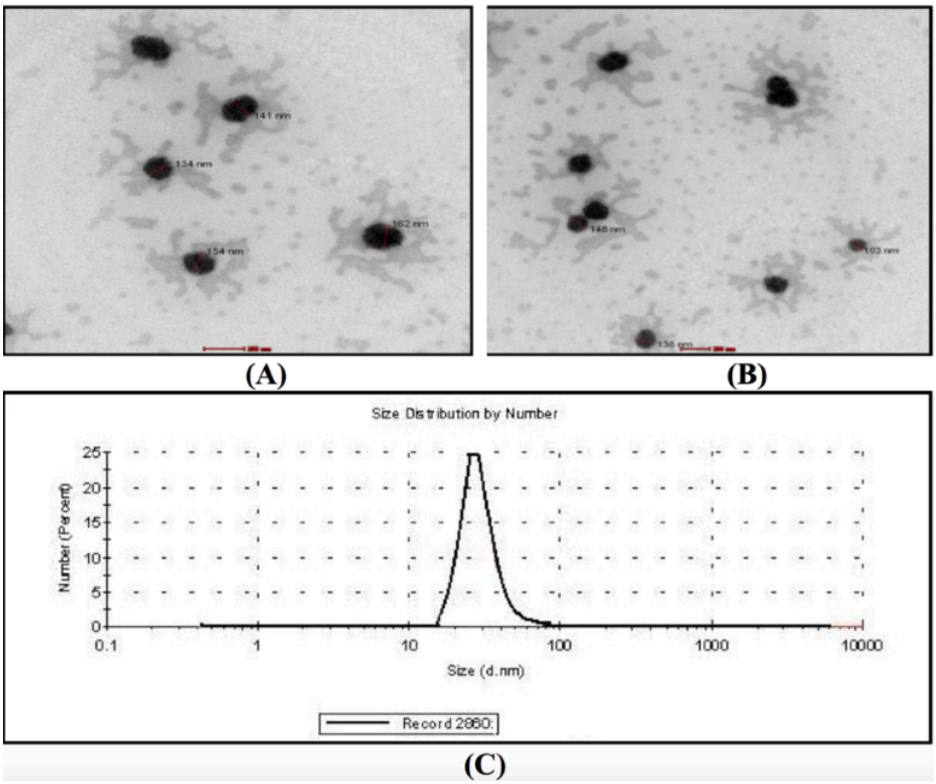

Figure 1. QF-loaded nanoparticles. (**A,B**) QFSLN under Transmission Electron Microscope (TEM), (**C**) particle size distribution.

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Animal behavior

The schizophrenic symptoms of rats were demonstrated using open field test (Fig. 2A) and passive avoidance test (Fig. 2B). The number of squares in the open field test were significantly increased ($p<0.05$) in the group of rats treated with ketamine; in addition the time spent in the dark room (latency time) in the passive avoidance test was significantly increased ($p<0.05$) with respect to the control group. These effects were more pronounced ($p<0.05$) in rats treated with QFSLN compared to QF. The data revealed that the observed effects were dose-dependent since the greatest effect was observed in the higher dose of treatments.

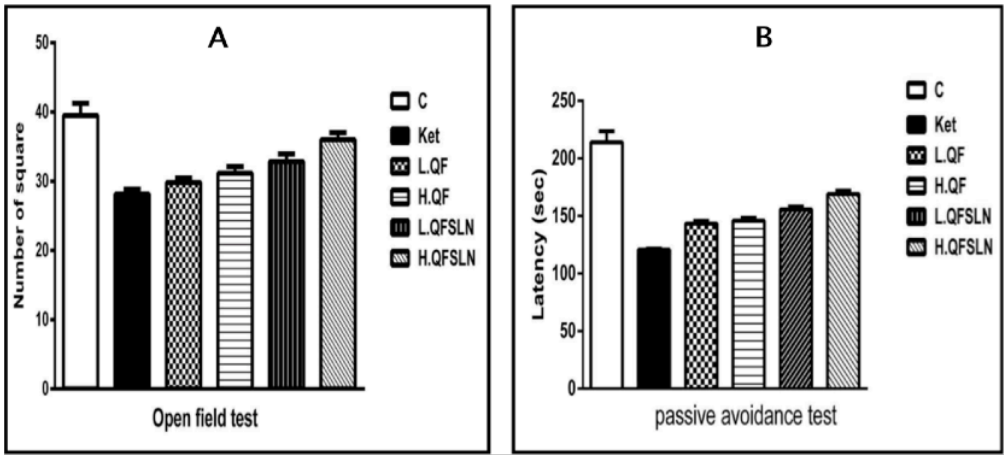

Figure 2. Effects of QF and QFSLN on the schizophrenic symptoms of rats. (**A**) Effect of QF and QFSLN on locomotors activity in open field test against ket-induced schizophrenia-like symptoms in rats. (**B**) Effect of QF and QFSLN on latency time in passive avoidance test against ket-induced schizophrenia-like symptoms in rats.

Biochemical analysis

The effects of the different treatments on DA, NE and 5-HT in brain hippocampus and cortex are represented in Table 1. It is clear that administration of ketamine caused a significant ($p<0.05$) elevation of DP in both regions. NE was significantly ($p<0.05$) reduced in the cortex but unchanged in the hippocampus region, 5-HT was slightly ($p>0.05$) reduced in the hippocampus and significantly ($p<0.05$) increased in the cortex region, compared to the control group. On the other hand, QF administration ameliorated the disturbance of the neurotransmitters either at low or high doses while the effect was even more noticeable in rats treated with QFSLN, in a dose-dependent manner.

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Table 1. Effect of QF and QFSLN on DA, NE and 5HT in brain areas against ket-induced schizophrenia-like symptoms in rats

Groups	DA (µg/g tissue)		NE (µg/g tissue)		5HT (µg/g tissue)	
	Cortex	Hippocampus	Cortex	Hippocampus	Cortex	Hippocampus
C	1.20±0.11	0.92±0.20	3.59±0.35	3.38±0.12	0.84±0.04	1.03±0.01
Ket	1.69±0.13*	1.47±0.01*	2.33±0.06*	3.37±0.02	1.58±0.14*	0.99±0.015
L.QF	1.21±0.05*	1.02±0.05	3.75±0.36*	3.15±0.15	1.04±0.01*	1.17±0.03
H.QF	1.23±0.04*	0.93±0.03*	3.80±0.47*	3.17±0.27	1.21±0.04*	1.17±0.03
L.QFSLN	1.11±0.11*	0.83±0.03*	3.45±0.44*	3.11±0.17	1.16±0.08*	0.98±0.03
H.QFSLN	0.85±0.08*	0.77±0.02*	3.68±0.35*	3.01±0.20*	0.84±0.07*	0.89±0.03

Data expressed as mean ± S.E. Changes are considered significant when p< 0.05.
** Significant difference from Ket group. Ket group was compared against C group and the other groups where compared against Ket group.*

Table 2. Effect of QF and QFSLN on GABA and Glutamate in brain areas against ket-induced schizophrenia-like symptoms in rats

Groups	GABA (µg/g tissue)		Glutamate (µg/g tissue)	
	Cortex	Hippocampus	Cortex	Hippocampus
C	3.48±0.13	3.67±0.12	14.1±0.38	13.4±0.19
Ket	2.88±0.13*	3.32±0.14	10.5±0.15*	14.3±0.44*
L.QF	3.57±0.11*	3.76±0.12*	13.5±0.14*	12.5±0.52*
H.QF	3.51±0.13*	3.66±0.12*	13.7±0.09*	12.9±0.29*
L.QFSLN	3.21±0.30*	3.67±0.08*	14.0±0.13*	14.0±0.13
H.QFSLN	3.33±0.28*	4.01±0.12*	14.4±0.19*	14.1±0.09

Data expressed as mean ± S.E. Changes are considered significant when p< 0.05.
**Significant difference from Ket group. Ket group was compared against C group and the other groups where compared against Ket group.*

Table 2 illustrates the effect of different treatments on the level of GABA and glutamate in the cortex and hippocampus of brain tissues. From the table, it is clear that ketamine caused a reduction in the level of GABA in the cortex and hippocampus regions. Glutamate was reduced in the cortex and significantly ($p<0.05$) increased in the hippocampus. The different treatments ameliorated these values.

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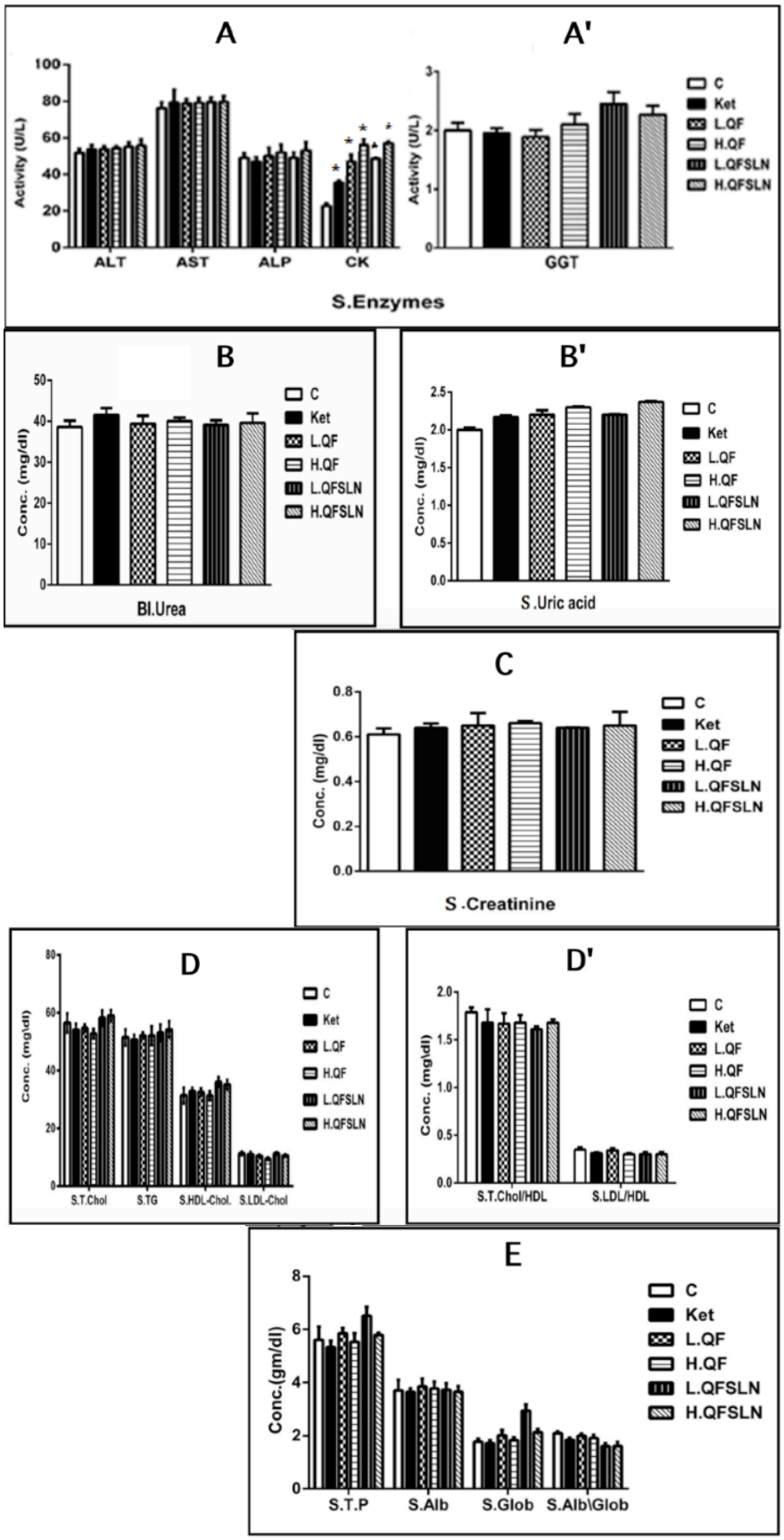

Figure 3. Effect of the various treatments on hepatorenal function, serum proteins and serum lipids after 3 weeks of treatment. (A,A') Effect of QF and QFSLN on liver enzymes (ALT, AST, ALP, CK and GGT) against k e t-i n d u c e d schizophrenia-like symptoms in rats. **(B,B')** Effect of QF and QFSLN on Bl.Urea and S.uric acid against ket-induced schizophrenia-like symptoms in rats. **(C)** Effect of Q F and QFSLN on S.creatinine against ket-induced schizophrenia-like symptoms in rats. **(D,D')** Effect of QF and QFSLN on lipid profile parameters against ket-induced schizophrenia-like symptoms in rats. **(E)** Effect of QF and QFSLN on S. total protein, albumin, globulin and albumin/globulin ratio against ket-induced schizophrenia-like symptoms in rats.

