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ARYA Atherosclerosis

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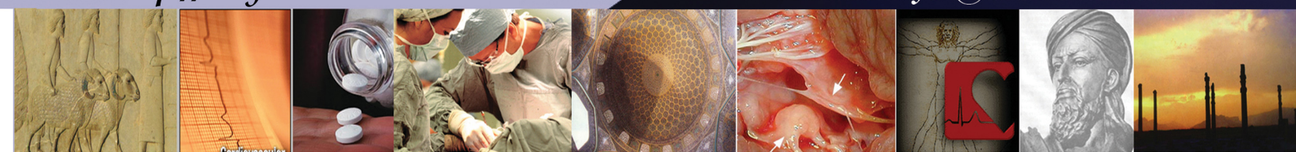

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2. Bonow RO, Mann DL, Zipes DP, Libby P. Braunwald's Heart Disease E-Book: A Textbook of Cardiovascular Medicine. 7th ed. Philadelphia, PA: Elsevier Health Sciences; 2007. p. 1976, 1981, 1982.

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miR-33 inhibition attenuates the effect of liver X receptor agonist T0901317 on expression of liver X receptor alpha in mice liver

Abbas Mohammadi[(1)], Hossein Fallah[(2)], Beydolah Shahouzehi[(3)], Hamid Najafipour[(4)]

Original Article

Abstract

BACKGROUND: microRNAs play pivotal roles in metabolism and other aspects of cell biology. microRNA-33 and liver X receptor (LXR) affect lipid metabolism and cholesterol trafficking. In this study, we evaluated effects of co-administration of miR-33 inhibitor and LXR activator on LXR-α and adenosine triphosphate-binding cassette transporter A1 (ABCA1) expression in mice liver.

METHODS: Twenty-four mice were randomly allocated into four groups (n = 6). Group 1 mice received standard chow diet without any treatment, group 2 received 30 mg/kg/48 hour LXR agonist (T0901317), group 3 received 1 mg/kg/48 hour in vivo locked nucleic acids (LNA) anti-miR-33 and group 4 received both T0901317 and in vivo LNA anti-miR-33. All treatments were administrated through intraperitoneal injection (IP). After 7 days and at the end of the study, mice were sacrificed, liver tissues were excised and blood samples were collected. LXR-α and ABCA1 genes and protein expression were quantified by real-time polymerase chain reaction (PCR) and western blotting, respectively.

RESULTS: LXR activation caused LXR-α and ABCA1 mRNA ($P < 0.050$) and protein elevation as compared to control ($P < 0.001$). miR-33 inhibition attenuates T0901317 effect on LXR-α expression in group IV. Co-administration of T0901317 and anti-miR-33 remarkably elevated high-density lipoprotein cholesterol (HDL-C) levels, compared to control group ($P = 0.001$). Separate administration of T0901317 and anti-miR-33 also elevated HDL-C levels ($P < 0.010$).

CONCLUSION: Co-administration of T0901317 and anti-miR-33 can be considered as a good therapeutic alternative for atherosclerosis because miR-33 inhibition reduced lipogenic effects of LXR-α activator and also helps LXR-α agonist to increase reverse cholesterol transport (RCT) and also HDL-C as antiatherogenic effects.

Keywords: Atherosclerosis, mir-33 Human, T0901317, Liver X Receptor-Alpha, ABCA1 Protein

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Introduction

Dysregulation of lipid homeostasis is related to cardiometabolic and cardiovascular diseases.[1] Atherosclerosis is a progressive and multi-factorial disorder. Cholesterol-laden macrophages are the hallmark of atherosclerotic plaques.[2,3] Finding strategies in order to reduce the risk of cardiometabolic disease is the main goal of researchers. To achieve this goal, considerable attention has been paid to elevate the high-density lipoprotein cholesterol (HDL-C) levels and improve the reverse cholesterol transport (RCT).[4-6] Cholesterol removal from tissues occurs via the liver. The process of cholesterol efflux from peripheral tissues and transport to the liver by HDL-C is known as RCT.[1,7] adenosine triphosphate-binding cassette transporter A1 (ABCA1) is necessary for cholesterol trafficking in RCT process. It also has a pivotal role in HDL-C formation.[1,4,8]

Liver X receptors (LXRs) are nuclear transcription factors which regulate lipid homeostasis. LXRs are comprised of two isoforms, designated as LXR-α and LXR-β, which are

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identified as members of orphan receptors superfamily. Recently there have been some reports showing cholesterol derivatives, such as oxysterols, act as physiological ligands of LXRs.[9,10] Synthetic LXR agonist, T0901317, is also capable to bind to and activating LXRs in the liver.[11] LXRs target genes are including sterol regulatory element-binding proteins (SREBPs), ABCA1, ABCG1, ABCG5, ABCG8, and cholesterol 7-α-hydroxylase (CYP7A).[10-13] LXRs can be encouraging therapeutic targets for cardiovascular disease prevention and treatment because there are beneficial effects following LXR activation on lipid homeostasis.[9,10,12] LXRs play a pivotal role in RCT regulation which occurs by direct LXR effects on ABCA1 and ABCG1.[13] LXR activation results in HDL-C elevation and reduced cholesterol levels.[14] Some studies have shown that LXR activation, by a synthetic ligand, increased expression of SREBP-1c and fatty acid synthase (FAS) and cause elevation of triglyceride (TG) levels.[9,10,13,15] Due to the lipogenic properties of synthetic agonists of LXR, their utilization as a therapeutic target in atherosclerosis would be in doubt.

microRNAs are endogenous, short (18-25 nucleotides) non-coding RNAs which bind to their target mRNA in order to inhibit its translation or degrade it.[1] Najafi-Shoushtari discovered that there are two microRNAs in the introns of SREBP-1 and SREBP-2 genes known as miR-33b and miR-33a, respectively.[1] miR-33a/b have very similar sequence and their seed sequence is exactly the same. Therefore, both have the same mRNA targets. miR-33 is involved in cholesterol and lipid metabolism and regulates ABCA1 and adenosine monophosphate-activated protein Kinase (AMPK). miR-33 inhibits ABCA1 expression and results in increased intracellular cholesterol levels.[1] Also, it has been demonstrated that miR-33 antisense oligonucleotides and genetic depletion of miR-33a in mice increased HDL levels.[1]

AMPK is an energy sensor which elevates fatty acid β-oxidation and also reduces synthetic pathways such as cholesterol and TG synthesis. AMPK activation attenuates LXR-induced steatosis in mice liver.[16] Moreover, AMPK activation reduces SREBP-1c mRNA expression which is increased by T0901317.[17,18] miR-33 elevation increases intracellular cholesterol levels and reduce RCT which results in generation and deterioration of atherosclerosis and plaques formation.[1,15] In this study, we evaluated the effects of miR-33 inhibition and LXR activation and the combination of both on LXR-α and ABCA1 expression in mice liver and circulating HDL levels.

Materials and Methods

T0901317, as LXR agonist, was obtained from Cayman Chemical (Ann Arbor, Michigan, USA, Cat no. 71810), in vivo locked nucleic acids (LNA)™ miR-33 inhibitor was purchased from Exiqon (Woburn, MA, United States). EZ-10 spin column total RNA mini-prep super kit (Bio Basic Inc., Markham, Canada), Primescript reverse transcriptase (RT) reagent kit (Takara Bio Inc., Japan), SYBR Premix Ex Taq II (Tli RNase H Plus) Takara Bio Inc., Japan), LumiFilm Chemiluminescent detection film (Roche, Germany), Phenylmethanesulfonyl Fluoride (Sigma, USA), sodium orthovanadate (Sigma, USA), protease inhibitor cocktail (Sigma, USA), and Amersham enhanced chemiluminescence (ECL) prime western blotting detection reagent (GE Healthcare UK Ltd.) were also used in this study. ABCA1 (sc-20794), β-Actin (sc-130656), LXRα/β (sc-13068), and goat anti-rabbit IgG-HRP (sc-2004) were purchased from Santa Cruz (Sana Cruz Biotechnology, USA). Real-time polymerase chain reaction (PCR) was performed on ABI StepOnePlus instrument (Applied Biosystem, Step One Plus, USA).

Mice were purchased from Animal Care Center of Kerman Physiology Research Center, Iran. Animals were kept for a week in an animal care facility for acclimatization. Then, mice weighing 22 ± 2 g were selected for the study and housed at a temperature of 22 °C, 12-hour light/12-hour darkness cycle. Animals had open access to standard chow diet and water. Selected mice were randomly allocated into four groups (n = 6) as follow: group 1, the control untreated group, received standard chow diet, group 2 received 30mg/kg/48 hour of T0901317, group 3 received 1mg/kg/48 hour of LNA miR-33 inhibitor, and group 4 received combination of LXR agonist and LNA miR-33 inhibitor as groups 2 and 3. Duration of the study was a week and animals in treatment groups received 3 intraperitoneal injections (IP) every two days. At the end of the study, after 10-hour fasting overnight, mice were sacrificed, blood samples were collected and serum was separated for HDL-C measurement. Also, liver tissues were excised and washed with cold saline and frozen in liquid nitrogen immediately and maintained at -80 °C for further examination. All procedures were approved by the Animal Research Ethics

Table 1. Sequences of real-time polymerase chain reaction (PCR) primers

Gene	Forward primer 5' to 3'	Reverse primer 5' to 3'	Product size (kb)
ABCA1	GCTCTCAGGTGGGATGCAG	GGCTCGTCCAGAATGACAAC	81
β-Actin	CAACGAGCGGTTCCGATG	GCCACAGGATTCCATACCCA	90
LXR-α	AGGAGTGTCGACTTCGCAAA	CTCTTCTTGCCGCTTCAGTTT	101

ABCA1: Adenosine triphosphate-binding cassette transporter A1; LXR-α: Liver X receptor alpha

Committee of Kerman University of Medical Sciences, Iran (ethics committee permission number: IR.KMU.REC.1394.319).

Total RNA was extracted by EZ-10 spin column total RNA mini preps super kit according to company's instructions. For this purpose, 75 mg of liver tissue was excised from frozen storage and used for RNA extraction. For cDNA synthesis, we used 250 ng of total RNA and PrimeScript RT reagent kit, using Oligo-dT and random 6mer primers. Real-time PCR was performed by SYBR Premix Ex Taq II (Tli RNase H Plus). The reactions contained ROX (6-Carboxyl-X-Rhodamine), forward and reverse primers, sterile water, and 100 ng cDNA. Real-time PCR was performed on ABI StepOnePlus instrument. The thermal reaction was stage 1 denaturation, 95 °C for 10 minutes, stage 2, 40 cycles of 95 °C for 20 seconds and 60 °C for 30 seconds. Furthermore, the melt curve analysis was performed which started from 60 °C and increased by 0.3 °C. Primers (Table 1) were purchased from Macrogen (Macrogen Inc., Seoul, South Korea). Finally, the expression level was determined by the $2^{-\Delta\Delta Ct}$ method and normalized to β-actin as the housekeeping gene.[19,20]

For tissue homogenization and protein extraction, we used RIPA buffer (containing protease inhibitor cocktail, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM sodium orthovanadate, pH 7.4). About 50 mg of liver tissue was excised and homogenized in ice-cold RIPA buffer by sonication (Hielscher Ultrasound, UP200H, Germany). Homogenates were centrifuged at 15000 rpm, 4 °C for 20 minutes and supernatant was removed for further study. Total protein concentration in the supernatant was measured by the Bradford method. In order to run sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), an equal volume of 2× sample buffer was added to each sample and incubated at 95 °C for 5 minutes. Then, 100 µg of total protein was loaded into each well of a 12.5% gel and separation was conducted by a constant voltage (120 V, 80 min, Tris-Gly buffer, pH 8.3). After electrophoresis, proteins were transferred to a Polyvinylidene Difluoride (PVDF) membrane at a constant current 220 mA in cold transfer buffer. The membrane was incubated with blocking buffer (5% skim milk in Tris-buffered saline and Tween 20) overnight at 4 °C. In the next step, the membrane was washed 4 times, 5 minutes each. Washed membrane was incubated with primary antibody for 1 hour, followed by a washing step for 4 times. Eventually, incubation with secondary antibody for 1 hour was carried out, followed by 4 times washing, and the PVDF membrane was incubated with Western Lightning Plus ECL for 1 minute. The antigen-antibody complex was detected by enhanced chemiluminescence detection film in a dark room. Band densities were analyzed by the ImageJ software. β-Actin was used as the housekeeping gene and control.[21]

Real-time PCR and western blotting data, and HDL-C levels were reported as a mean ± standard error of the mean (SEM). Data were analyzed by SPSS software (version 19, SPSS Inc., Chicago, IL, USA). Comparison between groups was conducted by analysis of variance (ANOVA) and pair-wise comparison was done by post hoc Tukey's test. $P \leq 0.050$ was considered as statistically significant.