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Figure 3 illustrates the effect of the various treatments on hepatorenal function, serum proteins and serum lipids after 3 weeks of treatment. In general, there was a slight ($p>0.05$) increase on the different parameters after treatment with H.QF and QFSLN (low & high doses); however, only treatment with ketamine induced a significant ($p<0.05$) increase in the level of serum CK.

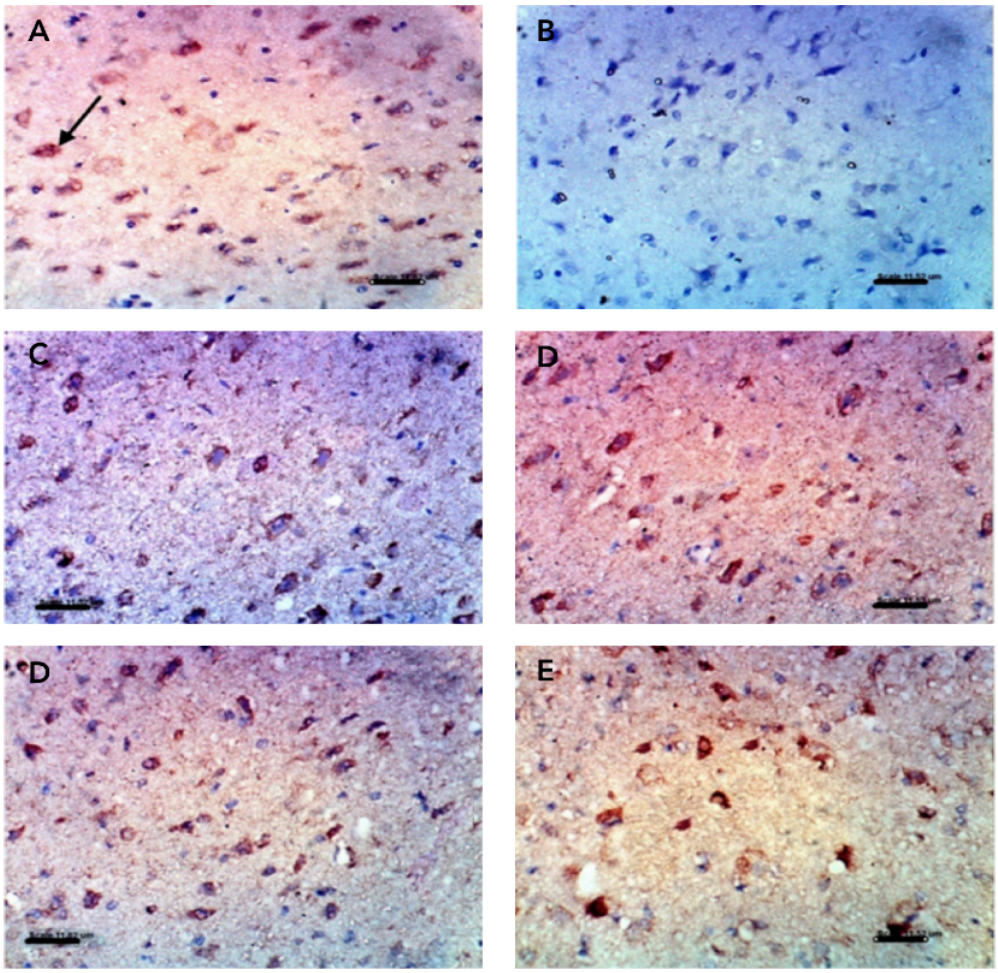

Figure 4. Immunohistochemical staining of BCL2 in cerebral cortex of rat from some groups. (**A**) Control negative group: strong positive expression of BCL2 (immuno positivity indicated by brown colour). (**B**) Group 2: negative expression of BCL2. (**C**) Group 3: moderate expression of BCL2. (**D**) Group 4: strong positive expression. (**E**) Group 5: strong positive expression. (**F**) Group 6: strong positive expression. All figures were captured at X400.

Immunohistochemical (IHC) study

IHC analysis of Bcl2 in both the cortex and hippocampus of rats treated with QF showed a moderate positive expression of Bcl2, compared to the ketamine group that had negative expression in the cortex (Fig. 4). On the other hand,

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this effect was increased in rats treated with QFSLN, which showed strong positive expression in the hippocampus (Fig. 5). Moreover, the observed expression was dose-dependent, i.e. expression was strongest at the highest dose (30 mg/kg b.w.).

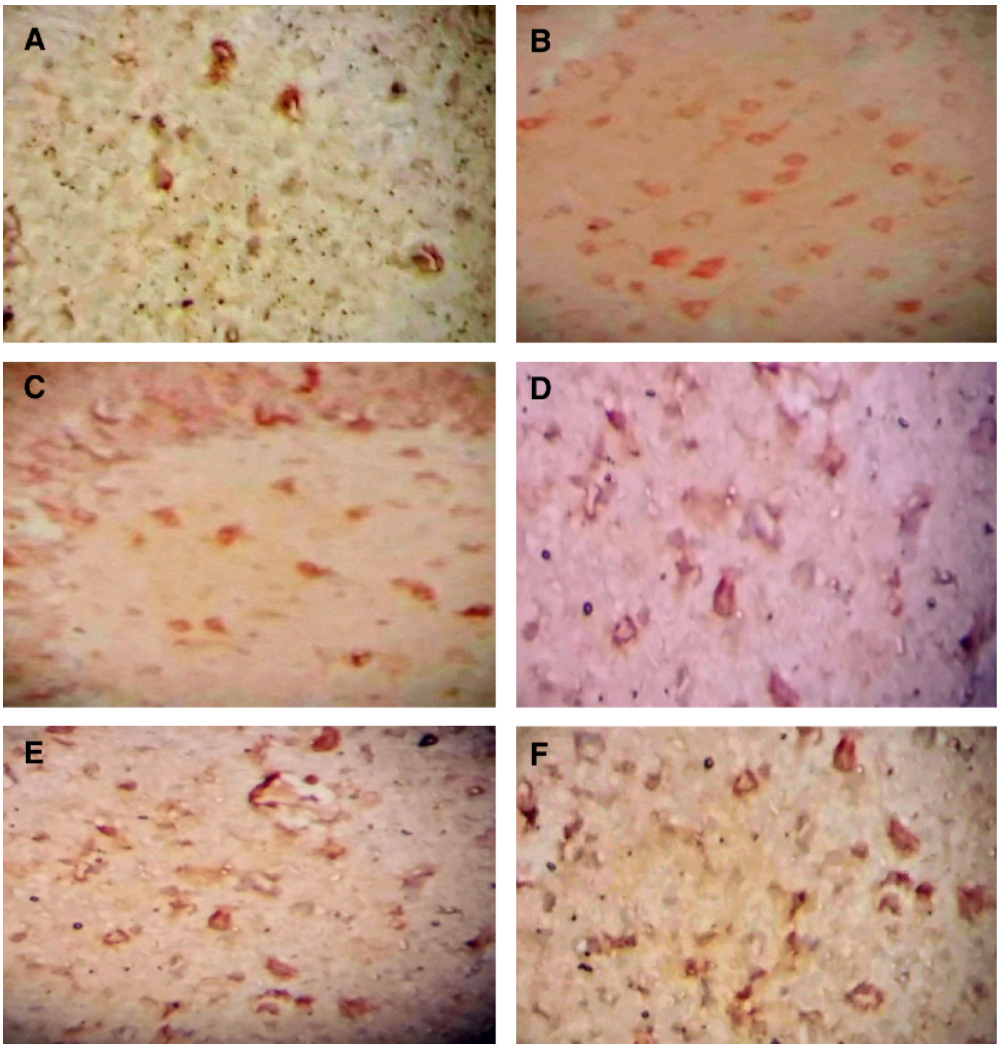

Figure 5. Immunohistochemical staining of BCL2 in hippocampus of rat from some groups. (**A**) Control negative group: strong positive, (**B**) Group 2: negative expression, (**C**): Group 3: moderate expression, (**D**) Group 4: strong positive expression, (**E**): Group 5: strong positive expression, (**F**) Group 6: strong positive expression.

Discussion

Schizophrenia is a chronic and severe disorder of the brain that results from disturbance in the levels of neurotransmitters (Wang et al., 2012).

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Psychotomimetic effects of NMDA receptor antagonists in humans suggest that reduced NMDA receptor function may contribute to the pathophysiology of schizophrenia. The antagonists of the NMDA receptor used in this study was ketamine, known to induce a spectrum of behavioral effects that mimic positive, negative and cognitive symptoms of schizophrenia**.** The occurrence of schizophrenia was confirmed using passive avoidance test and open field test**.** Ketamine administration caused a significant increase in the locomotor activity of rats as shown in the open field test. Ketamine also caused depletion of memory as indicated by the increased time spent in the light box in the passive avoidance test (representing signs of positive symptoms and cognitive impairment). The data also showed a significant increase of CK enzyme activity after ketamine injection. CK is found mainly in brain, skeletal muscles and heart. Therefore, the increase of CK after 3 weeks of ketamine injection may be a result of the effect of ketamine on brain tissue and induction of schizophrenia. Furthermore, results from the study showed that administration of ketamine increased the levels of DA, NE, and 5-HT in areas of the brain. A reduction in the level of excitatory amino acids (e.g. glutamate) and inhibitory amino acids (e.g. GABA) were observed. Changes in dopaminergic activity are likely to be the primary cause of schizophrenia or positive symptoms of schizophrenia.

With respect to animal models, while the underlying neuropharmacological mechanisms may be the same in humans and rodents, changes in DA-related behaviors and behavioral consequences may be quite different. In this study, we focus mainly on the observation of locomotor hyperactivity to reveal underlying neurotransmitter changes. Due to the relative ease of quantification, locomotor activity testing has been widely used in modeling the positive symptoms of schizophrenia (Van den Buuse and de Jong, 1989). On the other hand, cognitive impairments constitute a central feature of schizophrenia and, indeed, schizophrenic patients are commonly described to possess abnormalities in attention and information processing (Tsai and Coyle, 2002). From observations of tests in the rats**,** it was clear that they exhibited schizophrenia-like symptoms after ketamine injection. The observations are in accordance with those reported by Olesen et al. (2012) & Hamon and Blier (2013) (Hamon and Blier, 2013; Olesen et al., 2012).

The present study also showed that treatment with QF or QFSLN, prior to ketamine injection, ameliorated ketamine-induced schizophrenic symptoms in rats. The effect of QFSLN was more pronounced. The original DA hypothesis states that hyperactive DA transmission results in schizophrenic symptoms. This hypothesis is the main theory related to the incidence of schizophrenic symptoms. The antipsychotic drug QF blocks DA transmission, thus alleviating the schizophrenic symptoms. Riedel et al. (2007) reported that QF is a dibenzothiazepine derivative with a relatively broad receptor binding profile. It has major affinity to cerebral serotonergic, histaminergic and dopaminergic D1 and D2 receptors, moderate affinity to adrenergic receptors 1 and 2, and minor affinity to muscarinergic M1 receptor (Riedel et al., 2007). The efficacy of QF in reducing the symptoms of schizophrenia has been demonstrated in several

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clinical trials; in these trials, QF showed efficacy in treating some of the aggressive symptoms. In accordance with our results, Duncan et al. (1998) reported that pre-treatment of rats with QF blocked metabolic activation in the brain induced by ketamine (Duncan et al., 1998).

To date, the mechanism of action of anti-psychotic drugs in schizophrenia have been linked to DA and its interaction with other neurochemicals including glutamate, GABA, 5-HT and acetylcholine (Redrobe et al., 2012). GABA and glutamate regulate the activity of DA. The activation of GABA receptors in the brain results in various receptor interactions with glutamate and modulation of GABA receptors improves cognitive deficits in rats. Maeshima et al. (2007) concluded that the atypical anti-psychotic drug QF electively binds to central DA D2 and 5-HT receptors, leading to reduction in the level of DA and symptoms of schizophrenia (Maeshima et al., 2007). Some theories suggest that the neurotransmitter serotonin may also play a role in causing the symptoms of schizophrenia (Riedel et al., 2007). QF antipsychotic drug can treat these symptoms by blocking DA and serotonin transmissions.

Since the antipsychotic drug QF has poor oral bioavailability (about 9% only) (Sweetman, 2007), it is important to find ways to increase its absorption and bioavailability; the use of SLN is one of these approaches. In this study we succeeded in generating QFSLN, with particle size ranging from 134-162 nm. Narala and Veerabrahma (2013), similarly, generated QFSLN with mean size of 200 nm (and entrapment efficiency between 80-92%) (Narala and Veerabrahma, 2013); they discovered that the relative bioavailability was increased by 3.7 fold compared to that for QF. In general, delivering QF to the brain is a great challenge due to the blood brain barrier and, therefore, SLN formulation has been exploited as a potential drug carrier for tissue targeting. In addition, SLN are composed of solid lipid matrix that provides an effective means for controlled release of the drugs. It also improves drug bioavailability in the brain.