Results

We showed that anti-miR-33 reduced miR-33 levels by real-time PCR (data are not shown here). LXR activation by LXR synthetic agonist T0901317 elevated LXR-α gene expression compared to control ($P = 0.046$) (Figure 1A). In contrast, anti-miR-33 reduced LXR-α gene expression which was attenuated by a combination of both T0901317 and anti-miR-33. ABCA1 expression was elevated by T0901317 ($P = 0.033$), anti-mR-33 ($P < 0.001$) and the combination of both, but only when they were administrated separately the elevation was significant compared to control group. In fact, a combination of T0901317 and anti-miR-33 insignificantly increased ABCA1 gene expression (Figure 1B).

LXR-α protein levels were significantly increased by T0901317 administration compared to control ($P < 0.001$). On the other hand, anti-miR-33 and combination of anti-miR-33 plus T0901317 significantly reduced LXR-α protein levels compared to T0901317 ($P < 0.001$) (Figure 2A).

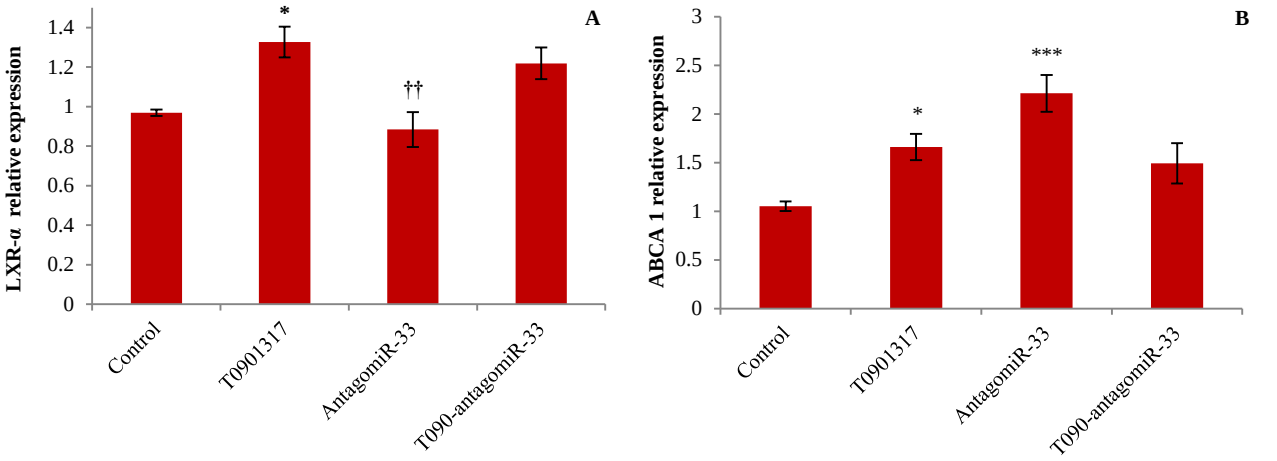

Figure 1. Expression of liver X receptor (LXR)-α (A) and adenosine triphosphate-binding cassette transporter A1 (ABCA1) (B) genes was evaluated by real-time polymerase chain reaction (PCR). Control group received standard mice chow diet, T0901317 group received T0901317 by intraperitoneal injection (IP), AntagomiR-33 group received anti-miR-33 by IP, and group T090-antagomiR-33 received combination of both. Data are expressed as mean ± standard error of the mean (SEM).
* statistically significant compared to the control group; † statistically significant compared to the T0901317 group (* $P < 0.050$, *** $P < 0.001$, †† $P < 0.010$)

ABCA1 protein expression was elevated in other three groups compared to control group ($P < 0.001$), also, anti-miR-33 therapy increased ABCA1 protein levels significantly compared to T0901317 group ($P < 0.050$) (Figure 2B). HDL-C increased in three other groups compared to control, T0901317 and antimiR-33 elevated HDL-C significantly ($P < 0.010$). However, the group that received antimiR-33 plus T0901317 caused more significant elevation of HDL-C ($P = 0.001$) (Figure 3).

Discussion

LXR activation by T0901317 elevated LXR-α expression and we found that anti-miR-33 administration attenuates T0901317 increasing effect on LXR-α expression. By increasing ABCA1 and ABCG1, LXR-α increases HDL-C which is an antiatherogenic factor.[2]

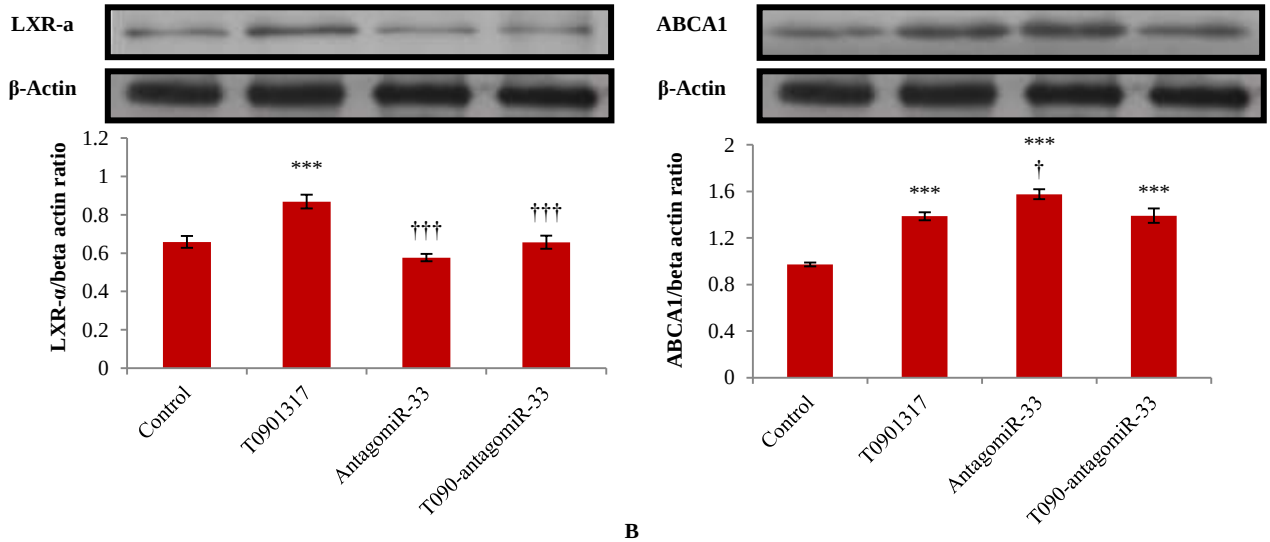

Figure 2. Protein expression of liver X receptor (LXR)-α (A) and adenosine triphosphate-binding cassette transporter A1 (ABCA1) (B) was evaluated by western blotting. Control group received standard mice chow diet, T0901317 group received T0901317 by intraperitoneal injection (IP), AntagomiR-33 group received anti-miR-33 by IP, and groupT090-antagomiR-33 received a combination of both. Data are expressed as mean ± standard error of the mean (SEM).
* Statistically significant compared to the control group, † Statistically significant compared to the T0901317 group (*** $P < 0.001$, ††† $P < 0.050$)

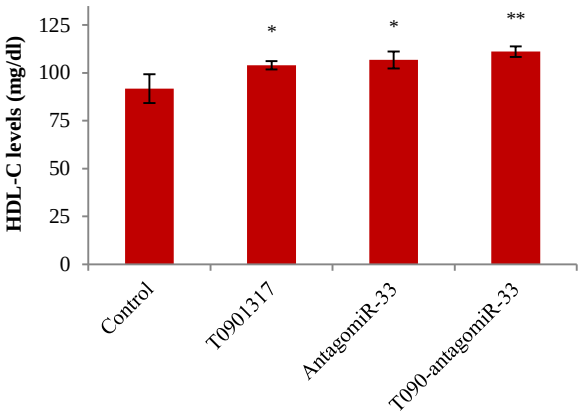

Figure 3. High-density lipoprotein cholesterol (HDL-C) levels were elevated by T0901317, anti-miR-33 and co-administration of T0901317-antagomiR-33. Control group received standard mice chow diet, T0901317 group received T0901317 by intraperitoneal injection (IP), AntagomiR-33 group received anti-miR-33 by IP, and groupT090-antagomiR-33 received combination of both. Data are expressed as mean ± standard error of the mean (SEM).
* statistically significant compared to the control group (* $P < 0.010$, ** $P < 0.001$)

The beneficial and therapeutic effects of LXR-α induction over atherosclerosis would be limited because of promotion of lipogenesis which may result in hepatic steatosis or possibility of exacerbating insulin resistance-related disease.[10,22] We showed that LXR activation and miR-33 inhibition have different effects on LXR-α expression. Thus, T0901317 increased and anti-miR-33 reduced LXR-α expression, while anti-miR-33 therapy along with T0901317 reduced LXR-α expression. Consistent with our results, Horie et al. showed that miR-33 deficiency elevated cholesterol efflux from macrophages and promote HDL-C production resulting in regression of atherosclerosis.[5] Finally, our findings showed that anti-miR-33 therapy helps T0901317 to increase HDL-C levels and also, miR-33 inhibition reduced LXR-α lipogenic effects. It has been showed that anti-miR-33 therapy increases AMPK and ABCA1 expression.[1] AMPK has negative effects on synthetic pathways through impeding cholesterol and TG synthesis by inhibition of LXR-α, SREBPs and 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR).[23] We conclude that anti-miR-33 administration, which is well-documented AMPK up-regulator, inhibits LXR-α/SREBP-1c lipogenesis pathway probably by an AMPK dependent mechanism.

ABCA1 helps cholesterol trafficking. It is also a major component of RCT and HDL-C production.[4,24] We found that separate administration of either T0901317 or anti-miR-33 increased ABCA1 expression. Co-administration of T0901317 and anti-miR-33 attenuated ABCA1 gene expression but still, ABCA1 expression is more than the control group. Previous studies have demonstrated that ABCA1 reduced intracellular cholesterol content which stimulates SREBP-2 expression.[6-8] SREBP-2 is a transcription factor increasing HMGCR expression and elevates cholesterol biosynthesis.[6] According to the recent reports, it has been proved that miR-33 is coexpressed along with SREBP-2 gene.[25,26] miR-33 inhibits ABCA1 mRNA and reduces ABCA1 levels.[27] Therefore, we conclude that this reduced ABCA1 expression by combination of T0901317 plus anti-miR-33, compared to either T0901317 or anti-miR-33, probably is a consequence of miR-33 slight elevation (miR-33 data are not shown) in this group. However, there was a significant elevation of ABCA1 protein expression in all three groups compared to the control.

miR-33 regulates intracellular cholesterol levels. Overexpression of miR-33 declines cholesterol efflux and in contrary, miR-33 inhibition causes elevation of cholesterol efflux by LXR activation.[9,27] LXR activation in peripheral tissues such as macrophages increases RCT and cholesterol transport to the liver. In liver, LXR synthetic agonists lead to cholesterol excretion.[7,10] Inhibition of miR-33 activity by anti-miR-33 in peripheral tissues helps LXR activation to increase ABCA1 levels and may improve RCT as a synergic effect.[28] Li et al. demonstrated that AMPK activation reduced hepatic steatosis by inhibition of SREBPs activity. They introduced this mechanism as a new therapeutic strategy for atherosclerosis and dyslipidemia.[29] We showed that miR-33 inhibition along with T0901317 administration improved ABCA1 protein levels significantly; on the other hand, anti-miR-33 therapy reduces LXR-α expression. Anti-miR-33 increases AMPK levels which inhibit LXR-α and SREBP-1c levels, so it seems that miR-33 suppression by anti-miR-33 administration in liver attenuates LXR agonist lipogenic effects and can help to reduce steatohepatitis.