Thus, our present study was designed to enhance the bioavailability of QF in the brain by incorporating a minimal dose of QF into SLN, which act as a carrier (Parvanthi et al., 2014). In addition, Rathi et al. (2013) showed that preparations of QFSLN were capable of promoting sustained drug release over a period of 48 h (Rathi, 2013). This may reduce the concentration of the drug to be administered along with frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug**.** With regard to the effects of QF and QFSLN on hepatorenal function, CK and lipid patterns were evaluated after 3 weeks of treatment. The results showed that there was a slight increase in hepatorenal function and lipid parameters in the QF treatment group. Since the dose of nanoparticles used in this study was the same dose as QF suspension, this suggests that the bioavailability of QFSLN was markedly increased. These results are in agreement with those reported by Goldstein (1999), who described an increased activity of enzymes, particularly of ALT (Goldstein, 1999). Meyer and Koro (2004) demonstrated that during QF treatment, the level of serum cholesterol and triglycerides were slightly elevated

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(Meyer and Koro, 2004). The slight effect of QF on hepatorenal function and lipid profile in the present study might be due to QF treatment, which is essential for optimal therapeutic response. Adaptation induced by the drug exposure could provide novel treatment strategies that would mimic or promote adaptive responses. In some preclinical models, different effects have been seen after chronic (compared to acute) antipsychotic administration. For example, acute treatment with atypical antipsychotic drugs enhance NMDA-induced electrophysiological responses but chronic treatment with drugs reduce NMDA receptor sensitivity (Yang and Reis, 1999).

Moreover, the results of the immunohistochemical assay showed negative or low Bcl2 expression after ketamine induction. Previous studies have shown that ketamine induces neuronal apoptosis through down-regulation of Bcl2 expression (Hardwick and Soane, 2013). The groups of rats treated with QF and QFSLN showed an increase of Bcl2 expression in the cortex and hippocampus of the brain tissue. Bcl2 is known to inhibit apoptosis by preventing the release of cytochrome C and the subsequent activation of caspases (Hardwick and Soane, 2013). Thus, the beneficial effects of QF and its nanoparticle form (QFSLN) on the aforementioned behavioral and biochemical assays may also be related to their potential effects on neuroprotection and/or neurogenesis beyond the DA and 5-HT receptor blockade effects (Lodge and Grace, 2011).

Conclusion

Ketamine induction caused schizophrenic-like symptoms in rats. QF-loaded nanoparticles (QFSLN) was more effective than QF in improving the ketamine-induced schizophrenic-like symptoms in rats. The effect for both QF and QFSLN was dose-dependent. Thus, the nanoparticle form of QF showed increased solubility and enhanced efficacy as an antipsychotic drug. The use of QF-loaded nanoparticles may be beneficial for schizophrenia and perhaps other related diseases.

Abbreviations

5-HT: Serotonin
ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
CK: Creatine Kinase
DA: Dopamine
GABA: Gamma-Aminobutyric Acid
GGT: Gamma-Glutamyl Transferase
HDL: High-Density Lipoprotein

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LDL: Low-Density Lipoprotein
NE: Norepinephrine
QF: Quetiapine Fumarate
QFSLN: Quetiapine Fumarate Solid Lipid Nanoparticles
S.Alb: Serum Albumin
S.Alb/Glob: Serum Albumin/Globulin Ratio
S.Glob: Serum Globulin
S.T.P.: Serum Total Protein

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Author Contribution

Gehan A. Elmenoufy performed behavioral tests, neurotransmitter, and amino acids analyses; Said M. M. performed the assessment of liver and kidney functions.

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Original Research

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Effect of IgG from multiple sclerosis patients on amidolytic activity of coagulation and anticoagulation factors of hemostasis

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Abstract

Background: Immunoglobulin G (IgG) is a major immunoglobulin (Ig) in blood that accumulates to a greater extent in the bloodstream of patients impacted by neuroimmunological disorders such as multiple sclerosis (MS). The aim of this study was to determine the effect of IgG obtained from MS patients on the amidolytic activity of coagulation and on anticoagulation factors, and to compare those effects to the effects of IgG from healthy donors. **Methods**: Spectrophotometric hydrolysis of specific chromogenic substrate by key haemostasis factors was examined. **Results**: Our study shows that unlike healthy individuals, patients suffering from MS express IgG which enhances the amidolytic activity of thrombin and protein C, but inhibits the activity of factor Xa. **Conclusion**: Our study shows that IgG and coagulation factors, indeed, interact with each other. IgG may be key mediators of neuroinflammation and, therefore, may serve as a potential target for therapeutic strategies for MS and other neuroimmunological diseases.

Keywords

Amidolytic activity, Factor Xa, IgG, Multiple sclerosis, Protein C, Thrombin

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Introduction

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system mediated by different molecular and cellular immune components, which lead to disseminated inflammatory lesions within the brain parenchyma and potential brain damage (Bhat and Steinman, 2009; Göbel et al., 2016a). The pathogenesis of MS has long been imparted to self-reactive T cells though B cells have also recently been found to play an important role in the development of MS (Disanto et al., 2012; Sospedra and Martin, 2005). Furthermore, it has been demonstrated that innate immunity plays a pivotal role in the initial pathogenesis as well as in advanced stages of MS (Gandhi et al., 2010; Mayo et al., 2012; Weiner, 2008).

Recent studies suggest that factors of the coagulation cascade traditionally described as an entirely separate entity of the immune system might also be involved in MS development (Delvaeye and Conway, 2009). Moreover, several extensive studies have demonstrated the association of disorders of hemostasis cascades and MS. Recent data point to a role of both the extrinsic and the intrinsic coagulation systems.

One factor that has been described within chronic active MS plaques is tissue factor, a glycoprotein considered to be the initiator of the extrinsic coagulation cascade leading to the activation of factor X directly or indirectly through activation of factor IX (Han et al., 2008). Activation of factor X mediates the cleavage of prothrombin to thrombin that is able to cleave fibrinogen to fibrin. Interestingly, both fibrinogen deposition and thrombin activation have been reported in human MS lesions or in animal models (Adams et al., 2007; Davalos et al., 2014; Gverić et al., 2003). Furthermore, degradation products of fibrinogen and fibrin (e.g. fibrinopeptide A and D-dimer) have been shown to be significantly upregulated in individuals suffering from MS, while fibrinogen levels were found to be unaltered (Aksungar et al., 2008; Ehling et al., 2011; Liguori et al., 2014). These alterations were also significantly increased in blood samples of MS patients compared to healthy controls (Göbel et al., 2016b).

Overall, data from the literature suggest that inhibition of components from both the intrinsic and extrinsic coagulation systems can protect against inflammatory neurodegeneration. For instance, multiple findings support the prominent role of the coagulation system in the development of MS (Aksungar et al., 2008; Han et al., 2008). Nevertheless, to date, the mechanisms of regulation of coagulation factors in blood of individuals suffering from neuroinflammatory disorders (especially MS) have not been evaluated in detail.

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Materials-Methods

Blood plasma samples were taken from 35 healthy donors and 20 patients with MS. Patients were hospitalized in the Neurological Department of Hospital №4 (Kyiv, Ukraine). All donors and patients (or their respective relatives) were informed about the clinical research protocol. Informed consent was obtained in accordance with the Declaration of Helsinki. The clinical research protocol was approved by the Ethics Committees of the ESC (Institute of Biology and Medicine) of Kyiv, Ukraine. Fasting blood samples were collected from the cubital vein of all patients on the first day of hospitalization. Blood was collected into 3.8% sodium citrate solution (at a ratio of 9:1).

IgG was separated by affinity chromatography on protein A sepharose. One ml of blood plasma was applied to the column of protein A Sepharose (total volume of the column was 5 ml). Non-specific bound proteins were washed with 50 mM Tris-HCl buffer containing 130 mM NaCl, pH 7.4. Elution was performed using 0.1 M glycine-HCl buffer, pH 2.2. The purity of separated IgG fractions was controlled by 7.5% PAGE using the following protein standards: myosin (200 kDa), b-galactosidase (116 kDa), phosphorylase b (97 kDa), albumin (66.2 kDa) and ovalbumin (45 kDa). Gels were stained with 0.125% solution of Coomassie Brilliant Blue G-250 in 25% isopropanol and 10% acetic acid. The concentration of the separated IgG was measured by spectrophotometer (Bio-Rad, Hercules, CA).

In order to investigate the influence of IgG on hemostasis *in vitro* experiments were conducted using a standard set of reagents; "Renam" Russia reagents were used according to the manufacturer's instructions. IgG obtained from patients with MS as well as from healthy donors were applied to a mixture in two concentrations: 100 and 300 μg/ml.

To examine the influence of IgG on key hemostasis enzymes (thrombin and Factor Xa) *in vitro* experiments were performed. The following mixture was prepared as follows: 25 μl of enzyme was mixed with 50 mM Tris-HCl containing 130 mM NaCl, pH 7.4 and then IgG was added. After 5 min incubation at 37°C, the corresponding specific chromogenic substrate (in a final concentration of 0.3 mM) was added to the mixture (**Table 1**).

To examine the influence of IgG on hemostasis enzymes (prothrombin and proenzyme of Protein C) during their zymogen activation the following mixture was prepared as follows: 25μl of healthy donor plasma was mixed with 50 mM Tris-HCl containing 130 mM NaCl, pH 7.4, then with 25 μl of corresponding activators for plasma zymogens, and finally IgG was added (**Table 1**). After 5min incubation at 37°C, the corresponding specific chromogenic substrate (in a final concentration of 0.3 mM) was added to the mixture (**Table 1**).

Absorption was measured in two-wave mode at the primary 405 and reference 492 nm wavelengths in a microplate spectrophotometer (QuantTM, BioTek

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Instruments, Inc) for 60 minutes. The activity of the evaluated process was proportional to color intensity following release of p-nitroaniline from the chromogenic substrate. The control sample contained the same components but with an equal volume of 50 mM Tris-HCl buffer containing 130 mM NaCl, pH 7.4, instead of IgG.

Table 1. Experimental design

Evaluations	Activator	Corresponding chromogenic substrate
Activation of protein C zymogen in plasma	Derived from the venom Agkistrodon blomhoffi ussuriensis (Renam, Russia)	S2366
Activation of thrombin zymogen in plasma	Ecamylin (Technology-Standard, Russia)	S2238
Activity of thrombin	-	S2238
Activity of factor Xa	Derived from the Russell viper venom (Renam, Russia)	S2765

Statistical analysis of the experimental results was performed in the Origin program. Mean (M) and standard deviation (SD) were calculated for each group. A statistically significant difference was set at $P<0.05$. Statistical analysis of electrophoregrams was performed in the TotalLab 2. 01 program.

Results

In this study, IgG from MS patients was shown to have the ability to affect the amidolytic activity of hemostasis enzymes such as thrombin, factor X and protein C. The effect of IgG from blood plasma of the MS patients was significantly greater compared to the effect of IgG from healthy donors.

Thrombin is a protease in blood that facilitates blood clotting by converting fibrinogen to fibrin. There was an increase of amidolytic activity of thrombin under influence of fractions of IgG in both concentration of 100 and 300 μg/ml (**Fig. 1**). In normal conditions without IgG in mixture, the level of amidolytic thrombin activity was equal to 0.485±0.013 relative units (r.u.). After applying IgG from healthy donors to the mixture, the level of thrombin activity after 60 minutes of incubation was 45% higher (for the 100 μg/ml IgG concentration) and 35% higher (for the 300 μg/ml IgG concentration). However, IgG obtained from blood plasma of patients with MS induced a stronger effect, after application of IgG from MS patients. Thus, IgG from MS patients, at a concentration of 100 μg/ml, increased thrombin activity by 30%; at a concentration of 300 μg/ml, IgG from MS patients increased the activity by 58%

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(**Fig. 1**). In comparison to the effect of IgG from healthy donors on thrombin activity, IgG from MS patients (at the same concentration of 300 µg/ml) was 17% greater.

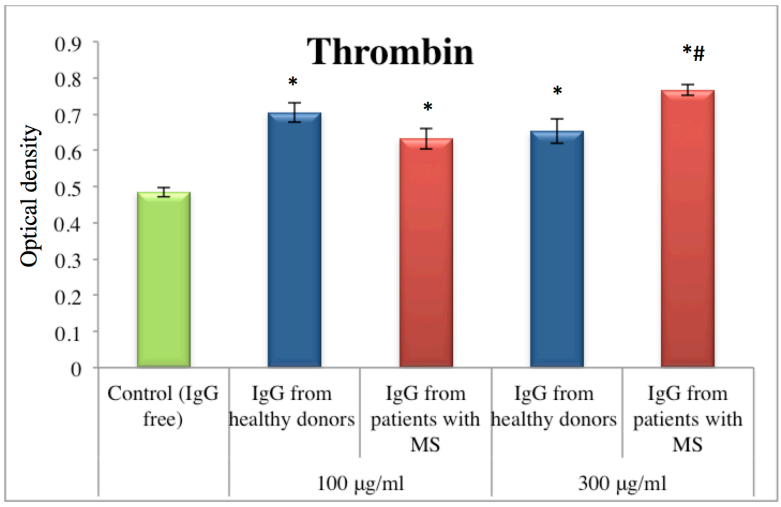

Figure 1. **Effect of IgG from blood plasma of MS patients on the amidolytic activity of thrombin** (healthy donors, n = 35; MS patients, n = 20). (*) statistical significance compared to control (IgG free); (#) statistical significance compared to IgG from healthy donors (at the indicated IgG concentrations).