We showed that LXR activation or miR-33 inhibition lead to HDL-C elevation ($P < 0.050$). Moreover, co-administration of anti-miR-33 and T0901317 remarkably increased HDL-C levels ($P < 0.001$). Yamamoto et al. showed that hepatic

ABCA1 inhibition reduced plasma HDL-C but increased RCT and cholesterol excretion as bile.[7] We also found that co-administration of T0901317 and anti-miR-33 reduced ABCA1 gene expression which is consistent with Yamamoto et al. findings. However, we did not observe this effect on ABCA1 protein levels.[7] Yamamoto et al. reported that HDL-C was reduced after ABCA1 decline.[7] On the contrary, we observed a significant elevation of HDL-C levels after co-administration of T0901317 and anti-miR-33 and slight reduction of hepatic ABCA1 expression. According to previous studies, this HDL-C increase could probably result from LXR activation and miR-33 inhibition in peripheral tissues, which return cholesterol by ABCG1 and ABCA1 to circulation.[10,28]

Conclusion

miR-33 inhibition is a potential therapeutic approach which elevates HDL-C levels. Thus, it may be a potential therapeutic target to reduce the risk of cardiovascular disease. Also, many studies have shown that LXR activation has beneficial effects on lipid metabolism and atherosclerosis. They have also suggested that this could be a novel target for cardiovascular disease treatment. Our results showed that combination of T0901317 and miR-33 suppression remarkably elevate HDL-C production and also reduce LXR-α expression, the latter being totally dependent on miR-33 inhibition. Furthermore, they also reduced LXR-α expression which is a master regulator of TG synthesizing genes. Therefore, administration of a combination of miR-33 inhibition and LXR activation can be considered as a therapeutic approach in cardiovascular and cardiometabolic disorders.

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Conflict of Interests

Authors have no conflict of interests.

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Air pollution and cardiovascular and respiratory disease: Rationale and methodology of CAPACITY study

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Original Article

Abstract

BACKGROUND: Considering the high level of air pollution and its impact on health, we aimed to study the correlation of air pollution with hospitalization and mortality of cardiovascular (CVD) and respiratory diseases (ResD) (CAPACITY) to determine the effects of air pollutants on CVD and ResD hospitalizations and deaths in Isfahan, Iran.

METHODS: Hourly levels of air pollutants including particulate matter (PM), carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3), information of CVD and ResD admissions and death certificate were obtained respectively from Department of Environment (DOE), Iran, hospitals and cemetery. Time series and case-crossover model were used to find the impact of air pollutants. This paper only summarizes the descriptive findings of the CAPACITY study.

RESULTS: The total number of hospitalized patients were 23781 in 2010 and 22485 in 2011. The most frequent cause of hospitalization and death was ischemic heart diseases in both years. While the mean annual levels of O_3, CO, and PM_{10} were lower in 2011 than in 2010, NO_2 and SO_2 levels higher in 2011. In both years, PM_{10} was similarly increased during last month of fall, late spring and early summer. In 2011, the $PM_{2.5}$ and PM_{10} monthly trend of change were similar.

CONCLUSION: The CAPACITY study is one of the few large-scale studies that evaluated the effects of air pollutants on a variety of CVD and ResD in a large city of Iran. This study can provide many findings that could clarify the effects of these pollutants on the incidence and burden of both disease groups.

Keywords: Air Pollution, Cardiovascular Diseases, Respiratory Tract Diseases

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Introduction

Air pollution is undoubtedly associated with a variety of diseases, and thus serves as an indirect cause of mortality.[1,2] According to the World Health Organization (WHO) report for 2012, three million deaths throughout the world were attributable to ambient air pollution. Cardiovascular diseases (CVDs) and respiratory disease (ResD) were responsible for respectively 72% and 14% of these deaths.[3]

Numerous studies, mainly in the developed countries, have evaluated the relationships between air pollution and death or hospitalization due to ResD

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and CVDs.[4-7] However, there is a shortage of similar research in developing countries where rapid population growth and its consequences such as an increase in air pollution generally raise the disease burden. The mentioned relationships can even be confounded by climatic factors,[8] poor air quality management policies, and inefficient monitoring of environmental factors, The absence of a comprehensive patient information systems have turned the evaluation of the health effects of air pollution in developing countries into a challenge and necessitated further large-scale studies in these areas.[9]

Iran, a developing country, has witnessed a rise in air pollution during the recent years. In 2012, 26267 Iranians died due to air pollution and WHO identified Iran as one of the most polluted countries in the world.[10] Although growing research attention has been paid to the subject, only few studies have been performed at large-scale level and have examined the simultaneous effects of pollutants on CVD- and ResD-related hospitalizations and deaths.[11,12]

As an industrial city and the third large metropolis in Isfahan, Iran, has to deal with the problem of air pollution. According to Isfahan cohort study, CVDs are more frequent in Isfahan, even among the youth and women, than in many other populations.[13] Moreover, the high levels of CVD risk factors, particularly unhealthy lifestyle, would gradually increase the incidence of these diseases in the city. The prevalence of diabetes and hypertension in Isfahan has been estimated at 5.6% and 17.3%, respectively[14] and 21.8% of the man population of the city are smokers.[15] The daily sodium intake in the city is 4309.6 ± 1344.4 mg[16] and over half of the population do not have adequate levels of physical activity.[17,18] The city is surrounded by numerous industrial factories including Mobarakeh Steel Company, Isfahan Steel Company, Isfahan Oil Refinery, and power plants. Following the population growth in recent years, the numbers of motor vehicles and daily commutes, and thus, the levels of air pollutants have increased in the city.[19]

Therefore, we performed the correlation of air pollution with hospitalization and mortality of CVD and ResD (CAPACITY). This study evaluated the effects of different pollutants including particulate matter (PM) 10 and 2.5, carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and ozone (O_3), on two major outcomes of CVD and ResD-related hospitalization and death, under controlled climatic conditions. In this article, we report the rational, methodology and basic descriptive results of the CAPACITY study.

Materials and Methods

The CAPACITY study was performed in Isfahan from March 2010 to March 2012. Two methods, time series and case-crossover designs, were adopted for the purpose of the study. It is noteworthy that this paper reported the methodology of the CAPACITY study, including data collection, management, and statistical models of data analysis, in detail. However, only descriptive findings of the CAPACITY study were discussed and all other findings will be presented in future publications.

The city of Isfahan (32.6546 °N, 51.6680 °E) covers an area of 493.82 km^2 in the center of Iran, along with the foothills of Zagros mountains and Zayanderud plain. With a population of 2240249 in 2016 (2067821 people in 2011), Isfahan is the third largest metropolis in Iran.[20,21] It is located 1574 m above sea level with a mean temperature of 15.6 °C, and a mean annual precipitation of 125 ml. It has an arid climate categorized as cold desert climates in the Köppen-Geiger system.[22] However, as Zayanderud has recently changed from a permanent to a seasonal and sometimes even dried out river, Isfahan currently has drier climate.[10]

Owing to its industrial conditions and large factories, Isfahan has a relatively high population density.[23] Two thermal power plants are located near Isfahan. Shahid Montazeri Power Plant in the north of the city is one of the largest power plants in the country and generates 1616 MW per day. Isfahan Power Plant, located 75 km southwest of Isfahan, generates 954 MW per day.[24] Two large steel companies are also located in the west and southwest of Isfahan. There is also a large cement plant in the west of the city and an oil refinery in the north[25] (Figure 1).

The studied population consisted of all residents of Isfahan who were admitted either to hospitals of Isfahan or died due to a confirmed diagnosis of CVDs or ResDs based on the International Classification of Diseases-10 (ICD-10).[26]

Hospitals with coronary care units (CCU), emergency wards for cardiac or respiratory patients or cardiology, internal medicine, and respiratory diseases wards (where the target patients were likely to be admitted) in Isfahan were identified in coordination with the Deputy of Treatment. A total of 15 eligible hospitals including 8 universities, 4 private, 1 military, and 2 social insurance hospitals, were identified at the time of the study.

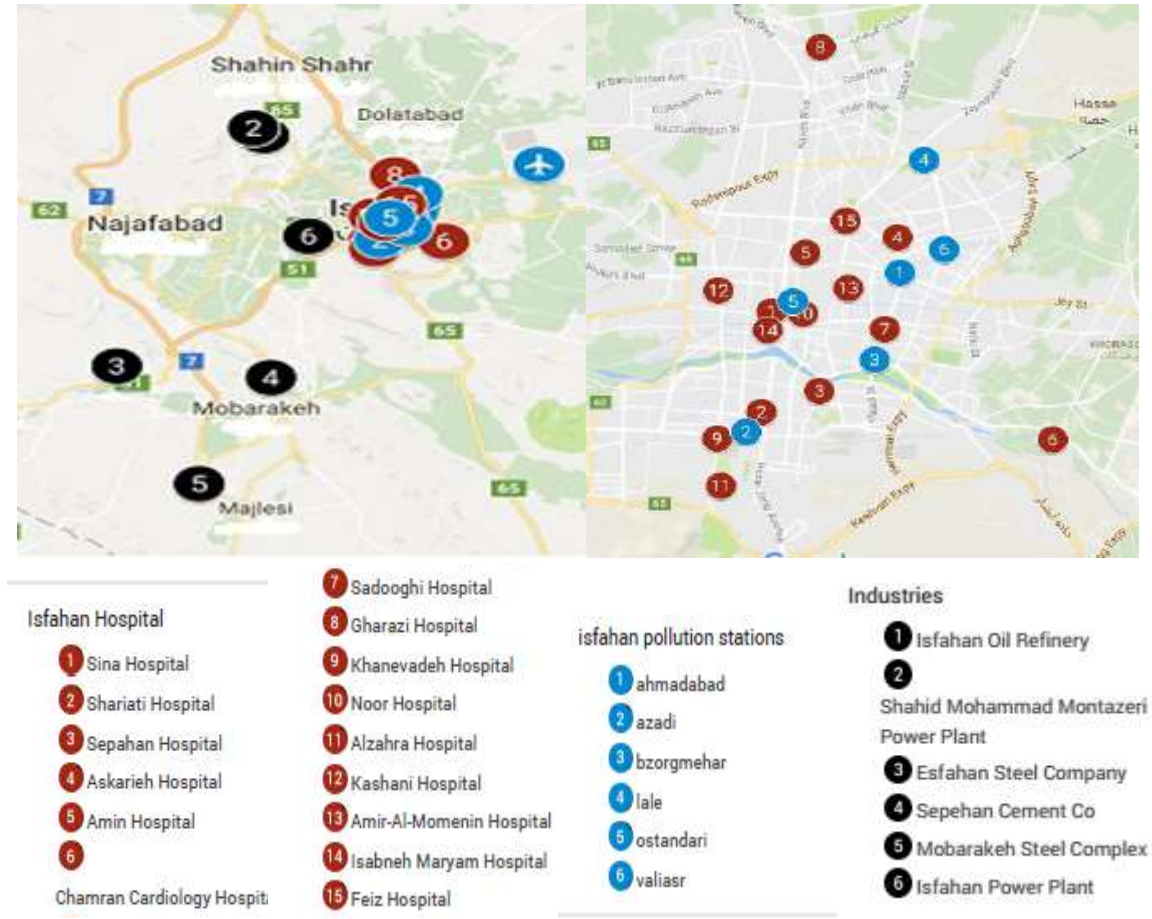

Figure 1. The location of air pollution monitoring stations in Isfahan, Iran, meteoritic data

Patients' information was collected through the hospital information system (HIS). Trained experts in medical records used patients' files to extract information like treatment, dates of admission and discharge, diagnosis, prescribed medications, lab results, and performed invasive procedures, as well as administrative and accounting information, such as incurred costs, insurance coverage, and health subsidies during the hospitalization period. Then, they entered the data into the HIS. However, clinical examination was not recorded in the system. Data entry was supervised by the treatment deputy of relevant university. The HIS of all hospitals in the country are constantly evaluated and monitored by the Office of Statistics and Information Technology of the Ministry of Health.[27]

15 hospitals in Isfahan met the criteria and considered in this study. However, only eight hospitals used the HIS in 2010. The medical record experts of these hospitals entered the final diagnosis for each patient (recorded in patient files by relevant physicians) in the HIS. The diagnoses were recorded based on ICD-10 codes. All emergency admitted patients diagnosed with codes I00-I99 (for circulatory diseases) and J00-J99 (for ResD) were enrolled. The demographic characteristics of the patients (name, father's name, file number, national identification number, sex, age, occupation, home and work address, and insurance coverage) and disease-related information (admission time and unit, type of referral, and final diagnosis) were then extracted by the hospital medical records experts.