The opposite trend was observed for factor Xa (also known as the eponym Stuart–Prower factor). Unlike IgG from healthy donors, IgG from MS patients inhibited amidolytic activity of factor Xa. For example, IgG from MS patients (at a concentration of 100 µg/ml) inhibited factor Xa activity by 23%. At a concentration of 300 µg/ml, IgG from MS patients inhibited factor Xa activity by 5% (**Fig. 2**). Overall, compared to the effect of IgG from healthy donors, the inhibitory effect of IgG from MS patients was 28% greater (at the 100 µg/ml IgG concentration) and 14% greater (at the 300 µg/ml IgG concentration).

Next, the amidolytic activity of hemostasis enzymes was measured after activation of the corresponding zymogens in blood plasma. Activation was achieved by addition of the specific endogenic activators in medium (**Table 1**). This technique can help address questions about specificity of the tested reactions. The effect of IgG in the blood plasma remains unclear. To address this, we applied the IgG from healthy donors and the corresponding activators instead of key enzymes in the medium during incubation.

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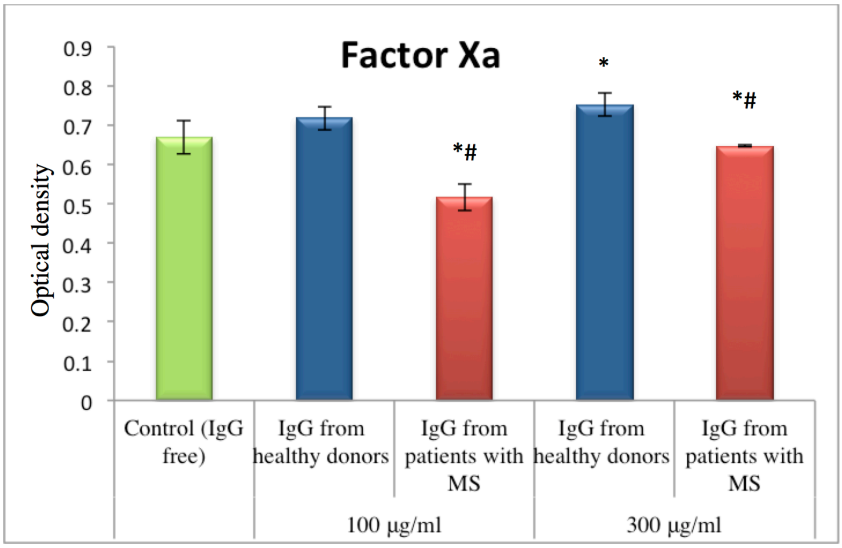

Figure 2. **Effect of IgG from blood plasma of MS patients on the amidolytic activity of factor Xa (healthy donors, n = 35; MS patients, n = 20)**. * statistical significance compared to control (IgG free). # statistical significance compared to IgG of healthy donors (at the indicated IgG concentrations)

The results showed an effect of IgG on thrombin followed by prothrombin activation in plasma under the influence of activator derived from the venom *Echis multisquamatus* (ecamylin). Thus, IgG obtained from MS patients as well as IgG from healthy donors both showed activation of amidolytic activity of thrombin after its activation of prothrombin in plasma. In the control probe without IgG, the level of amidolytic thrombin activity was equal to 0.458±0.021 r.u. After applying healthy donor IgG to the mixture, the level of thrombin activity after 60 minutes of incubation was 42% (for the 100 µg/ml IgG concentration) and 22% (for the 300 µg/ml IgG concentration) greater compared to the control. Meanwhile, the level of thrombin activity after applying IgG from MS patients was 49% (for the 100 µg/ml IgG concentration) and 32% (for the 300 µg/ml IgG concentration) greater compared to the control (**Fig. 3**).

Protein C, also known as autoprothrombin IIA and blood coagulation factor XIV, plays an important role in regulating anticoagulation, inflammation and cell death, and in maintaining the permeability of blood vessel walls. The amidolytic activity of Protein C was measured in plasma after activation of its zymogen under the influence of activator derived from the venom *Agkistrodon blomhoffi ussuriensis*. A statistically significant difference was observed under the influence of MS-derived IgG at a concentration of 300 µg/ml. The level of Protein C activation was elevated by 21% compared to the control (**Fig. 4**).

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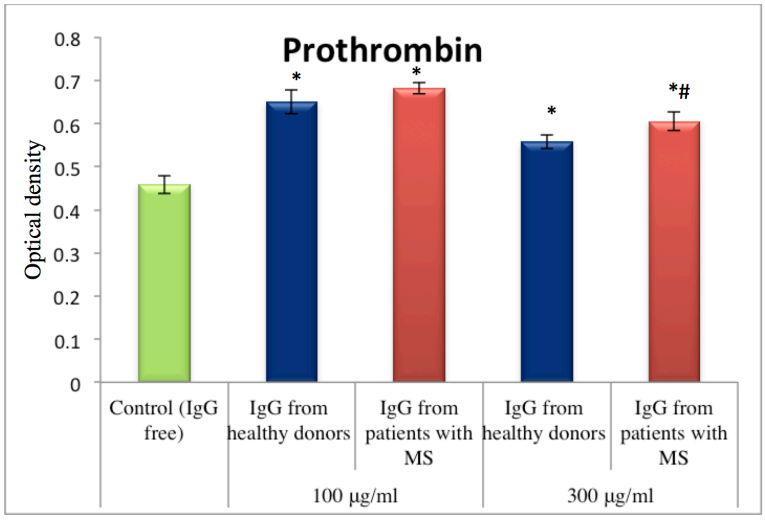

Figure 3. **Effect of IgG from blood plasma of MS patients on the amidolytic activity of thrombin after activation of prothrombin (healthy donors, n = 35; MS patients, n = 20)**. (*) statistical significance compared to control (IgG free), (#) statistical significance compared to effect of IgG from healthy donors (at the indicated IgG concentrations)

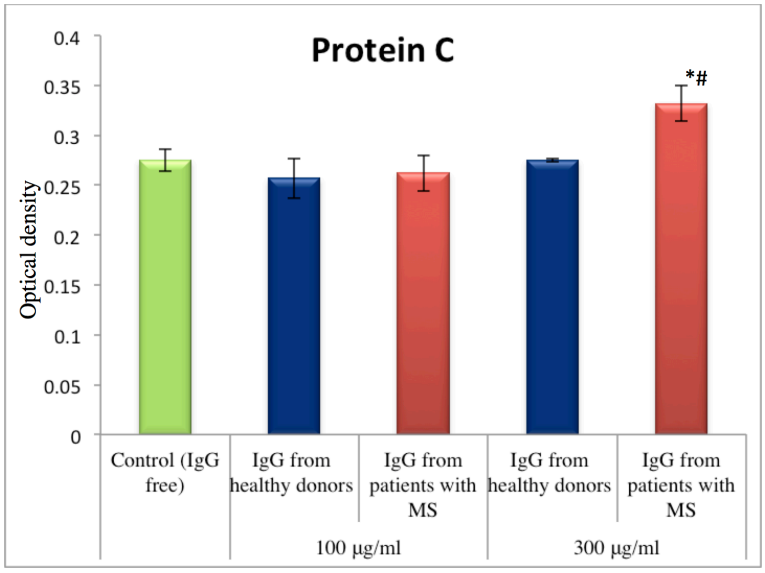

Figure 4. **Effect of IgG from blood plasma of MS patients on the amidolytic activity of Protein C after activation of its zymogen (healthy donors, n = 35; MS patients, n = 20)**. (*) statistical significance compared to control (IgG free), (#) statistical significance compared to IgG from healthy donors (at the indicated IgG concentrations)

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Discussion

In this study, we showed that the amidolytic activity of coagulation as well as anticoagulation factors were altered under the influence of IgG obtained from patients with MS (a prototypic neuroinflammatory disease). As comparison, the effect of IgG obtained from healthy donors was also evaluated. For the most part, an increase of amidolytic activity for both thrombin and protein C were observed. However, an inhibition of factor Xa activity was also observed. According to the literature, prothrombin and factor Xa are strongly elevated in individuals suffering from MS (Bhat and Steinman, 2009). Prothrombin and other hemostasis factors have been described to potentially enhance inflammation in artherosclerotic plaques, sepsis, endotoxemia and encephalomyelitis (Borissoff et al., 2011; Schoenmakers et al., 2005; Strukova, 2001). We showed an effect of IgG on the activity of coagulation as well as anticoagulation factors.

Although the role of hemostasis enzymes in the interactions with IgG need to be further studied, the described coagulation factors may represent key mediators in neuroinflammation (Bhat and Steinman, 2009; Göbel et al., 2016b). Accordingly, the interactions between IgG and coagulation factors may provide new targets for the development of future therapeutic strategies for MS and possibly other neuroimmunological diseases. Moreover, they may serve as possible biomarkers for disease monitoring.

Conclusion

In this study, we demonstrated that IgG is able to exert an effect on the enzymes of hemostasis system. There was an observed elevation of amidolytic activity of thrombin, as well as thrombin and protein C activated from their zymogens in blood plasma, after addition of IgG from MS patients (at a concentration of 300 µg/ml). The influence of MS-derived IgG fractions (at 100 and 300 µg/ml concentrations) on thrombin, in a system without plasma or after its activation in plasma, provide evidence of a potentially direct impact of IgG on thrombin. These revelations may influence future therapeutic strategies for MS. Moreover, inhibition of amidolytic activity of factor Xa was also observed. However, correlation between the level of inhibition and IgG concentration was absent. IgG obtained from healthy donors also affected the tested reactions in some measure too. A mechanism of concurrent inhibition might be occurring, but future investigations are needed to evaluate that.

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Abbreviations

IgG: Immunoglobulin G
MS: multiple sclerosis

Author Contribution

All authors contributed in manuscript preparation. Katrii T.B., Shandyuk V.Yu. obtained data and analyzed it. Vovk T.B., Halenova T.I., Raksha N.G. interpreted of data analysis. Katrii T.B. designed of figures. Shershnov O.V., Melnyk V.S, Savchuk O.M., Ostapchenko L.I. performed designed the study. All authors drafted the first version and approve the final draft.

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Original Research

Assessment of workload effect on nursing occupational accidents in hospitals of Kashan, Iran

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Abstract

Background: Nurses often experience occupational accidents and injuries. The mental workload is one of the factors that often cause tiredness. Perilous behaviors possibly occur more in tired persons resulting in injuries. The aim of the study was to evaluate the correlation between mental workload and nursing occupational accidents in public hospitals of Kashan, as well as factors affecting them. **Methods:** The analytic-descriptive study described herein consisted of nursing staff from public hospitals of Kashan, Iran. Information was collected using a three-part questionnaire from a selected clustery of 406 nursing personnel in 2016. The questionnaire was used to collect demographics, assess mental workload (per the National Aeronautics and Space Administration Task Load Index (NASA-TLX)), and assess frequency of accidents. The data were analyzed using SPSS software version 16. **Results:** A total of 455 occupational accidents were recorded for 2016 with about 2.27±1.21 accidents per nurse.

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The minimum and maximum scores for frustration and effort were 47.82 ± 30.91 and 78.61 ± 18.15, respectively. The mental workload mean was calculated as 69.49 ± 15.69. In total, 120 (32%) nurses experienced occupational accidents. There was a significant positive correlation between mental workload, mental need and physical need with occupational accidents (P<0.05). **Conclusion:** Occupational accidents are affected by mental workload. Taking actions to decline mental workload may result in a decrease of occupational accidents.

Keywords

Mental Workload, NASA-TLX, Nursing Staff, Occupational Accident

Introduction

The Texas Therapeutic Institute has classified hospitals as one of five occupational dangerous settings in terms of injuries and occupational diseases (Owens, 2007). Overall, there are numerous biologic dangers, such as from contact with infectious factors (e.g. transmitted by blood or air), and from contact with chemical materials (e.g. anesthesiology and sterilizing gases, antiseptics, etc.). Additionally, there are physical dangers (e.g. from contact with ionizing rays), immune and ergonomic factors (which can lead to musculoskeletal injuries and disorders), as well as socio-psychotic and organizational factors (such as mental and shift stresses). All the aforementioned can threaten health of personnel in health care centers (Alavi, 2014; Levy, 2006). In health care systems, the nursing cadre is under greater danger than other groups due to high demands of patient care and administration of treatments (sometimes biologically risky) to patients.

In Iran, 80% of occupants of the health care system are nurses. According to the National Association of Professional Safety in the United States of America, nursing is among the top 40 professions in which staff develop diseases related to work-related pressure (Safari et al., 2013). Nursing is a stressful job with a high rate of occupational accidents and injuries (Alavi, 2014).

According to a report by the Bureau of Labor Statistics, a high rate of occupational accidents was allocated to nursing in the United States; approximately 8.7 occupational accidents occur annually for every 100 permanent nurses. In fact, nursing profession ranks 7th among professions to have the greatest loss of working days due to accidents (Gershon et al., 2007). Some studies have been conducted in Iran which are related to safety and health of nurses (including nursing students). These studies have reported that the frequency of needle stick injury, contact with blood, and contact with bodily

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fluids are approximately 31.1%, 41.7%, and 84.8%, respectively, each year (Aghajanlou et al., 2007). Moreover, other studies have reported that the work environment for nurses can be dangerous, and that nurses are at significant risk for on-the-job accidents and injuries.

One of the vital factors affecting behavior and performance of nurses is mental workload (Kohn et al., 2002). Mental workload is the mental effort or concentration required during tasks (Kohn et al., 2002). It has also been defined as the mental/cognitive need or analytic effort to deal with the needs of workers/staff and performance under time-driven, physical and environmental demands (Neill, 2011). If the mental workload is more than the normal workload, individual performance deficit will emerge (Ozkan et al., 2015). High workload and long hours of overtime are the two key factors leading to tiredness (Young et al., 2008). Studies indicate that occupations with high workload can cause impaired memory, damage to thinking processes, irritability, and decreased learning due to tiredness and inappropriate working schedules (Mohammadi et al., 2013). Ozkan et al. (2014) showed that there is a significant positive correlation between mental workload and the signs of embarrassment and interpersonal sensitivities (Ozkan et al., 2015). Di Stasi (2008) reported that increase of mental workload caused attitudes and beliefs to the mental workload of nursing staff is one of the most important indices for safety assessment in special wards (Di Stasi et al., 2009). The main cause of more than 90% of occupational hazards (and 70% to 90% of work-related accidents) is human error (Ghasemi et al., 2011). When the mental workload is more tolerable than one's tolerance ability, the thinking process will be affected. Thus, the possibility of taking perilous behaviors will increase, as will the exposure and frequency of accidents.

Findings of previous studies have shown that mental workload and occupational accidents are high among nurses. Therefore, this study aims to evaluate the correlation between mental workload and occupational accidents among nurses of public hospitals in Kashan, Iran. As well, this study seeks to understand the factors and variables (e.g. demographics) which may affect mental workload (as categorized by the National Aeronautics and Space Administration Task Load Index (NASA-TLX)).

Materials-Methods

This analytic-descriptive study was conducted in Kashan, Iran in 2016. The study population consisted of nursing staff from all public hospitals in Kashan with at least one year of experience. The following formula was used to determine the sample volume:, in which Z is confidence coefficient (equal to 95% confidence), δ is standard deviation (estimated as 20.54 in pilot studies performed), and d is measurement accuracy (equal to 2 units). The required sample size was estimated as 406. A three-part questionnaire was used to collect data. The first part of the questionnaire related to demographic information, consisting of

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gender (male/female), age (year), marital status (single/married), experience (year), occupational status (official, contractual, formal, and staffing plan), and servicing department (general nursing, anesthesia, surgery, or services (e.g. nurses' aides or aides' assistants)).

The second part of the questionnaire assessed the mental work load status using the assessment scale of the NASA-TLX, one of the most commonly used tools to evaluate mental workload in terms of individual perspective (Cao et al., 2009; Holden et al., 2010; Holden et al., 2011; Safari et al., 2013). Several reports have confirmed the reliability and validity of NASA-TLX to evaluate mental workload (Hoonakker et al., 2011; Rubio et al., 2004); for instance, Hoonakker et al. (2011) reported the appropriateness of convergent and discriminant validities of the NASA mental workload scale (Hoonakker et al., 2011). Mohammadi et al. (2013) approved the validity of the Persian version of the questionnaire, and its internal consistency reliability (Cronbach's alpha) of 0.847 was estimated to be suitable (Mohammadi et al., 2013).

NASA-TLX evaluates the six sub-scales of mental demands, physical demands, time demands, performance, effort, and frustration using a visual scale divided into 0 to 100 as 5-unit sections. Thus, the minimum and maximum scores of each sub-scale are 0 and 100, respectively. The definition and concept of each sub-scale was presented in the questionnaire; the respondents reviewed the definitions to determine the appropriate sub-scale to mark. The mean of the sub-scales represents the mental workload amount and ranges from 0 to 100. A mean <50 is considered acceptable and >50 is considered high. The third part of the questionnaire (two questions) primarily evaluates whether occupational accidents have occurred during the last year and secondly, the number of occupational accidents that have occurred (Huang et al., 2006; McCaughey et al., 2013; Vinodkumar and Bhasi, 2009).

The questionnaire were distributed amongst the participants; questions regarding the questionnaire were explained by the study researchers. The distribution of sub-scale scores were generated and reported as mean ± standard deviation (SD), and minimum (min) and maximum (max) values. Finally, the data obtained were analyzed by SPSS software version 16 via independent t-test, one-tailed variance analysis, chi-square, and Pearson's correlation coefficient. P values < 0.05 were considered to be statistically significant.

Results

Of the 406 questionnaires distributed, 375 questionnaires were returned (response rate was estimated as 92.36%). The mean age of participants was 32.23 ± 7.26 years. Additionally, the mean experience was calculated as 9.04 ± 6.45 years. One hundred and thirty-nine (37.1%) and 236 (62.9%) participants were male and female, respectively. Two-hundred and seventy-eight were

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nurses, 33 were surgical technicians, 15 were anesthesiology technicians, and 40 were nurses' aides or aides' assistants. Three-hundred and twenty-six participants had irregular shifts compared to 49 with regular shifts. In terms of occupational status, 110 (29.3%) nurses were official, 77 (20.5%) were contractual, 107 (28.5%) were formal, and 81 (21.6%) were part of staffing plan. The mean overtime period was 42.27 ± 20.09 hours.

Table 1 shows the descriptive statistics relating to the mental workload and its subscales, together with occupational accident experience and mean score of accidents. According to **Table 1**, the minimum and maximum scores for frustration and effort were 47.82±30.91 and 78.61±18.15, respectively. The mental workload mean was calculated to be 69.49±15.69. In total, 120 (32%) nurses confirmed they had experienced occupational accidents, while 255 (68%) did not experience any occupational accident. Altogether, 455 occupational accidents occurred over the previous year; the average number of accidents per nurse was 2.27 accidents (range of 0 to 11).

Table 1. Mean, standard deviation, min and max of mental workload and occupational accidents

Variable		Mean±SD	Min	Max
Mental demands		71.80±21.18	20.00	100.00
Physical demands		70.68±22.86	0.00	100.00
Time demands		72.63±22.27	10.00	100.00
Efforts		78.61±18.15	20.00	100.00
Performance		75.41±18.23	10.00	100.00
Frustration		47.82±30.91	0.00	100.00
Sum of mental workload		69.49±15.69	30.0	100.00
Occupational Accident Experience	**Yes (%)**	120(32.00)	-	-
	No (%)	255(68.00)	-	-
Mean Score Occupational Accident		2.27±1.21	0	11

Table 2 depicts the correlation between mental workload and individual characteristics (i.e. demographic variables). According to **Table 2**, there was an insignificant difference between mental workload in men versus women (p=0.128). However, there was a significant difference in mental workload among the various age groups (p=0.049); the greatest significant difference was observed in higher aged groups. There was an insignificant difference

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between mental workload and experience (p=0.204) or hours of time in the week (p=0.735). The mean of mental workload in personnel with regular shifts (70.25 ± 15.66) was significantly higher than in personnel with irregular shifts (64.40 ± 15.05) (p=0.015). A higher mental workload was observed for anesthesiology technicians (73.33 ± 12.50), but no significant difference was observed for the other occupational groups (p=0.288).

Table 2. Mental workload by demographic variables

Variable		Number (%) n=375	Mean±SD	P-value
Gender		-	-	0.128
	Male	139(37.1)	71.11±15.93	-
	Female	236(62.9)	68.54±15.50	
Age		-	-	0.049
	20-30	136(36.3)	67.20±15.07	-
	30-40	173(46.1)	69.70±16.20	
	40-50	59(15.7)	73.97±15.03	
	>50	7(1.9)	71.19±14.13	
	Experience Mean±SD	9.26±6.99	-	0.204
Work Time in Week Mean±SD		42.28±20.09	-	0.735
Shift working	**Yes (%)**	326(86.9)	70.26±15.66	0.015
	No (%)	49(13.1)	64.40±15.05	
Work Unit		-	-	0.288
Nursing		287(76.5)	68.83±16.05	-
Surgery		33(8.8)	68.96±15.51	
Anesthesia		15(4.0)	73.33±12.50	
Nursing Services		40(10.7)	73.27±13.94	

Table 3 indicates the correlation between individual characteristics (i.e. demographic variables) with occupational accident experience and mean of accidents. According to **Table 3**, there was a significant difference in occupational accident experience in men versus women (p=0.012). The mean of accidents for men (1.30) was significantly higher than for women (1.16)

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(p=0.032). Moreover, a significant difference was observed in occupational accident experience (p=0.049) and occupational accident number (p=0.043) for the different occupational groups. There was an insignificant (p>0.05) correlation of accident experience to nursing years (experience), or to hours of time per week. Also, there was no significant correlation between occupational accident and shift (p>0.05), and no correlation to work unit (p=0.081). However, there was a significant difference in the mean of occupational accidents in the different wards (p=0.034).

Table 3. Occupational accident by demographic variables

Variable			Accident experience			Mean score of occupational accident per person	
			P-value	Mean±SD	P-value	No N=255	Yes N=120
Gender			-	-	0.012	-	
	Male	n=139	50(35.9)	89(64.1)	-	1.30±2.27	0.032
	Female	n=236	70(29.7)	166(70.3)	-	1.16±2.28	
Age		-	-	-	0.049	-	
	20-30	n=136	53(38.9)	83(61.1)	-	1.33±2.15	0.043
	30-40	n=173	43(33.1)	130(66.9)	-	0.92±2.00	
	40-50	n=59	21(35.6)	38(64.4)	-	1.66±2.89	-
	>50	n=7	3(42.8)	4(57.2)	-	2.28±4.11	
Experience		-	9.60±8.72	9.10±6.03	0.514	-	
Work Time in Week		-	41.97±10	42.42±23.38	0.839	-	0.058
Shift work	**Yes**	n=326	101(30.1)	255(69.9)	0.171	1.19±2.30	0.590
	No	n=49	19(38.7)	30(61.3)		1.32±2.15	0.710
Work Unit of nurses in parts of hospital		-	-	-	0.081	-	
	Part of Nursing Care	n=287	91(31.7)	196(68.3)	-	1.09±2.02	0.034
	Part of Surgery	n=33	11(33.3)	22(66.7)	-	1.60±2.97	-
	Part of Anesthesia	n=15	4(26.7)	11(73.3)	-	0.93±1.62	-
	Part of Nursing Services	n=40		26(65.0)	-		-

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Table 4 shows the correlation between mental workload (and sub-scales) and occupational accidents. According to **Table 4**, the mean scores of mental demands, physical demands, time demands, efforts, and sum mental workload in personnel who have experienced occupational accidents were significantly higher compared to personnel without accident experience ($p<0.05$). Moreover, there was a significant positive correlation of occupational accidents to mental demands ($p=0.041$), to physical demands ($p=0.032$), to time demands ($p=0.033$), to efforts ($p=0.048$), and to sum mental workload ($p=0.014$). No significant correlation was observed for occupational accidents with performance ($p=0.165$) or with frustration ($p=0.548$).

Table 4. Correlation between mental workload and sub-scales to occupational accidents

Variable Mean ± SD	Occupational accident experience			Mean score of occupational accident	
	Yes	No	P-value	r	P-value
Mental demands	76.25±20.54	69.70±21.75	0.005	0.281	0.041
Physical demands	75.54±21.75	68.39±23.05	0.005	0.310	0.032
Time demands	75.50±22.70	71.27±21.98	0.048	0.251	0.033
Efforts	81.41±18.84	77.29±17.71	0.040	0.195	0.048
Performance	76.50±18.64	74.90±18.05	0.429	0.072	0.165
Frustration	46.15±32.38	48.68±30.23	0.471	0.031	0.548
Sum workload	71.89±15.82	68.36±15.52	0.042	0.412	0.014

Discussion

The aim of the current study was to evaluate the correlation between mental workload and occupational accidents in nurses, as well as the factors affecting accidents. The rate of estimated mental workload (69.54 ± 15.71) of the nurses in the study was greater than that for bank staff (Giahi et al., 2014), indicating the extent of their mental workload. The estimated amount of mental workload in the current study was lower than the amount reported by Malekpour et al. (2014) for nurses working in the Intensive Care Unit (82.33) and higher than the value reported for nurses working in the Orthopedics Ward (63.5) (Malekpour et al., 2014). The difference between these results could possibly be due to the difference between the occupational groups. The population surveyed in the

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present study was a nursing cadre, while in other studies the participants surveyed were nurses occupying different wards of the hospital.