In the remaining seven hospitals without HIS, patient files were archived based on the final diagnosis (ICD-10 codes). Therefore, medical record experts were asked to extract the paper files of all patients with CVD or ResD with a diagnosis of I00-I99 or J00-J99. The above-mentioned information was then collected and entered into a designed reporting software package.

In 2011, all hospitals used the HIS, and thus the described procedure used in hospitals with HIS was followed to extract data.

Since all patients with I00-I99 and J00-J99 diagnoses were included, the search results were filtered based on the address (packages) to identify Isfahan residents. Patients' files in each hospital were re-evaluated by two experts to ensure that only Isfahan

residents were included. After extracting the data from all hospitals, the address of 1500 randomly selected patients were checked to confirm not only their place of residence, but also the data management adopted by hospital medical records experts.

In order to obtain mortality data of 2010 and 2011 categorized by cause of death, data were collected from Isfahan's cemetery. The cause of death recording and categorization system in Iran was developed in 2004. In this system, the main cause of death is extracted from death certificates issued by relevant physicians and recorded (as ICD-10 codes) in collaboration with health deputies of universities of medical sciences, the Forensic Medicine Organization, municipalities, and organizations for civil registration.[24] In this study, the characteristics (name, father's name, sex, dates of birth, date and cause of death, and address) of individuals who died of CVDs (I00-I99) and ResDs (J00-J99) were obtained.

Hourly records of air pollutants were collected from air pollution monitoring stations and managed in Microsoft Excel files by the lab experts of Isfahan's Department of Environment. In 20th March 2010, to 19th March 2012, PM_{10}, CO, NO_2, O_3, and SO_2 levels were automatically measured and recorded every hour. $PM_{2.5}$ data were also recorded on 20th March 2011, to 19th March 2012. All stations were constantly monitored and calibrated by the lab experts of Isfahan's Department of Environment. SO_2, O_3, NO_2, and CO levels were determined by ultraviolet (UV) fluorescence, UV absorption, chemiluminescence, and infrared absorption spectroscopy methods, respectively. PM_{10} and $PM_{2.5}$ measurements were performed using beta attenuation monitoring.[28]

The experts of Isfahan's Department of Environment (DOE) examined the collected data and eliminated the outliers by checking the correlation of trend of data in all stations. In order to determine the mean daily levels of pollutants, the average 24-hour levels at each station were first separately calculated. The obtained values in all stations were then averaged to calculate the mean levels in Isfahan. Files related to 24-hour levels at different stations and the whole city were finally used to develop time series and case-crossover files.

Daily temperature, dew point, wind speed, sea level pressure, and visibility data were also collected from Isfahan's Meteorological Organization to eliminate their confounding effects on air pollution and the consequent hospitalization. In order to ensure the accuracy of the collected data, they were compared with available satellite data (an archive of all climatic data recorded at meteorological stations since 1950).[29]

In order to create the required files, identification (ID) code was assigned to each row of data in all files (including the patients, deceased, pollutants, and meteorological data). The IDs were developed based on dates, in a way that a specific yyyy/mm/dd/hh code was allocated to each point of pollutant data (8760 points of data in each year). Considering the nature of the data, the IDs were unique in the air pollutants and meteorological data files. However, since several individuals could die or be hospitalized on any particular day, the IDs could be repeated in the patients and deceased files. One-to-many merging methods were thus administered to build the files. Based on the created file the missing data for air pollutants were shown.

Time series and case-crossover methods were simultaneously applied for data analysis for all objectives of the CAPACITY study. All data analyses were conducted using R version 3.2.3. A confidence interval (CI) of 95% was considered in both Poisson and conditional regression methods. In this paper, we presented only the descriptive findings of the CAPACITY study.

Results

The mean annual O_3, CO, and PM_{10} levels were lower in the second year. In contrast, NO_2 and SO_2 levels increased by 1.5 and 1.8 times, respectively (Table 1). In both years, the highest mean levels of NO_2 and SO_2 were detected in January. The greatest mean levels of PM_{10} in the first and second years of study were seen in January and April, respectively (Figure 2). In the second year, the mean NO_2 and SO_2 levels were higher than 50 part per billion (ppb) on 182 days. These levels were higher than 100 ppb in about 30 days (in fall and winter in case of SO_2 and sometimes in spring in case of NO_2). In the first year, however, the mean levels of these two pollutants were higher than 50 ppb only on 50 days. Furthermore, the SO_2 and NO_2 levels were higher than 60 ppb on only one and five days, respectively. In both years, PM_{10} increased on similar days of the last month of fall, late spring, and early summer. In the second year, the increments in $PM_{2.5}$ had a similar trend to that of PM_{10}.

The total number of hospitalized patients in Isfahan was 23781 in the first year and 22485 in the second year of study. The mean age of the selected individuals in the first and second years was 52.55 ± 25.26 and 54.98 ± 23.59 years, respectively.

Table 1. Descriptive data of the pollutants in 20th March 2010 to 19th March 2012 in Isfahan, Iran

Variable	Observed data (%)	Mean ± SD	Min	Max
First year				
O_3 (ppb)	91.53	36.40 ± 6.33	20.87	51.98
CO (ppm)	94.30	6.07 ± 1.57	2.49	9.99
SO_2 (ppb)	92.52	27.26 ± 9.30	9.00	69.91
NO_2 (ppb)	91.40	33.48 ± 7.04	17.38	67.59
PM_{10} (µg/m^3)	67.42	161.98 ± 40.91	39.63	358.10
Second year				
O_3 (ppb)	46.23	28.17 ± 21.15	1.33	132.24
CO (ppm)	80.50	3.97 ± 2.13	0.01	31.84
SO_2 (ppb)	94.36	49.06 ± 37.51	0.02	284.00
NO_2 (ppb)	67.43	48.36 ± 32.95	0.01	377.65
NO (ppb)	70.19	79.58 ± 39.59	1.70	545.60
PM_{10} (µg/m^3)	46.23	117.94 ± 70.24	7.67	1227.50
$PM_{2.5}$ (µg/m^3)	75.32	54.64 ± 37.10	1.66	500.23

SD: Standard deviation; O_3: Ozone; CO: Carbon monoxide; SO_2: Sulfur dioxide; NO_2: Nitrogen dioxide; PM: Particulate matter; ppb: Part per billion; ppm: Part per million

Moreover, 53% of the patients in both years were men. The frequency of CVDs in the mentioned years were 18397 (77.36%) and 16990 (75.56%) individuals, respectively. Table 2 presents the frequency of different causes of hospitalization and the mean age of the patients. Ischemic heart disease was responsible for the greatest numbers of hospitalizations in both years. Among the ResDs, chronic lower ResDs was the most frequent cause of hospitalization in both 2010 (n = 1583, 29.40%) and 2011 (n = 1,415, 25.75%). In both years, all diseases, except for hypertension, were more prevalent in men than in women.

The largest numbers of hospitalization for ischemic heart diseases, hypertension, and heart failure were seen in winter 2011 and fall 2011. The greatest hospitalization frequency for all ResDs was in the winters of both years (Figure 3).

Discussion

The world's first severe air pollution and smog events occurred in Pennsylvania and London in the mid-20th century, after World War II.[30,31] Increased mortality following these events highlighted the health effects of air pollutants and motivated health policymakers to perform epidemiological studies to determine such impacts. As a result, the first studies on air pollution were conducted.[29,32,33] Over time, similar studies were performed in different developed countries and air pollution reduction strategies were implemented accordingly. Considering the trend of population growth in Iran and the consequent increase in the number of motor vehicles, changes in lifestyle, and industrial developments in Iran, further researches are required to clarify the health effects of population growth and industrial development, particularly air pollution.

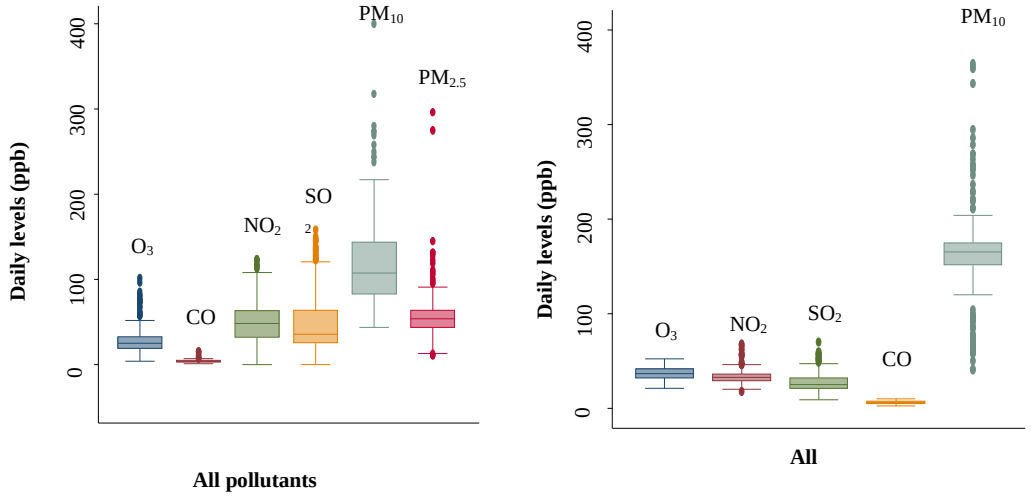

Figure 2. The distribution of daily levels of pollutants 20th March 2010 to 19th March 2012
O_3: Ozone; CO: Carbon monoxide; NO_2: Nitrogen dioxide; SO_2: Sulfur dioxide; PM: Particulate matter

Table 2. Frequency of patients admitted to hospitals of Isfahan, Iran, due to cardiovascular and respiratory diseases