Evaluation of the sub-scales of mental workload showed that maximum frequency for effort score was 78.61 ± 18.15. This finding implies that nurses consume a lot of energy to carry out their job functions. Zheng et al. (2012) reported that effort score as the highest score of the sub-scales of mental workload (Zheng et al., 2012). Additionally, Safari et al. (2013) reported that the sub-scale of mental workload is the highest sub-scale (83.5) for nurses (Safari et al., 2013). In our study, the lowest score belonged to frustration (69.54 ± 15.71); this finding concurs with findings from a study by Zheng et al. (2012) (Zheng et al., 2012).

There was no significant difference in mental workload in men and women, which corresponds to the study by Malekpour et al. (2014) (Malekpour et al., 2014) and Safari et al. (2013) (Safari et al., 2013). The job duty for male and female nurses is the same, so there should be no difference. The mean mental workload for the higher aged groups was significantly higher than for the lower aged groups, which may reflect the increase of responsible duties and workload for nurses between 40-50 years of age. Safari et al. (2013) reported a significant correlation between mental workload and age in nurses (Safari et al., 2013). Clearly, with increasing age, job concentration and performance may decrease. Thus, the significant correlation between age and mental workload in nurses could be affected by that factor.

Nurses are constantly exposed to stress and burnout (Malekpour et al., 2014; Morris et al., 2007). This could also affect their mental power. Our results demonstrated that there is no significant correlation between experience and shift hours with workload; other studies have reported an insignificant correlation amongst these variables too (Hoonakker et al., 2011; Malekpour et al., 2014; Safari et al., 2013). The mean of mental workload in personnel with regular shifts was significantly higher than in personnel with erratic shifts. Hoonakker et al. (2011) also reported a significant correlation between shifts and mental workload (Hoonakker et al., 2011) and, indeed, shifts have always been considered a key factor for mental workload in a job (Costa, 2010).

Studies indicated that there is a significant correlation between tiredness and workload; shift workers with circadian problems or those receiving inadequate rest are more inclined to be tired. The accumulative tiredness could affect overall mental workload (Rahimi et al., 2012). The mean number of occupational accidents in the current study was greater than those for the chemical industry (Vinodkumar and Bhasi, 2009), indicating that the nursing work environment is a perilous and risky one. The percentage of injured individuals in this study was lower than that reported by McCaughey et al. (2013), which evaluated nurses in hospitals of India. Indeed, the rate of accidents in our current study was greater than McCaughey's study (McCaughey et al., 2013). This indicates that a certain group of people are

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more inclined to be injured at these hospitals, and it will be useful to identify them immediately.

The number of injured people and rate of accidents for men was significantly higher than for women, which is consistent with observations by Bakhtiyari et al. (2012) (Bakhtiyari et al., 2012). The higher rate of accidents among men is conceivable since men typically perform heavy work and movements in the wards. The mean number of accidents in younger and elder groups were significantly greater than that for the middle-age group, which concurs with the study by Ghamari et al. (2013) (Ghamari et al., 2013). The main cause of the high rate of accidents in the younger group could be due to less experience and unfamiliarity with dangers of safety in the setting. Thus, the cause of a high rate of accidents in elder group might be due to decreased focus and ability.

There was no significant correlation between hours of work time per week and accidents. This could relate to the equal hours of work time among staff. As aforementioned, the mean number of accidents in nursing wards is significantly higher than other occupational groups. Personnel working in the servicing ward (typically lower education) have less information about necessary actions to take when confronting different situations. Also, they transfer patients and, accordingly, accidents are among the highest in this group than other groups.

Moreover, our results showed that mental workload in nurses who have experienced occupational accidents is greater than that of nurses with no experience. Furthermore, there was a positive correlation between mental workload and mean of accidents. Mazur et al. (2012) reported that there is a significant correlation between radiotherapy accidents and mental workload score (Mazur et al., 2012). Additionally, there is a significant correlation between the subscales of mental need, physical need, effort score, and frequency of accidents. If the mental and physical needs of an occupation are high, the staff is obliged to do his/her job duties with greater effort. This affects the thinking process and concentration, and increases the number of errors. Holden et al. (2011) showed that there is a significant positive correlation between mental workload and possibility of creating errors (Holden et al., 2011). In fact, human error is one of the major causes of occupational accidents (Wiegmann and Shappell, 2001; Young et al., 2008).

Correspondingly, Di Stasi et al. (2009) showed that people with higher mental workload tend to exhibit perilous behaviors (Di Stasi et al., 2009), which can increase occupational accidents. Nurses are among professionals who are constantly under risk of occupational accidents, such as needle stick injuries, exposure to chemical materials, and acquisition of musculoskeletal injuries (Alavi, 2014). The high level of mental workload not only affects occupational accidents but also increases the prevalence of depression, occupational stress, and burnout. Furthermore, the high mental workload affects health and welfare of personnel as well as safety of patients (Holden et al., 2011).

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Indeed, occupational accidents are affected by various factors, including physical and psychological conditions of the staff, organizational factors (such as organizational culture), and safety conditions. Further studies are needed to investigate the effects of these factors on mental workload and occupational accidents. In the future, it will be important to better understand the factors affecting mental workload and accidents, evaluate more subjects, and possibly investigate the impact of adequate rest at home.

Conclusion

There is a significant positive correlation between mental workload and occupational accidents. Taking actions to prevent or decrease mental workload can possibly lead to a decrease in occupational accidents for nurses.

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Author Contribution

Milad Derakhshanjazari and Hamid Salehiniya was all responsible for the study conception and design. Fatemeh Honarjoo performed the sampling and data collection and prepared the draft of the manuscript. Vali sarsangi made critical revisions to the paper for important intellectual content, performed the data analysis and supervised the study. All authors reviewed and commented on final draft.

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Original Research

Variations in the Incidence and Mortality of Ovarian Cancer and Their Relationship with the Human Development Index in European Countries in 2012

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Abstract

Background: Ovarian cancer (OC) has high incidence and mortality rates among the reproductive system cancers. This study investigated the relationship between the age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) of OC and Human Development Index (HDI) in European countries in 2012. **Material and methods:** This ecological study assessed the correlation between the ASIR and ASMR of OC and HDI and its components including life expectancy at birth, average years of schooling, and gross national income (GNI) per capita. Bivariate correlation analysis was used for assessing the correlation between the ASIR and ASMR of OC and HDI and its components. All reported P values were two-sided. Statistical analyses were performed using SPSS (Version 15.0, SPSS Inc.). **Results:** The maximum ASIR of OC was observed in Latvia, Bulgaria, and Poland. The highest ASMR of OC was observed in Latvia, Lithuania,

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and Poland. The incidence and mortality rates of OC are expected to increase between 2012 and 2035. This increase will be more pronounced in women ≥ 65 years. HDI had a weak negative correlation with the ASIR of OC (r=- 0.213; P=0.186) and a weak positive correlation with the ASMR of OC (r=0.072; P=0.659). **Conclusion:** According to the results of this study, health policy makers must make appropriate decisions to deal with the increasing morbidity and mortality of OC, especially in women over 65 years of age, in regions with lower access to prevention and treatment services.

Keywords

Europe, HDI, Incidence, Mortality, Ovarian Cancer

Introduction

Ovarian cancer (OC) is the seventh leading cancer diagnosis and the fifth leading cause of cancer-related mortalities (Coburn et al., 2017). OC has high incidence and mortality rates among the reproductive system cancers (Ferlay et al., 2010). Based on the global estimates, about 225,000 new cases of OC are diagnosed every year and 140,000 women annually die from this disease worldwide (Ferlay et al., 2010). In 2014, the lifetime risk of OC among women in the United States was ranging from 4% to 9%, 73% of women had no family history of ovarian cancer (Pearce et al., 2015).

The incidence and mortality rates of OC vary in different areas of the world (Coburn et al., 2017). Although the incidence and mortality rates of OC are globally high, the cause and etiology of the disease are not completely understood (Razi et al., 2015). However, several factors associated with OC have been identified and classified into three categories of protective factors, e.g. parity and contraceptive use, risk factors, e.g. no history of a full-term pregnancy, a positive family history of OC, and age, and other factors, e.g. such as lactation, age at menopause, and age at menarche, whose relations with OC are not clearly determined (Poorolajal et al., 2014). One of the significant factors related with OC is the Human Development Index (HDI) which reflects the socioeconomic position of individuals living in different countries (Mahdavifar et al., 2016; Mohammadian et al., 2015; Shuja et al., 2017).

Socioeconomic factors affect cancer incidence and mortality through complex and changeable mechanisms. In a study in Asian countries in 2012, Razi et al. found a positive correlation between age-standardized incidence rate (ASIR) of OC and HDI and its components including life expectancy at birth, average years of schooling, and gross national income (GNI) per capita. However, no significant correlation was observed between age-standardized mortality rate (ASMR) of OC and HDI and its components (Razi et al., 2016). While several

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other studies have evaluated the relationship between HDI and various cancers, especially in Asian countries (Ghoncheh et al., 2015; Hassanipour-Azgomi et al., 2016; Mohammadian et al., 2015; Pakzad et al., 2015a, b; Pakzad et al., 2016; Rafiemanesh et al., 2015), the relationships between the incidence and mortality of OC and HDI have not been assessed in European countries. Therefore, this study aimed to investigate the relationship between the ASIR and ASMR of OC with HDI and its components, i.e. life expectancy at birth, the average years of schooling, and the country's GNI per capita in 2012. We used HDI as an indicator of socioeconomic development (Bray et al., 2012).

Materials-Methods

This ecological study examined the relationship between ASIR and ASMR of OC and HDI and its components including life expectancy at birth, average years of schooling, and GNI per capita. The 2012 ASIR and ASMR data for each European country were collected from the GLOBOCAN project (available at http://globocan.iarc.fr/Default.aspx) (Ferlay et al., 2015). Data about the HDI were extracted from the Human Development Report 2013 (Malik, 2013). The details of the methods to estimate the ASIR and ASMR in GLOBOCAN project have been provided in previous reports (Ferlay et al., 2010; Ferlay et al., 2015; Foulkes and Cooney, 2010; Pakzad et al., 2015b; Pakzad et al., 2016).

Statistical analysis: Pearson's correlation analysis was used to assess the correlations between the ASIR and ASMR of OC and HDI and its components. All reported ASIR and ASMR were per 100,000. Statistical significance was considered at $P \leq 0.05$. All P values reported in this study are two-sided. All statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 65584 cases of OC were recorded in European countries in 2012. Five countries, including the Russian Federation (13,373 cases), the United Kingdom (6,692 cases), Germany (6,673 cases), Italy (5,911 cases) and France (metropolitan; 4,592 cases), had the highest numbers of OC. Overall, 37,241 cases (56.78%) of OC were reported in these five countries.

The highest ASIRs of OC were seen in Latvia (14.2 per 100,000), Bulgaria (14 per 100,000), Poland (13.6 per 100,000), Serbia (12.8 per 100,000), and Lithuania (12.2 per 100,000). The lowest ASIRs of the OC belonged to Albania (3.2 per 100,000), Ireland (6 per 100,000), Portugal (6.2 per 100,000), the Netherlands (6.5 per 100,000), and Cyprus (7 per 100,000). The number, crude incidence rate, and ASIR of the OC based on sex are presented in **Table 1**. The

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countries with the highest and lowest ASIR in both sexes are shown in **Table 1**, **Fig. 1**, and **3**.