Diseases (ICD codes)	First year (March 20, 2010, to March 19, 2011)			Second year (March 20, 2011, to March 19, 2012)		
	Frequency [n (%)]	Age (mean ± SD)	Men [n (%)]	Frequency [n (%)]	Age (mean ± SD)	Men [n (%)]
All cardiovascular diseases (I00-I99)	18397 (77.36)	57.53 ± 21.39	9642 (52.41)	16990 (75.56)	59.63 ± 19.15	8813 (51.87)
Hypertensive diseases (I10-I15)	1805 (7.59)	64.61 ± 14.65	667 (36.95)	2046 (9.10)	64.34 ± 14.86	785 (38.10)
Ischemic heart diseases (I20-I25)	8810 (37.05)	56.83 ± 20.02	4763 (54.07)	8075 (35.91)	58.39 ± 17.30	4276 (53.10)
Conduction disorders,cardiac arrest (I44-I46)	458 (1.93)	62.14 ± 24.40	266 (58.08)	507 (2.25)	62.25 ± 23.85	279 (55.03)
Cardiac arrhythmias (I47-I49)	839 (3.53)	52.89 ± 23.73	401 (47.79)	761 (3.38)	56.58 ± 23.43	352 (46.44)
Congestive heart failure (I50.0)	958 (4.03)	61.90 ± 23.91	537 (56.05)	840 (3.74)	65.51 ± 20.41	483 (57.57)
Cerebrovascular diseases (I60-I69)	2088 (8.78)	66.31 ± 18.95	1077 (51.58)	1766 (7.85)	69.25 ± 15.00	934 (53.01)
Other cardiovascular diseases	3439(14.46)	49.61 ± 23.74	1931 (56.15)	2995 (13.32)	52.75 ± 22.45	1714 (57.32)
All respiratory disease (J00-J99)	5384 (22.64)	35.50 ± 29.64	3088(57.36)	5495 (24.44)	40.60 ± 29.49	3178 (57.83)
Acute upper respiratory infections (J00-J06)	358 (1.51)	12.60 ± 16.65	207 (57.98)	286 (1.27)	12.08 ± 17.52	169 (59.30)
Influenza due to seasonal influenza virus (J10)	5 (0.02)	27.40 ± 4.28	3 (60.00)	4 (0.02)	46.00 ± 22.72	3 (75.00)
Pneumonia (J12 , J13, J14, J15, J16, J17, J18)	1085 (4.56)	39.27 ± 32.20	607 (55.94)	1126 (5.01)	40.20 ± 32.71	621 (55.45)
Acute bronchitis and bronchiolitis (J20- J21)	143 (0.60)	22.86 ± 26.05	81 (56.64)	184 (0.82)	14.14 ± 23.05	111 (60.33)
Chronic lower respiratory disease (J40-J47)	1583 (6.66)	49.66 ± 27.15	856 (54.11)	1415 (6.29)	52.77 ± 25.32	800 (56.62)
Other respiratory diseases	2210 (9.29)	28.09 ± 26.76	1334 (60.39)	2046 (9.10)	64.34 ± 14.86	775 (38.10)
Total	23781 (100)	52.55 ± 25.26	12730 (53.54)	22485 (100)	54.98 ± 23.59	11991 (53.49)

ICD: International classification of diseases; SD: Standard deviation

Owing to considerable development in Isfahan, a major employment and industrial hub in Iran, during the past 50 years, the city is now facing several problems including air pollution. Studies to quantify the health impacts of air pollution in this city are hence critical. In the CAPACITY study, we aimed to determine the effects of air pollutants on various diseases. Therefore, we collected the CVD and ResD-related mortality and hospitalization data (a total of 54266 cases) over a two-year period from relevant organizations. We also evaluated 266926 hourly records from six air pollution monitoring stations.

According to the initial results of CAPACITY study, the mean annual levels and most mean daily levels of pollutants in 2010 and 2011 were higher than the standard levels determined by the WHO.[34] While the WHO allows $PM_{10} > 50$ μg/m^3 on only 35 days of the year, the mean daily levels of this pollutant were higher than 50 μg/m^3 on over 80% of the days in both years. Moreover, SO_2 and NO_2 levels were considerably increased in 2011. Based on the WHO guidelines, the standard 24-hour and annual SO_2 levels should be respectively below 48 and 19 ppb. The mean annual levels of this pollutant were higher than the allowed level in both years. It was actually 2.5 times higher than the allowed level in 2011. The mean daily levels of this gas were also higher than the allowed level in over half the year in 2011. In 2010, the number of days with high SO_2 levels was much lower than that in 2011.

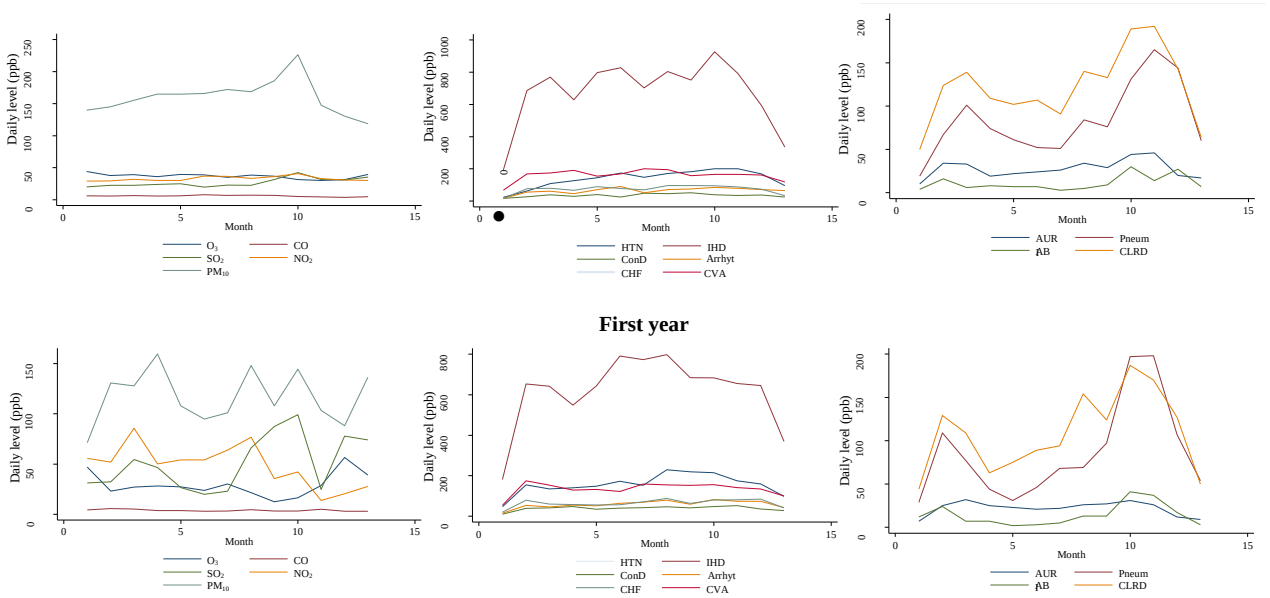

Figure 3. Trends of monthly changes in the mean levels of pollutants and frequency of hospitalizations in 20th March 2010 to 19th March 2012

O_3: Ozone; CO: Carbon monoxide; NO_2: Nitrogen dioxide; SO_2: Sulfur dioxide; PM: Particulate matter; HTN: Hypertensive diseases; IHD: Ischemic heart diseases; ConD: Conduction disorders and cardiac arrest; Arrhyt: Cardiac arrhythmias; CHF: Congestive heart failure; CVA: Cerebrovascular diseases; AURI: Acute upper respiratory infections; Pneum: Pneumonia; AB: Acute bronchitis and bronchiolitis; CLRD: Chronic lower respiratory disease

As stated by the WHO, the mean annual NO_2 levels should be less than 21 ppb. The mean level of this pollutant was 34 ppb in 2010 and 49 ppb in 2011. Therefore, the air pollution status in 2010 and 2011 was worse than the WHO recommendations. According to the available statistics, fuel oil and diesel consumption were higher and gas consumption was lower in 2011 compared to 2010. Since the largest amounts of SO_2 are produced in power plants run on fuel oil and high-sulfur diesel, higher levels of this pollutant in 2011 can be related to the increased use of these two fossil fuels.

Similar to previous research in other countries, the CAPACITY study used the HIS to collect the frequency of the selected diseases. Since health and mortality-related data are recorded in various national systems including the civil registration and hospital records, benefiting from the existing data seems to be the optimum and cost-effective choice for many studies. Registration systems have also been used in a large number of studies on air pollution. For instance, a study in Taiwan used the National Health Insurance (NHI) Program, which covers over 98% of the population, and the case-crossover design to evaluate the relationship between PM_{10} levels and ischemic heart disease hospitalizations.[35] A case-crossover study in South Africa on the relationship between respiratory, cardiovascular, and cerebrovascular mortality and different pollutants obtained mortality data from the Health Information and Technology Department.[36] In an eight-year study, Wichmann et al. examined the relationship between PM_{10} levels and hospitalization for myocardial infarction. They extracted the diagnoses, as well as patients' age and sex, from the National Ambulatory Care Reporting System (NACRS) database maintained by the Canadian Institute for Health Information (CIHI).[36]

The HIS has introduced to Iran's health system about 10 years ago. The Ministry of Health has evaluated and monitored the system three times ever since. Owing to the data recorded in the HIS, the system can serve as an invaluable source of information in various studies. Nevertheless, the possible use of different approaches to data entry in 2010 and 2011 might have been responsible for the decreased rate of hospitalization in 2011.

Research on the health effects of pollutants is relatively new in Iran. Recent increases in pollutants in different cities in the country, especially in the western and southwestern areas, have necessitated studies on the relationship between pollutants and disease incidence, mortality and generally its burden on health. Most previous studies, however, have adopted the WHO's air quality (AirQ) model to examine the burden of disease caused by air

pollutants in various cities of Iran. A previous study estimated the proportion of cardiovascular and respiratory mortality attributable to air pollutants in 2011 in Tehran.[37] A series of studies in Ahwaz used the AirQ model to calculate the proportion of cardiovascular and respiratory mortality attributable to PM_{10}, CO, SO_2, and NO_2.[38,39] Another study in Sanandaj, Iran, used the same software to determine the proportion of cardiovascular and respiratory mortality attributable to PM_{10} in 2013.[40]

One of the few efforts to directly measure health impacts was performed by Ebrahimi et al. who evaluated the relations between dust storm and cardiovascular and respiratory admissions. The study subjects were patients with cardiovascular and ResDs who received medical services from the Center for Disaster Management and Medical Emergencies (CDMME) in Sanandaj during dust event days. According to Pearson correlation analysis, the incidence of dust storm significantly increased the frequency of emergency admissions.[41] While the mentioned study only evaluated the effects of PM_{10} during dust event days on emergency admissions, the present research applied the time series and case-crossover designs to examine the effects of all pollutants during the whole year.

Qorbani et al. were among the first to use the case-crossover design in Iran. They sought to clarify the relationship between admissions for the acute coronary syndrome and CO and PM_{10} levels 24 hours before admission among 250 patients in a hospital in Tehran. Their findings indicated a significant association between the risk of admission and CO levels 24 hours before admission [odds ratio (OR) = 1.18]. However, no such a relation was observed in case of PM_{10}.[12] Despite its novelty in 2012, this study had a small sample size. Khamutian et al. adopted the time series design and found significant relationships between CO, NO_2, and O_3 levels and admissions for asthma to seven large hospitals in Kermanshah.[42] Although the study evaluated all emergency admissions to seven large hospitals during two years, they only studied the relation between PM_{10} levels and asthma.

A number of studies have also assessed the associations between various diseases and air pollution in Isfahan. Mansourian et al. conducted a time series analysis to determine the relationship between ResD admissions in children and different air pollutants during 2005 and 2006. They randomly selected 120 children who were hospitalized for ResDs. Poisson regression analysis revealed significant associations between the admission rate and SO_2-CO, SO_2-PM_{10}, and PM_{10}-CO levels (β = 0.937, 0.930, and 0.952, respectively).[43] Meanwhile, in the CAPACITY study, the relationship between five air pollutants with all CVD and ResD hospitalization and mortality in all age groups were measured. A case study during 2005-2009 reported the higher frequency of cardiovascular mortality in more polluted cities of Isfahan Province.[44]

Apparently, previous studies in Iran have highlighted limited associations between a number of diseases and air pollution indices or some pollutants. However, to the best of our knowledge, this study was the first in Iran and the Middle East region to examine the relationship between cardiovascular and respiratory mortality and hospitalization in general and levels of six pollutants (as quantitative independent variables). The large sample size, the diversity of the pollutants and outcomes, and the simultaneous administration of time series and case-crossover methods have added to its strengths.

This study, however, has some limitations. First, the data was previously collected by the HIS. Although we believe that the emergency and elective admissions could be easily distinguished in the HIS, the systems used in the selected hospitals were not designed by a single company. Thus, it led to differences in the recorded data. Therefore, after descriptive analysis of the collected data, we realized that we should delete the elective patients of our database and consider the only emergency admitted patients. Based on our experience and the increasing interest in the use of data registration systems in scientific research, data entry and usage in the HIS have to be updated.

Conclusion

In conclusion, the CAPACITY study showed that performing large-scale studies, at the level of cities or even the country, requires coordination between and data from several organizations. However, data collection using field questioning at any single organization is both costly and time-consuming. Data collection at this level facilitates the development of various models and the application of different analytical methods. Therefore, the results of the CAPACITY study can be beneficial to the design of future studies, development of research policies aiming at the use of national registration systems, and modification of the existing policies for the extraction of health indices. Moreover, our findings can provide health researchers, authorities, and policymakers with fresh insight into the health impacts of air pollution and

thus facilitate the development of effective preventive strategies. These results can also be compared with the results of similar research in other countries.

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Conflict of Interests

Authors have no conflict of interests.

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An investigation of the psychological experiences of patients under mechanical ventilation following open heart surgery

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Original Article

Abstract

BACKGROUND: Breathing and living on mechanical ventilation develops a different feeling in patients. Most of such feelings and experiences are not pleasant and can lead to psychiatric disorders in the patients after they are detached from the ventilator. The aim of this study is to explore the psychological experiences of patients under mechanical ventilation.

METHODS: This qualitative study was conducted according to an interpretive epistemological approach in 2016. Fifteen participants were selected according to purposive sampling. Data were drawn from the transcripts of in-depth, semi-structured interview that were not discontinued until data saturation was ensured. The participants were asked to share what they experienced when they were under mechanical ventilation and intubation. Data analysis was conducted according to Diekelmann method.

RESULTS: Altogether, 2 themes, 7 subthemes, and 27 sub-subthemes were drawn from the data. Two themes were dread (a horrible experience) and hope (an inspiring experience). Dread consisted of anxiety, hopelessness, and dependency. Hope consisted of spiritual connection as the only possible effort, the presence of health team the source of comfort, the family looking forward, and overcoming the illness (a step to life).

CONCLUSION: The psychological experiences of patients under mechanical ventilation are specific, and nurses can play an important role in decreasing tension and increasing hope among them through gaining knowledge about their experiences.

Keywords: Mechanical Ventilation, Psychology, Heart Surgery

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Introduction

It is necessary to use mechanical ventilation during major surgeries.[1] The main aim of mechanical ventilation is to assist gas exchanges until the patient's breathing problem is resolved. Different advanced devices, models, and techniques are used to conduct ventilation and oxygenation. Basically, these techniques are aimed to help patients initiate ventilation and support their breathing completely or partly. Despite the availability of such advanced devices, mechanical ventilation is associated with numerous physical and mental complications in patients.[2] Tsay et al. pinpoints that although the mortality rate has declined considerably among the patients under mechanical ventilation, the complications due to undergoing this device persist even after discharge.[3] Recent studies have indicated that the mechanical ventilation-associated complications decrease as the duration of ventilation is shortened and the patient is detached from the device sooner.[4] Such complications include damage to mouth, throat, and trachea, respiratory function, reducing the need for analgesics, mortality, and psychiatric complications

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such as stress, anxiety, sensory deprivation, length of hospital stay, and reducing costs.[5]

For early consciousness and sooner detachment of these patients, sedatives and sleep aids are less frequently used; and therefore, they need specialized nursing care.[6] Nurses play an important role in taking care of these patients and detaching them from mechanical ventilation and reducing their physical and psychiatric complications.[6-8] Holistic care contributes effectively to recovery and rehabilitation of the patients; therefore, nurses should pay special attention to these patients' psychological, in addition to physical, needs. Intubation causes certain psychological problems for the patients via limiting their verbal communication[9]. Therefore, a big challenge may be developed between physiological and psychosocial issues in taking care of these patients.[10] Elder et al.[11] reported that the disrupted process of communication and mistakes due to lack of understanding patients' needs in intensive care units (ICUs) cause increase in cost and mortality, and disruption in treatment course and also influence nurses' capabilities and even services delivered by other healthcare divisions.

Therefore, nurse and medical team members should take into account all the patients' needs from a holistic perspective and pave the way to detach them from mechanical ventilator as soon as possible.[11] Eckerblad et al.[12] argues that it is necessary for ICU nurses to be familiar with the patients' physiological and psychological states as they are under mechanical ventilation to detach them from the device appropriately and prevent associated physical and psychological complications after discharge.[12] Physiological and mental states are unique experiences that vary among different people depending on their backgrounds and individual differences.

Qualitative studies mainly have investigated the physiological and physical aspects of patients under mechanical ventilation, and few studies have already been conducted on these patients' psychological aspects as they are under mechanical ventilation. The experiences and psychological needs of these patients have not yet been sufficiently explained for ICU and especially cardiac care unit nurses, while emotional stress and psychological complications of this period persist in the patients even years after discharge that influence the health of the patients especially those undergoing heart surgery.

Studies have indicated that despite extraordinary developments in mechanical ventilators technology and medical treatments pharmacotherapies, the process of detachment from the ventilator is still a stressful, frightening, and disappointing experience from the patients' perspectives.[13-17] In principle, 11% of the patients have been reported to experience certain psychological problems such as amnesia, difficulty concentrating, and delusion after detachment from mechanical ventilator. However, if the patient is better supported under the ventilator, these complications develop less frequently.[1] Despite many studies on mechanical ventilation, it seems necessary to conduct a study to exclusively investigate the psychological experiences of the patients under mechanical ventilation so that their psychological needs can be addressed to prevent subsequent associated problems. This study was conducted to investigate the psychological experiences of patients following open heart surgery as they are under ventilation.

Materials and Methods

This qualitative study was conducted according to the interpretive phenomenological approach. The phenomenological study refers to the close, systematic, and critical of phenomena.[18] This study was conducted to discover the lived psychological experiences of patients under mechanical ventilation and at detachment from the ventilator following open heart surgery in 2016. The study setting was three ICU of the Shahid Chamran Hospital, Isfahan, Iran, and 15 participants were selected purposively from patients undergoing open heart surgery. The participants were interviewed after full recovery and in the surgical ward. To conduct interviews, the participants were invited to the meeting room after their conditions became stable. To achieve this purpose, the patients were visited after recovery and asked to provide consent to participation in the study after an introductory conversation and giving explanations about the research purposes. Then, the patients were asked to describe their experiences from recovery until the tracheal tube was taken out from their mouths. Data collection was conducted through individual, in-depth, open, and semi-structured interviews. The interviews were started with this question: "May I ask you talk about your experiences from the time when you recovered and found out that you were under mechanical ventilator until you were detached from it."

The patients whose conditions did not become stable after the surgery, and needed other invasive interventions or re-intubation after extubation were excluded from the study. The interviews lasted for 25-40 minutes and were not discontinued until in-

depth data could be drawn. All interviews were recorded and transcribed. Meanwhile, the participants were observed from the development of the first recovery symptoms under mechanical ventilation until the extubation and as they were being interviewed. Moreover, field-notes was taken.

Data analysis was conducted by Diekelmann et al. method that is used to analyze the data of qualitative, hermeneutic phenomenology epistemological studies.[19] First; the interviews transcripts were read several times to achieve a general perception of the phenomenon in question. After the researcher achieved such perception and felt comfortable with the data, the transcripts were separately examined according to text interpretation method. Explicit and implicit meanings offered by the participants drawn from the transcripts, coded and categorized. The categories were coined. The summary of the codes and categories was reviewed by other researchers to reach a common understanding of the participants' descriptions. Then, the contradictions in the interpretations were resolved after all researchers shared their viewpoints. To achieve this purpose, the original transcripts were reviewed or if necessary, the participant in question was called for further explanations. At this step of the study, two participants were called, the categories were determined and described by comparison and contrasting of the texts. Finally, the data were drawn and categorized accompanied by a selection of the transcripts and examined by the researcher to develop the final scheme of the findings.

To enhance the internal consistency, in-depth questions and semi-structured interviews were used. Participants enrolled in this study had a comprehensive range of ages and educational levels and were from both sexes. In addition, a number of the participants were re-interviewed, the data confirmed by continuous comparison and exploring into negative meanings, and categorization conducted appropriately. Besides, the interviews were continued until saturation of the data was ensured the participants selected according to purposive sampling, and maximum diversity of the samples sought out to provide external reliability.

In addition, a number of the participants were asked to review the data to investigate their reliability and acceptability. To do this, a sample of the data was offered to five participants and they were asked to share their viewpoints on the compatibility between the data and their experiences. To determine whether the data are generalizable or not, the participants' situations were completely and elaborately described so that others could judge and assess the findings. In addition, the researchers did their best to remain neutral in reporting the data through conducting an open interview and narrating the participants' experiences, reflecting and being present for the long-term, working in the study setting, and keeping and reporting the evidence tactfully.

To observe research ethics, researchers then achieving authorization from the Ethics Committee of Nursing and Midwifery University, Faculty of Medical Sciences, Isfahan, Iran (Number: 312-1-1394) approved the study protocol and the authorities of Shahid Chamran Hospital provided consent to conduction of this study. In addition, the patients were informed about the anonymity and confidentiality of the data drawn from the transcripts as well as voluntary withdrawal from the study at any step of the study without any penalty. Then, they were asked to provide consent to participate in the study if they were willing to do it.

Results

15 patients undergoing open heart surgery in Shahid Chamran Hospital participated in this study. Table 1 shows the participants' demographic characteristics.

Overall, 2 themes, 7 subthemes, and 27 sub-subthemes were drawn from the data. The two themes were dread (a horrible experience) and hope (an inspiring experience) (Table 2). Dread consisted of three subthemes, namely anxiety and fear, despair, and dependency. The participants reported that anxiety and fear occurred following the transition from unconsciousness to consciousness. Two patients described the stressful environment of the ICU as follows:

"... When I woke up, I did not know where I was. First, I thought I slept on the furniture of our home. Gradually, I realized that here is somewhere else. I could not move at all. I thought I had a myocardial infarction (MI) because my tongue got numb and I could not talk. I realized that a tube had been put in my mouth. I had got so worried ..." (p3-i1).

"... To be honest, everyone who wakes up and does not know where he is and once sees all these devices hooked to him and cannot do anything, is definitely going to have another MI from fear. I could not move at all. The nurse told never move at all because this tube would be taken out and you could not breathe. I had been more scared. I was afraid that this tube would be taken out and I could not breathe..." (p7-i1).

Table 1. Participants' demographic characteristics and frequency distribution

Variable		Frequency (%)
Sex	Man	7 (46.7)
	Woman	8 (53.3)
Marital status	Married	10 (66.7)
	Divorced	1 (6.7)
	Widow/widower	4 (26.6)
Age	< 40 years	3 (20.0)
	40-60 years	7 (46.7)
	> 60 years	5 (33.3)
The number of children	≤ 2	5 (33.3)
	> 3	10 (66.7)
Education level	Elementary and lower	5 (33.3)
	Secondary	6 (40.0)
	Higher than high school completion	4 (26.6)
Type of surgery	Coronary grafting	8 (53.3)
	Heart valve replacement	4 (26.6)
	Atrial and ventricular wall repair	3 (20.0)

As a vital sign of living, breathing is not only important for the treatment team, but also critical for the patient's perspective. The participants in this study were clearly worried about the possibility of discontinuing breathing and being dependent on mechanical ventilator such that a patient said:

"... To be honest, I gradually got disappointed. I wondered maybe I would be breathing with this device until the end of [my] life, maybe I would not be able to get rid of here once again..." (p4-i1).