Table 1. Number, crude incidence rate, and age-standardized incidence rate (ASIR) of ovarian cancer

Estimated incidence, all ages				Estimated mortality, all ages			
Population	Numbers	Crude Rate	ASIR (W)	Population	Numbers	Crude Rate	ASMR (W)
Latvia	304	25.2	14.2	Latvia	223	18.5	8.8
Bulgaria	899	23.5	14.0	Lithuania	301	17.1	8.4
Poland	4456	22.5	13.6	Poland	2692	13.6	7.3
Serbia	935	18.8	12.8	Ireland	264	11.5	6.9
Lithuania	369	20.9	12.2	Malta	32	15.2	6.8
Montenegro	51	15.8	12.0	Croatia	321	14.1	6.6
Malta	46	21.8	11.8	Denmark	401	14.2	6.5
Estonia	156	21.6	11.8	Slovenia	150	14.4	6.4
United Kingdom	6692	21.0	11.7	Norway	326	13.2	6.4
Slovakia	518	18.4	11.6	Czech Republic	708	13.2	6.3
FYR Macedonia	169	16.4	11.3	Serbia	530	10.7	6.2
Russian Federation	13373	17.4	11.3	Russian Federation	7971	10.4	5.9
Ireland	380	16.6	11.2	Ukraine	2454	10.1	5.9
Czech Republic	1092	20.3	11.1	Bulgaria	440	11.5	5.9
Belarus	844	16.5	10.9	Estonia	97	13.4	5.8
Ukraine	4032	16.6	10.7	Montenegro	28	8.7	5.8
Hungary	999	19.1	10.6	United Kingdom	4040	12.7	5.7
Slovenia	192	18.4	10.4	Hungary	644	12.3	5.6
Denmark	544	19.3	10.3	Slovakia	280	9.9	5.6
Croatia	428	18.8	10.3	Sweden	609	12.8	5.6
Romania	1850	16.8	10.3	The Netherlands	1019	12.1	5.5

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Italy	5911	19.0	10.2	Belarus	484	9.5	5.5
Norway	418	16.9	9.5	Belgium	731	13.3	5.5
Finland	457	16.6	8.4	FYR Macedonia	91	8.8	5.4
Greece	915	15.9	8.4	Luxembourg	27	10.3	5.1
Bosnia Herzegovina	245	12.6	8.1	Romania	1020	9.3	5.0
Belgium	840	15.3	8.1	Finland	329	12.0	4.9
France (metropolitan)	4592	14.1	7.9	Switzerland	436	11.1	4.8
Switzerland	621	15.8	7.9	Italy	3617	11.6	4.7
Spain	3236	13.7	7.7	Germany	5379	12.9	4.7
Sweden	659	13.8	7.5	Austria	504	11.7	4.6
Republic of Moldova	196	10.6	7.5	France (metropolitan)	3389	10.4	4.3
Germany	6673	16.0	7.4	Greece	578	10.0	4.2
Austria	636	14.8	7.3	Republic of Moldova	117	6.3	4.2
Luxembourg	36	13.7	7.3	Bosnia Herzegovina	148	7.6	4.1
Cyprus	56	10.1	7.0	Cyprus	37	6.7	3.9
The Netherlands	1025	12.2	6.8	Spain	1878	7.9	3.7
Portugal	616	11.2	6.2	Iceland	10	6.1	3.5
Iceland	15	9.2	6.0	Portugal	381	6.9	3.1
Albania	62	3.8	3.2	Albania	30	1.9	1.5

A total of 42,749 deaths due to OC were recorded in 2012. The Russian Federation (7,971 cases), Germany (5,379 cases), the United Kingdom (4,040 cases), Italy (3,617 cases) and France (metropolitan; 3,389 cases) were the five countries with the highest numbers of deaths. Overall, 57.06% of deaths due to OC occurred in these five countries.

Latvia (8.8 per 100,000), Lithuania (8.4 per 100,000), Poland (7.3 per 100,000), Ireland (6.9 per 100,000), and Malta (6.8 per 100,000) had the highest ASMRs of OC. Albania (1.5 per 100,000), Portugal (3.1 per 100,000), Iceland (3.5 per 100,000), Spain (3.7 per 100,000), and Cyprus (3.9 per 100,000) had the lowest ASMRs of OC (**Table 2**, **Fig. 2** and **3**).

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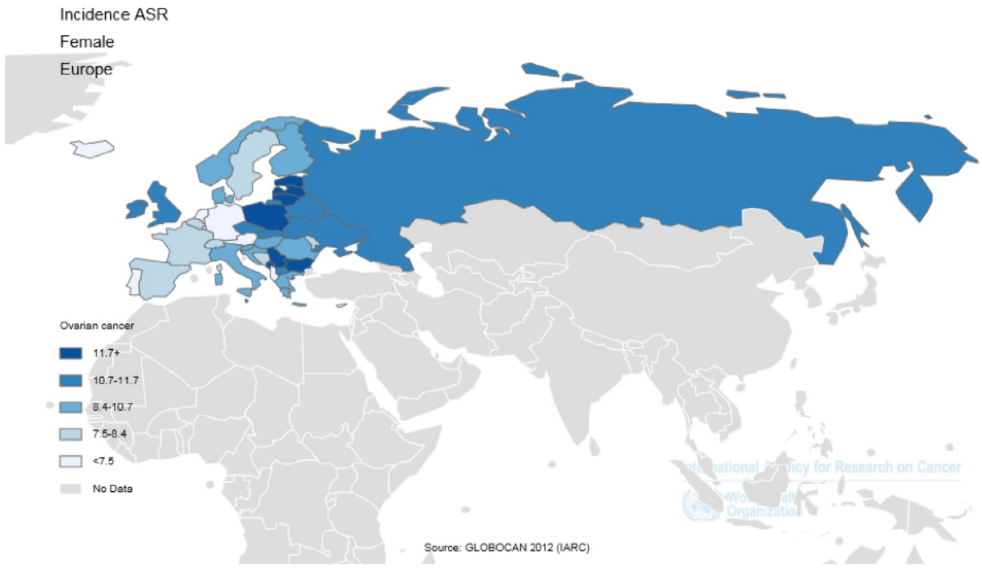

Figure 1. Distribution of the age-standardized incidence rate (ASIR) of ovarian cancer.

Of the 65,584 new cases of OC reported in 2012, 36,175 cases (55.15%) were in women below 65 years old and 29,409 cases (44.85%) were in those 65 years old or older. The numbers of new cases of OC in 2015, 2020, 2025, 2030, and 2035 are predicted to be 67,397, 69,663, 71,821, 73,730 and 74,982, respectively. These numbers are respectively 1,813 (2.76%), 4,079 (6.21%), 6,237 (9.50%), 8,146 (12.42%), and 9,398 (14.32%) higher than the new cases in 2012. While the number of new cases of OC is expected to increase in women ≥ 65 years old during 2012-2035, it is predicted to decrease in the age group below 65 years.

Of the 42,749 deaths due to OC in 2012, 16,547 (38.70%) and 26,202 deaths (61.30%) belonged to the age groups < 65 years and ≥ 65 years, respectively. The numbers of deaths due to OC are expected to reach 44,235, 46,217, 48,420, 50,622, and 52419 in 2015, 2020, 2025, 2030, and 2035, respectively. These numbers are 1,486 (3.47%), 3,468 (8.11%), 5,671 (13.26%), 7,873 (18.41%), and 9,670 (22.62%) higher than the rate reported in 2012. Apparently, the number of deaths due to OC is expected to increase between 2012 and 2035 and this increase is predicted to be more pronounced in women ≥ 65 years old.

Table 3 shows the HDI and its components in 2012. Accordingly, the European countries were classified to have very high HDI (n = 29), high HDI (n = 9), and moderate HDI (n = 2).

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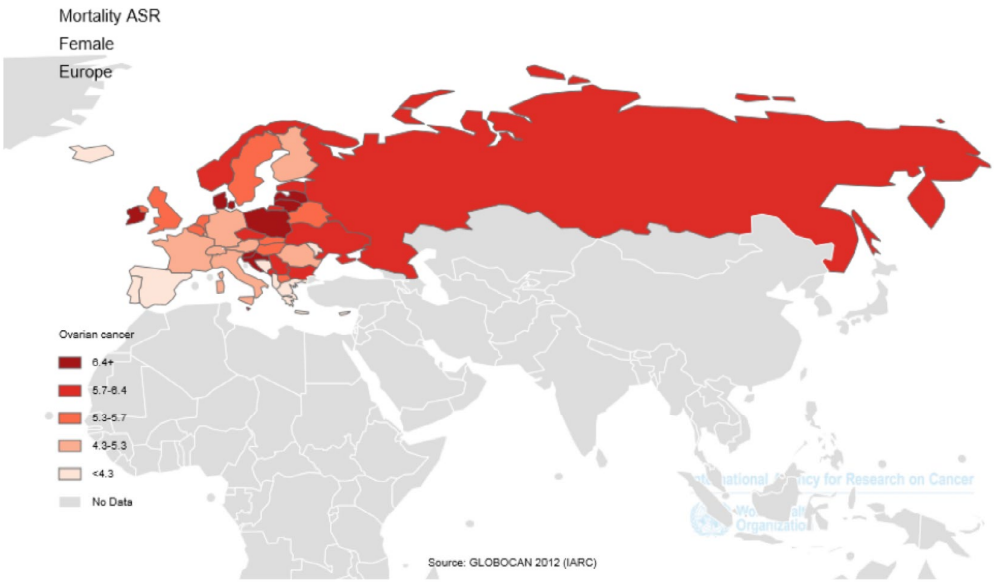

Figure 2. Distribution of the age-standardized mortality rate (ASMR) of ovarian cancer.

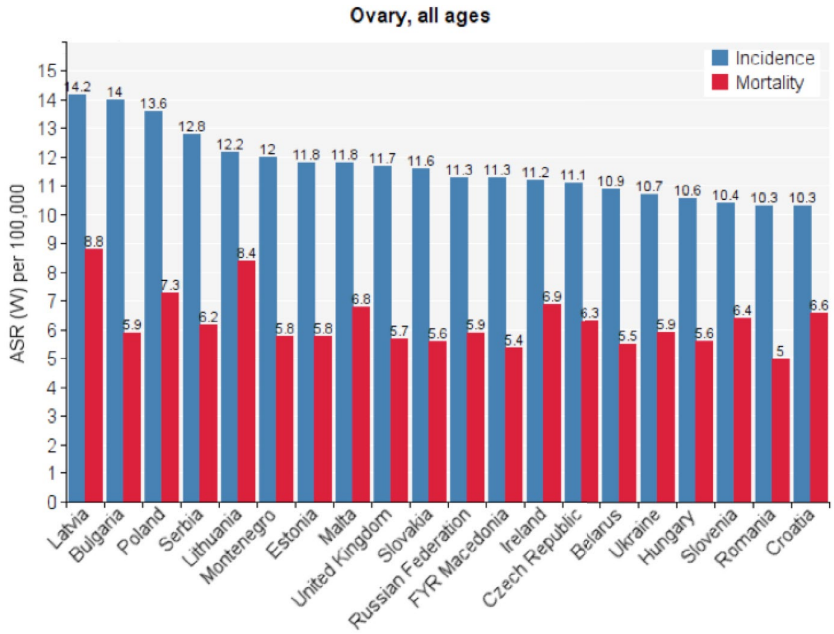

Figure 3. The age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) of ovarian cancer.

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Table 2. Estimated morbidity and mortality rates from ovarian cancer in 2012-2035

Year	Age group	Estimated number of new cancers	Age group	Estimated number of cancer deaths
2012		65584		42749
	ages < 65	36175	ages < 65	16547
	ages >= 65	29409	ages >= 65	26202
2015		67397		44235
	ages < 65	36730	ages < 65	16961
	ages >= 65	30667	ages >= 65	27274
	Demographic change	1813		1486
	ages < 65	555	ages < 65	414
	ages >= 65	1258	ages >= 65	1072
2020		69663		46217
	ages < 65	36472	ages < 65	16969
	ages >= 65	33191	ages >= 65	29248
	Demographic change	4079		3468
	ages < 65	297	ages < 65	422
	ages >= 65	3782	ages >= 65	3046
2025		71821		48420
	ages < 65	35920	ages < 65	16759
	ages >= 65	35901	ages >= 65	31661
	Demographic change	6237		5671
	ages < 65	-255	ages < 65	212
	ages >= 65	6492	ages >= 65	5459
2030		73730		50622
	ages < 65	35100	ages < 65	16351
	ages >= 65	38630	ages >= 65	34271
	Demographic change	8146		7873
	ages < 65	-1075	ages < 65	-196
	ages >= 65	9221	ages >= 65	8069
2035		74982		52419
	ages < 65	34437	ages < 65	16125

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	ages >= 65	40545	ages >= 65	36294
	Demographic change	9398		9670
	ages < 65	-1738	ages < 65	-422
	ages >= 65	11136	ages >= 65	10092

Population forecasts were extracted from the *United Nations, World Population prospects, the 2012 revision. Numbers are computed using age-specific rates and corresponding populations for 10 age-groups. GLOBOCAN 2012 (IARC) - 15.3.2016*

ASIR and HDI

A weak negative correlation was seen between the HDI and the ASIR of OC (r = -0.213; P = 0.186). There was a strong negative correlation between life expectancy at birth and the ASIR of OC (r = -0.480; P = 0.002). The mean years of schooling and the ASIR of OC had a weak positive correlation (r = 0.115; P = 0.481). Moreover, the GNI per capita and the ASIR of OC had a strong negative correlation (r = -0.348; P = 0.028) (**Fig. 4**).

ASMR and HDI

There was a weak positive correlation between the HDI and the ASMR of OC (r = 0.072; P = 0.659). Meanwhile, life expectancy at birth and the ASMR of OC were negatively correlated (r = -0.275; P = 0.086). A strong positive correlation was seen between the mean years of schooling and the ASMR of OC (r = 0.325; P = 0.041). The GNI per capita and the ASMR of OC had a weak negative correlation (r = -0.088; P = 0.591) (**Fig. 5**).

ASIR and ASMR

There was a strong positive correlation between the ASIR and ASMR of OC (r = 0.824; P ≤ 0.001) (**Fig. 6**).