Table 2. Sub-subthemes, subthemes, and themes of psychological experiences of patients under mechanical ventilation following open heart surgery

Theme	Subtheme	Sub-subtheme
Dread: A horrible experience	Anxiety and phobia	Ambiguous condition
		Fear of connections and devices
		Unsafety
		Respiratory illness
		Understanding error
		Fear of death
		Environmental risks
	Despair	Sense of loneliness
		Inability to communicate
		Inability to move
		Distracting thoughts
	Dependency	Dependency on device
		Dependency on personnel
Hope: An inspiring experience	Spiritual connection as the only possible effort	Praying recital
		Cordial resort to God
		Trust in God
	The presence of treatment team, the source of comfort	Sense of attracting others' attention
		Access to treatment team
		Communication with treatment team
	Family looking forward	Praying family
		Worried family
		The presence of family
	Overcoming illness: One step to life	Family, pursuing the patient's state
		Comfort after receiving care
		Becoming free from dependency
		Stable state
		Treatment team insurance

Stressful environment of the coronary care unit (CCU), the type of the staff's treatments with the patients, and everything that occurs in the CCU is interpreted differently by the patients. More clearly, each patient perceives the CCU environment depending on his own circumstances. These perceptions occasionally lead to the horrible experiences of despair and disappointment or the recovering experiences of understanding the staff's presence and their attention to the patients.

In this regard, a participant said:

"... I was terrified by the personnel's talks. They said that old man was very critically ill and there was no hope [for his survival] and we did not think that he would be detached from the device once again. I thought they were talking about me, my heart palpitations increased; two other nurses in the station were talking with each other and did not pay any attention to me at all. I wondered if this device did not give [me] puff, what would happen, if this tube was taken out, what would happen. there was not anyone else to help…" (p11-i1).

Inadequate interactions and lack of providing necessary explanations for the patients as they are under circumstances in which the situation is difficult to be understood by them lead to certain horrible state ambiguities such that a participant said:

"... My nurse told [me] to be calm, [as] your test result was received, we would take out that tube, and they did not give me water. I did not know what this test was [for], maybe its result would take several more days to come ..." (p6-i1).

According to the data, the theme hope represents an inspiring experience consisting of the subthemes spiritual connection as the only possible effort, the presence of health team the source of comfort, the family looking forward, and overcoming the illness (one step to life). Regarding the promising effect of nurses' presence and communication with them, a participant said:

"... A woman nurse came to me and told me where I was and I had undergone a heart surgery and my surgery had been done well. At that moment, I just realized where I was and why I had come here ..." (p3-i1).

Spiritual connection is one of the promising factors that the patients establish when they are alone and have no other ways out. The patients seek to restore the lost hope through spiritual connection. In this regard, a participant said:

"... I was so alone. Sometimes I closed my eyes [and] mentioned and I asked God to help me and sought for God's assistance…" (p15-i1).

Knowing the outcome of treatment and giving hope for recovery and overcoming the illness represents a path to hope among the patients under mechanical ventilation:

"... My nurse came and told me [that] I was well and to be calm for the moment until your test result would be received and if it was good, we would gradually take out the tube from your mouth ..." (p10-i1).

The patients who perceive themselves to be completely dependent on others' assistance achieve calmness after receiving physical care, which is one of their expectations from the healthcare system such that a patient said:

"... When the tube was in my mouth, my mouth got too dry and the end of my throat burned so much, a man [nurse] and a woman nurse came over my head. I pointed them with my head that I was thirsty, I took out my tongue, but that woman told [me] to be calm. After some minutes, that man nurse came and poured a few drops of water into my mouth, my mouth got wet, with difficulty I gulped the water, the burning of the end of my throat was diminished, I got very comfortable ..." (p8-i1).

Family's role, looking forward, and presence, and the special attention paid by the patient's spouse and children contribute greatly to restore lost hopes. In this regard, a patient said:

... At that time when I was disappointed by anything and anyone, I did not know how my son realized that I needed him. Through much insistence, he finally got the permission of the nurses and came over my head, I got much glad as if they had given me the world, I became relieved [when I realized that] the kids kept a close eye on me and did not abandon me and I became relieved [when I realized that] they realized that I was getting better and they did not need to be worried about me…" (p2-i1).

Discussion

The aim of this study was to gain an in-depth perception of the psychological experiences of patients undergoing open heart surgery as they are under mechanical ventilation. Although it is not conventional to generalize the findings of qualitative studies rather these studies are aimed to generate and describe important experiences correctly to increase insight, certain findings of the present study may lead to further understanding of the concept of care with reference to the evidence of other studies. In this study, fear and hope were

derived to be two explicit experiences in the conscious patients hooked to the mechanical ventilator, because the patients under mechanical ventilation need to expend a great deal of energy to survive and have reached a stage in which they feel dreadfully frustrated and that they need assistance. Such needs make them turn to spiritual sources. Meanwhile, caregivers and therapists seek to provide material and spiritual welfare for the patients through understanding such frustration, being present and displaying effective spiritual and physical reactions. In the light of the findings of the present study, these patients' psychological experiences represent that there are certain issues in such situation, some of which enhance spirit and hope and some of which cause fear and intensify worries among them. According to Loghmani et al.[20] study, the experiences of nurses and families of patients in ICUs were categorized into five themes, namely spiritual attention, emotional reaction, recruitment of participants, interactive education, and counseling and guidance. Loghmani et al. reported that giving hope, directing attention toward God, and resorting to religious rituals were drawn as the subthemes of spiritual attention,[20] which is in agreement with the current study. Inducing hope is, therefore, one of the most important care-related needs of the patients under mechanical ventilation.

Rezaee et al.[21] study on hope for the patients' recovery among ICU nurses demonstrated that the nurses' knowledge about hope themes can lead to taking care of the patients (the most important responsibility of the nurses) peacefully and in the best possible manner. The current study indicated that although hope concept has many applications in nursing dialogue, its specific domains are taken into account in different people. Through detecting these four themes, we can argue that increasing nurses' (professional) knowledge, as well as their knowledge about hope-related themes, can contribute to promoting the quality of effective care and developing intrinsic pleasant emotions, optimistic attitude, and sensible expectations (recovery, death with dignity, and gaining experience).[21] This argument is consistent with the present study as hope was derived to be an important experience among the studied patients.

Although the transplantation of cardiac vessels is a treatment that causes increase in hope for outliving and quality of life among the patients, these patients are faced with numerous mental problems that may remain unresolved even many years after discharge.[22] As the present study demonstrated, one of the main needs of these patients was the need for peace as most of them reported the need for mental peace in this period.

In the current study, fear (a horrible experience) was another theme drawn from the data. The patients under mechanical ventilation have to experience stressful moments. Being worried about the understanding of the risk of disease and its consequences, sense of being neglected and paid inadequate attention as well as being worried about one's own conditions in the future are some of the specifications of this theme. The patients' experiences in this study demonstrated that physical problems, fears and tensions related to breathing and discomfort have caused the perception of risk of the disease and its consequences among the patients. This fear or worry can be due to the equipment hooked to the patient.

Schou and Egerod[14] demonstrated lived experiences of the patients undergoing open heart surgery during detachment from ventilator consisted of three themes as general phenomena such as discomfort and disrupted communication, psychological phenomena including reduced control and loneliness, and existential phenomena such as transient interactions and human interactions. Regarding the psychological study, that study is consistent with the current study. The psychological findings of that study represented the experience of losing control as unawareness of time and location, losing self-confidence, being dependent on others, and feeling lonely reported by a large number of the patients.[14] As studies have indicated, this experience is completely dependent on the environment and nurses' communication with the patients. It seems that the nurses can help reduce this negative experience to a large extent through expending more time to be with the patients and communicating with them via speech, touching, and even eye contact.

In a study of Foster[23] on patients undergoing tracheostomy in a hospital in England, it has been reported six themes of the principal domains of the experiences, namely need for communication, achieving normal state, psychiatric disorders, painful procedures, unknown fears, and communicating with personnel. The researcher in that study arrived at this conclusion that this domain of the patients' experiences should be completely taught to the nurses so that the patients can pass this period more comfortably. Foster recommended conducting larger studies on psychiatric disorders and associated

causes.[23] In the current study, the patients who were under mechanical ventilation described their psychological experiences. Consistent findings were obtained with different conceptualization.

Arslanian-Engoren and Scott investigated the lived experiences of the patients under mechanical ventilation using the phenomenological methodology, and reported fear, stress, feeling dependent on the device, and pain to be the experiences of these patients as they were under long-term mechanical ventilation.[24] In the current study, the patients were under mechanical ventilation for the short-term, and if the patients knew the psychological problems associated with this period, they could detach the patients from the ventilator even sooner and with fewer complications such that the patients would pass this period pleasantly.

Wang et al. studied the lived experiences of the patients under mechanical ventilation in the ICU and reported five themes consisting of being in a new environment, physical suffering, mental suffering, self-encouragement, and self-reflection. Furthermore, four subthemes were drawn from mental suffering theme, consisting of experienced nightmares and delusions, fluctuation of consciousness level, and dependency on technology and others to survive,[25] which is consistent with the subthemes, namely dependency on the personnel and device and distracting thoughts, drawn in the current study.

As several studies on cultural and educative issues have indicated, both positive and negative psychological issues may occur among the patients under mechanical ventilation. These issues, in the current study, were explained in a specific cultural and educative context in Iran, i.e. Isfahan subculture, characterized by extensive interpersonal interactions, religious beliefs. Therefore, the findings of the current study cannot be generalized to other contexts but can serve as a guideline for the health team to satisfy the patients' expectations in similar circumstances in different sociocultural context, which can be considered a limitation of the present study. According to the findings of this study and other studies, psychological factors play an important role in healing and disease.[26]

This study had some limitations. The golden time to interview with the participants was immediately after detachment from the mechanical ventilation because they can remember those moments very well and can express their feelings more accurately. However, the participants in this study were not sufficiently conscious at those moments and we had to wait for them to recover fully and then interview them.

Conclusion

Irrespective of diagnosis and severity of disease, patients have fundamental needs. They expect specialized staff in the hospital to deliver full and early healthcare services continuously and kindly. This requirement is considerably more urgent for patients under mechanical ventilation. The findings of the current study and other studies indicated the ICU personnel should take into account the psychological, in addition to physical issues of the patients so that they can address the psychological needs of these patients more than ever, and therefore, deliver the best and highest quality care to them and prevent subsequent problems.

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Conflict of Interests

Authors have no conflict of interests.

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The effect of atorvastatin treatment duration on oxidative stress markers and lipid profile in patients with coronary artery diseases: A case series study

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Original Article

Abstract

BACKGROUND: The major aim of this study was evaluating the effect of atorvastatin treatment on thiobarbituric acid reactive substances (TBARS), ferric reducing the ability of plasma (FRAP), small dense low-density lipoprotein cholesterol (sdLDL) and lipid profile in coronary artery disease (CAD) patients.

METHODS: This study was carried out on 83 patients with angiographically proven coronary artery stenosis (52 men and 31 women) at Shahid Madani Hospital, Khorramabad, Iran, in 2015. The patients were divided into the 3 groups. 27 patients were classified statins consumption less than 6 days, 28 patients for 6 to 90 days, and 28 patients for more than 90 days. The level of sdLDL, lipid profile, TBARS and FRAP were assayed.

RESULTS: FRAP levels of patients that received atorvastatin for more than 90 days (832 ± 101) were significantly elevated (P = 0.01) compared to the patients received atorvastatin less than 6 days (688 ± 75), whereas the levels of TBARS diminished significantly (P = 0.04). Also, the levels of total cholesterol (TC) and LDL-C were significantly decreased after 3 months of atorvastatin receiving (158 as compared to patients that consumed atorvastatin less than 6 days), (P = 0.02 and 0.03, respectively). The level of sdLDL was slightly increased with long-time consumption of atorvastatin (37 ± 14) in patients in comparison with patients that received atorvastatin less than 6 days (32 ± 15) (P = 0.06), but was not significant.

CONCLUSION: The serum level of TBARS decreased and the serum level of FRAP increased in patients with long-time receiving atorvastatin. Therefore, atorvastatin contributes to the lowering oxidative stress in these patients.