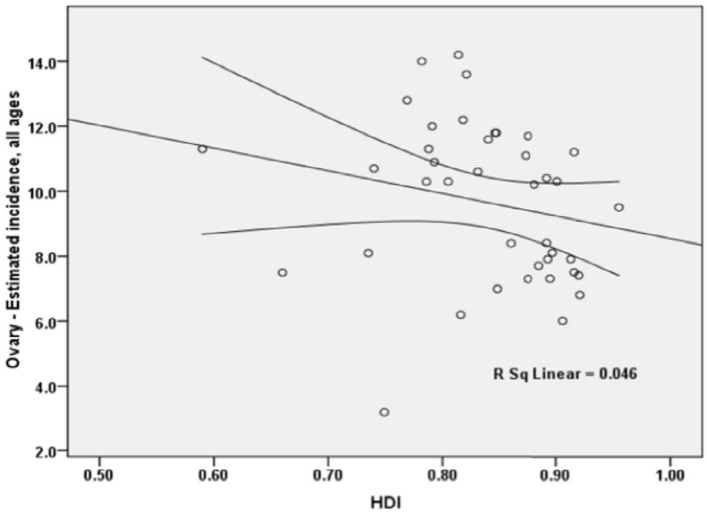

Figure 4. The correlation between the Human Development Index (HDI) and age-standardized incidence rate (ASIR) of ovarian cancer.

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Table 3. The Human Development Index (HDI) in 2012

HDI status	POPULATION	Human Development Index(HDI)	Life expectancy at birth	Average Year of schooling	Gross national income (GNI) per capita
Very high human development	Norway	0.955	81.3	12.6	48688
	The Netherlands	0.921	80.8	11.6	37282
	Germany	0.92	80.6	12.2	35431
	Ireland	0.916	80.7	11.6	28671
	Sweden	0.916	81.6	11.7	36143
	Switzerland	0.913	82.5	11	40527
	Iceland	0.906	81.9	10.4	29176
	Denmark	0.901	79	11.4	33518
	Belgium	0.897	80	10.9	33429
	Austria	0.895	81	10.8	36438
	France (metropolitan	0.893	81.7	10.6	30277
	Finland	0.892	80.1	10.3	32510
	Slovenia	0.892	79.5	11.7	23999
	Spain	0.885	81.6	10.4	25947
	Italy	0.881	82	10.1	26158
	Luxembourg	0.875	80.1	10.1	48285
	United Kingdom	0.875	80.1	9.4	32538
	Czech Republic	0.873	77.8	12.3	22067
	Greece	0.86	80	10.1	20511
	Cyprus	0.848	79.8	9.8	23825
	Malta	0.847	79.8	9.9	21184
	Estonia	0.846	75	12	17402
	Slovakia	0.84	75.6	11.6	19696
	Hungary	0.831	74.6	11.7	16088
	Poland	0.821	76.3	10	17776
	Lithuania	0.818	72.5	10.9	16858
	Portugal	0.816	79.7	7.7	19907

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	Latvia	0.814	73.6	11.5	14724
	Croatia	0.805	76.8	9.8	15419
High human development	Belarus	0.793	70.6	11.51	13385
	Montenegro	0.791	74.8	10.5	10471
	Russian Federation	0.788	69.1	11.7	14461
	Romania	0.786	74.2	10.4	11011
	Bulgaria	0.782	73.6	10.6	11474
	Serbia	0.769	74.7	10.2	9533
	Albania	0.749	77.1	10.4	7822
	Ukraine	0.74	68.8	11.3	6428
	Bosnia Herzegovina	0.735	75.8	8.3	7713
Medium human development	Republic of Moldova	0.66	69.6	9.7	3319
	FYR Macedonia	0.59	69.6	5.6	3557

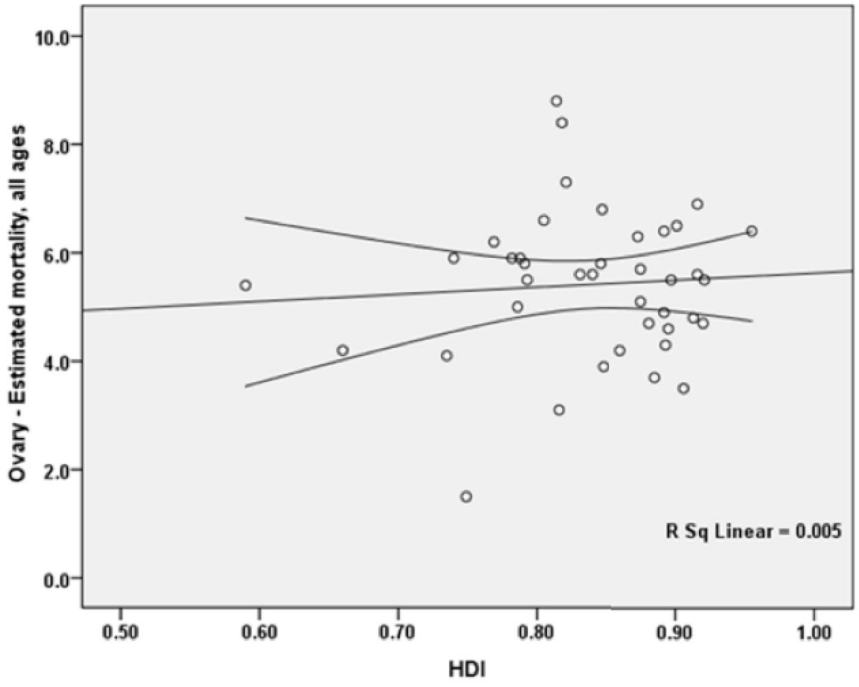

Figure 5. The correlation between the Human Development Index (HDI) and age-standardized mortality rate (ASMR) of ovarian cancer.

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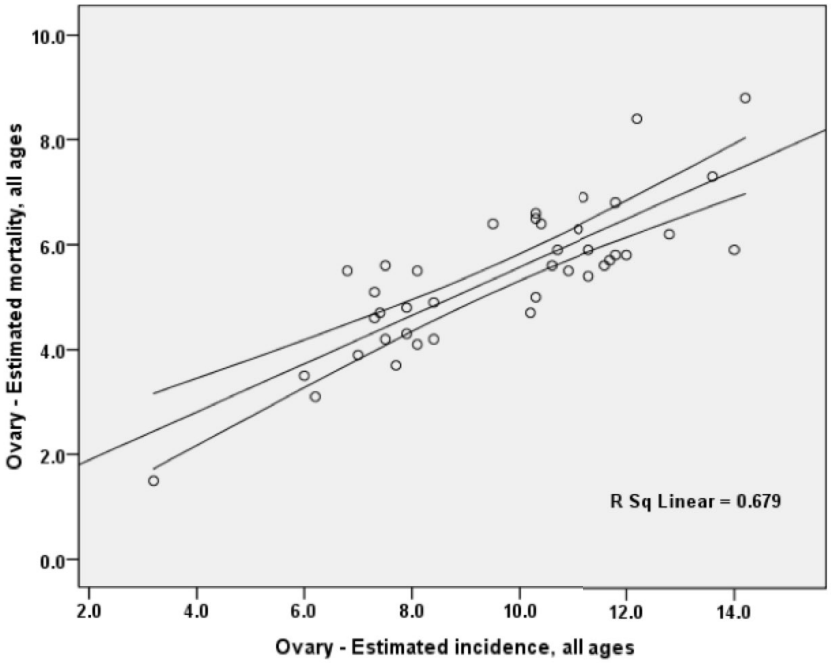

Figure 6. The correlation between age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) of ovarian cancer.

Discussion

According to the results of this study, among the European countries, Latvia, Bulgaria, Poland, Serbia, and Lithuania had the highest ASIR of OC and Latvia, Lithuania, Poland, Ireland, and Malta had the highest ASMR of OC. The incidence and mortality rates of OC are expected to increase between 2012 and 2035 and this increase is predicted to be more pronounced in women over 65 years of age. Moreover, our findings showed that increased HDI was associated with decreased ASIR and increased ASMR, but these relationships were not statistically significant.

According to the results of this study, European countries will have an increasing trend in OC morbidity and mortality. The morbidity and mortality caused by OC are expected to increase between 2012 and 2035 and this increase will be more significant in women over 65 years of age. In the past, the world's population used to consist mainly of teenagers and children. However, due to the aging phenomenon (following improved life expectancy) and decreased birth rate in recent decades, older adults currently constitute a high proportion of the world's population. With the aging of the population, an increase in the occurrence of non-communicable diseases, such as cancers, is predictable. Based on the World Health Organization (WHO) report in 2012, the aging of the population can increase the number of new cancer cases to

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19.3 million in 2025. The largest rates of new cancer cases (56.8%) and deaths (64.9%) will be seen in developing countries (Ferlay et al., 2013). Cancer is estimated to be responsible for 12.6% of all deaths. While cancer is now the second cause of death, after cardiovascular diseases, worldwide, its mortality rate is expected to exceed that of cardiovascular diseases in the coming years (Arab et al., 2014).

The incidence of cancer in diverse geographic areas can partly be attributed to variances in the lifestyle of their residents (Rohani-Rasaf et al., 2013). The incidence of cancer can be affected by numerous factors including work-related factors, alcohol and tobacco use, food and nutrition, pollution, water pollution, infectious agents, obesity, physical activity, and ultraviolet (UV) radiation (Jemal et al., 2010). Risk factors of cancer include type of nutrition, smoking, and reproductive behaviors in developed countries and infectious causes in developing countries (Rohani-Rasaf et al., 2013).

One of the significant factors in decreasing cancer morbidity and mortality might be HDI, which assesses long-term advancement in three areas of human development. It is a merged index of three rudimentary dimensions of human development including life expectancy at birth, access to education (according to a combination of adult literacy rate and primary to tertiary education enrollment rates), and income (based on per capita gross domestic product adjusted for purchasing power equality in US$).

According to the results of this study, among European countries, Latvia, Bulgaria, Poland, Serbia, and Lithuania had the highest ASIR of OC. Moreover, Latvia, Lithuania, Poland, Ireland, and Malta had the highest ASMR of OC. All these countries, except for Bulgaria and Serbia, are considered to have very high human development. Evaluating the associations between HDI and the ASIR and ASMR of OC revealed that an increase in HDI decreased the ASIR and increased the ASMR of OC. Likewise, in a study on Asian countries, the relationship between HDI and the ASMR of OC was negative and not significant. However, in contrast to our findings, the results of this study showed a significant positive correlation between HDI and the ASIR of OC (Razi et al., 2016).

Socioeconomic factors affect the incidence of cancer through complex and variable mechanisms. Fidler et al. reported positive correlations between HDI and the ASIR of leukemia, multiple myeloma, and lung, pancreas, gallbladder, brain/nervous system, colorectal, kidney, and thyroid cancer. Moreover, positive relations were detected between HDI and the ASIR of melanoma of the skin, Hodgkin lymphoma, and bladder, testicular, and lip/oral cavity cancers in males. HDI was also positively related with non-Hodgkin lymphoma, and breast, corpus uteri, and ovarian cancers in females. A negative correlation was detected between HDI and the ASIR of Kaposi sarcoma, and cervical and other pharyngeal cancers in females (Fidler et al., 2016). Moreover, Præstegaard et al.

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indicated a relationship between lower level of education and an increased risk of progressive tumor stage at diagnosis of OC (Præstegaard et al., 2016).

Limitations

Since this was an ecological study, its results are significant and interpretable at the population level and ecological fallacy will happen if the results are interpreted at the individual level. Some factors such as gynecological surgeries, tubal ligation, number of deliveries, gravidity, oral contraception and breastfeeding may reduce the risk of OC. On the other hand, some medical conditions and environmental factors such as endometriosis, hyperthyroidism, ovarian cysts, medical history and occupation hazards increase the risk of OC. However, in this study, we did not have access to adequate data on these factors to discuss their effects on the incidence and mortality of OC. Therefore, in addition to ecological research in each of the European countries, studies at an individual level with case-control or cohort designs are required to detect the role of factors associated with the incidence and mortality of OC at an individual level.

Conclusion

According to the results of this study, among European countries, Latvia, Bulgaria, Poland, Serbia, and Lithuania had the highest ASIR and Latvia, Lithuania, Poland, Ireland, and Malta had the highest ASMR of OC. Moreover, an increase in in HDI was associated with decreased ASIR and increased ASMR of OC. However, these relationships were not statistically significant. The morbidity and mortality of OC had an increasing trend in European countries. Therefore, it seems that health policy makers in these countries should make appropriate decisions to deal with the growth in the morbidity and mortality of OC, especially in women older than 65 years of age, in regions which may have lower access to prevention and treatment services.

Abbreviations

ASIR: Age-specific incidence rate
ASMR: Age-specific mortality rate
HDI: Human Development Index
OC: Ovarian Cancer

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Author Contribution

All authors contributed to the design of the research. MM, MG, BK and HS collected the data. HS, BK and AMH conducted analysis and interpretation of data. All authors drafted the first version. MM, MA, FAB, MG, AMH edited the first draft. All authors reviewed and commented on final draft.

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