Keywords: Atorvastatin, Coronary Artery Disease, Oxidative Stress

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Introduction

Coronary artery disease (CAD) is the consequence of atherosclerosis, a vascular disorder. CAD is the first cause of mortality and debility in the developed countries.[1] Atherosclerosis, the primary cause of CAD, is a heterogeneous disorder and a multifactorial disease. Oxidative stress is known to be involved in a number of human disorders including atherosclerosis.[2-4] Elevation of oxidants and reduction of antioxidants play the main role in the incidence and development of atherosclerosis. Hydroxymethylglutaryl-CoA reductase inhibitors (statins) promote reductions in plasma levels of low-density lipoprotein cholesterol (LDL-C), a primary risk factor for CAD. Statins raise a considerable study interest due to their pleiotropic effects such as antioxidative, anti-inflammatory and endothelium protective capabilities.[5,6]

LDL-C particles have two phenotypes: phenotype A consisting of large buoyant LDL-C, and phenotype B which contains small dense LDL-C (sdLDL).[5] sdLDL is a subfraction of LDL-C with smaller particle size and higher density that is strongly associated with the development of CAD. In addition, sdLDL has prolonged plasma residence time compared to native LDL-C, and had a lower affinity for the LDL-C receptor. On the other hand, sdLDL particles are highly susceptible to oxidation

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than large buoyant LDL-C. Their oxidation concept is related to the atherogenic potential of sdLDL particles and is associated with incident atherosclerosis.[6,7] Several studies found that sdLDL particles had lower affinity for the LDL-C receptor, and therefore have a longer residence time in plasma, which affects them to oxidative alteration and their increased affinity to bind to intimal proteoglycan.[8,9] In addition, other studies have highlighted that people with sdLDL particles are prone to the formation and development of atherosclerosis and sdLDL is more atherogenic than native LDL.

People with a predominance of sdLDL have a 3 fold increased risk of myocardial infarction. Relative risk is 4.5 fold for CAD and 7 fold for myocardial infarction when sdLDL > 100 mg/dl.[10,11] sdLDL was also found to be elevated in patients with diabetes, metabolic syndrome and other disorders such as preeclampsia. There is also an association between sdLDL and early atherosclerosis in menopausal women.[12-15]

Although atorvastatin clearly decreases total LDL-C particle level, it is unclear whether it can affect the concentration of sdLDL. Also, several studies have reported that statin treatment results in a reduction of malondialdehyde (MDA) and or lipid peroxidation products as oxidative stress-related markers.[16-18] However, there are several questions about the duration of intake, required dose of statin and response to the therapy in atorvastatin treatment of CAD. The main aim of this study was evaluating the serum levels of sdLDL, thiobarbituric acid reactive substances (TBARS) and ferric reducing the ability of plasma (FRAP) as total antioxidant capacity index after 20 mg/day consumption of atorvastatin in atherosclerosis patients.

Materials and Methods

This study was carried out on 83 subjects considered as CAD patients (52 men and 31 women, aged 60 ± 11 years) diagnosed with positive angiography as the golden standard at Shahid Madani Hospital, Khoramabad, Iran, in 2015.

All subjects were given written informed consents. The research protocol was approved by the Ethics Committee of Lorestan University of Medical Sciences, Lorestan, Iran, (Ethics Committee registration number was 200/72425).

Inclusion criterion was coronary heart disease (CHD) patients diagnosed by coronary angiography. Exclusion criteria were diabetic patients, patients with any concurrent illness like chronic liver disease, hypothyroidism and active rheumatologic disease, and patients on drugs like diuretics, steroids, oral contraceptives and beta blockers.

Fasting blood samples were collected into tubes. Serum was recovered after centrifugation at 3500 rpm for 10 minutes at 4 °C and stored at -80 °C until analysis. Atherosclerosis patients were divided into 3 groups on the basis of 20 mg/day consumption of atorvastatin. 27 patients were classified as atorvastatin consumption less than 6 days, 28 patients consumed 6 to 90 days and 28 patients received for more than 90 days.

Serum TBARS were measured by a modified spectrophotometric (Shimadzu Corp, Kyoto, Japan) method by using tetramethoxypropane as a standard and a standard curve was constructed using solutions of 0-100 µM. All measurements were carried out in duplicate. TBARS are expressed in terms of MDA equivalents.[19]

The level of FRAP was measured according to previously published methods.[20] In brief, three reagents were used including sodium acetate in acetic acid buffer (pH = 3.6), 10 mM solution of 2,4,6-tripyridyl-s-triazine in a 40 mM solution of hydrochloric acid, and 20 mM solution of ferric chloride. The FRAP reagent was prepared daily with 25 ml of reagent 1, and 2.5 ml reagent 2 and 3.

Serum FRAP was measured by adding FRAP reagent to serum and the absorbance at 540 nm was measured in a microplate reader. All measurements were carried out in duplicate. FRAP concentrations were calculated with a calibration curve of iron (II) sulfate ($FeSO_4$) (100-1000 µmol/l).

Serum sdLDL was measured by using a precipitate method described previously.[21] The precipitation reagent (0.1 ml) containing 150 U/ml heparin-sodium salt and 90 mM $MgCl_2$ was added to each serum sample (0.1 ml), mixed, and incubated for 10 minutes at 37 °C. Then, each sample was placed in an ice bath for 15 minutes. After centrifugation at 15000 rpm for 15 minutes at 4 °C, the precipitate was packed at the bottom of the tube and the clear supernatant consisted of sdLDL.

The serum levels of fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), LDL and high-density lipoprotein cholesterol (HDL-C) were analyzed. FBS, TC and TG concentrations were measured by biochemical analyzer using commercial kits (Olympus AU-600, Tokyo, Japan).

HDL-C was analyzed by a Pars Azmoon kit (Iran). All measurements were carried out in duplicate. LDL-C was determined by a calculation, according to our previous study.[22]

Table 1. Difference of baseline characteristics among three various durations of atorvastatin (20 mg/day) consumption in coronary artery stenosis patients

Variable	Groups of patients according to consumption of atorvastatin			P*
	< 6 days (n = 27)	6-90 days (n = 28)	> 90 days (n = 28)	
Sex (man)	17 (63.0)	20 (67.8)	18 (64.3)	0.20
Smoking	15 (55.6)	14 (50.0)	15 (54.0)	0.14

Data are shown as number (%); *Chi-square test

The data were analyzed by SPSS software (version 20, IBM Corporation, Armonk, NY, USA). Continuous variables were mentioned as mean ± standard deviation (SD) and categorical variables as number and percentage. Normality of the variables was confirmed by Kolmogorov-Smirnov test. Hence, comparison of mean values between studied groups was performed using analysis of variance (ANOVA) followed by post-hoc pairwise comparisons by Tukey's test. Discontinuous variables were analyzed with the chi-square test. P less than 0.05 was considered statically significant.

Results

The difference of baseline characteristics among three various durations of 20 mg/day atorvastatin consumption in coronary artery stenosis patients is shown in tables 1 and 2.

There was no significant difference in age (P = 0.12), sex (P = 0.20), body mass index (BMI) (P = 0.51) and smoking (P = 0.14) between three groups (Tables 1 and 2). Also, There was no significant difference in levels of FBS (P = 0.39), TG (P = 0.17), HDL-C (P = 0.48), erythrocyte sedimentation rate (ESR) (P = 0.24) and sdLDL (P = 0.24) among three groups (Table 3).

The levels of TC and LDL-C in patient groups with long-time receiving atorvastatin was significantly decreased compared to the group that consumed less than 90 days (P = 0.04 and P = 0.02, respectively), while TG levels were moderately decreased (P = 0.17). The levels of HDL-C were not significantly increased after 90 days of treatment compared to atorvastatin consumption less than 6 days (P = 0.48). There was not a significant difference in ESR levels among three groups. These differences were not significant (P = 0.24) (Table 2).

The serum FRAP index in patients received atorvastatin for more than 90 days was significantly elevated compared to the patients received atorvastatin less than 6 days (P = 0.04). While the levels of TBARS, an index of lipid peroxidation and oxidative stress, in atherosclerosis patients that consumed atorvastatin for more than 90 compared to patients taking atorvastatin less than 6 days showed a significant decrease (P = 0.04). The serum levels of sdLDL in patients that received atorvastatin for more than 90 were slightly increased compared to the patients that received less than 6 days. These differences were not significant (P = 0.24) (Table 3).

Discussion

In this study, atherosclerosis patients that received 20 mg/day atorvastatin for long-term had significantly decreased levels of cholesterol and LDL-C as compared to the patients that received atorvastatin less than 6 days. There was not any significant difference in HDL-C and TG levels among three groups. Oxidative environment develops oxidative stress because of the disparity between oxidative reactants, such as reactive oxygen species (ROS), and antioxidants.[23] Biological samples have a mixture of TBARS, including lipid hydroperoxides and aldehydes, which are an index of lipid peroxidation and oxidative stress.[24] In the present study, treatment with 20 mg/day atorvastatin for long-term in atherosclerosis patients with coronary artery stenosis led to a significant decrease in the levels of TBARS and a significant increase of serum FRAP index as total antioxidant capacity.

Table2. Difference of baseline characteristics among three various durations of atorvastatin (20 mg/day) consumption in coronary artery stenosis patients

Variable	Groups of patients according to consumption of atorvastatin			P*
	< 6 days (n = 27)	6-90 days (n = 28)	> 90 days (n = 28)	
Age (year)	62 ± 11	58 ± 10	60 ± 11	0.12
BMI (kg/m^2)	27 ± 3	26 ± 3	27 ± 2	0.51

Data are shown as mean ± standard deviation (SD); * Analysis of variance (ANOVA)
BMI: Body mass index

Table 3. Difference of fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), erythrocyte sedimentation rate (ESR), small dense LDL-C (sdLDL), ferric reducing the ability of plasma (FRAP) and thiobarbituric acid reactive substances (TBARS) among three various durations of atorvastatin (20 mg/day) consumption in coronary artery disease (CAD) patients

Variable	< 6 days (n = 27)	6-90 days (n = 28)	> 90 days (n = 28)	P*	P†	P‡	P§
FBS (mg/dl)	93 ± 22	96 ± 23	99 ± 25	0.39	0.90	0.53	0.92
TG (mg/dl)	161 ± 72	156 ± 50	141 ± 57	0.17	0.62	0.11	0.32
TC (mg/dl)	184 ± 43	178 ± 49	158 ± 26	0.04	0.39	0.03	0.08
LDL-C (mg/dl)	128 ± 33	109 ± 41	104 ± 25	0.02	0.06	0.02	0.84
HDL-C (mg/dl)	37 ± 8	39 ± 10	41 ± 7	0.48	0.79	0.38	0.79
ESR (mm/h)	17 ± 15	16 ± 9	12 ± 8	0.24	0.80	0.36	0.27
sdLDL (mg/dl)	32 ± 15	33 ± 16	37 ± 14	0.24	0.87	0.06	0.16
FRAP (µmol/l)	688 ± 75	760 ± 175	832 ± 101	0.04	0.12	0.01	0.13
TBARS (µmol/l)	2 ± 1	2 ± 1	2 ± 1	0.04	0.21	0.04	0.15

Data are shown as mean ± standard deviation (SD); * Analysis of variance (ANOVA); † Post-hoc pairwise comparisons (Tukey's test) (difference between subjects who received 20 mg/day atorvastatin less than 6 days and someone who used it 6 to 90 days); ‡ Post-hoc pairwise comparisons (Tukey's test) (difference between subjects who received 20 mg/day atorvastatin less than 6 days and someone who used it more than 90 days); § Post-hoc pairwise comparisons (Tukey's test) (difference between subjects who received 20 mg/day atorvastatin 6 to 90 days and someone who used it more than 90 days)

FBS: Fasting blood sugar; TG: Triglycerides; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; ESR: Erythrocyte sedimentation rate; sdLDL: Small dense LDL-C; FRAP: Ferric reducing the ability of plasma; TBARS: Thiobarbituric acid reactive substances

FRAP concentrations consider the cumulative action of all the antioxidants present in plasma and body fluids and provide an integrated parameter rather than the simple sum of measurable antioxidants.[25] Our results indicated that with long-time treatment with atorvastatin oxidative stress decreased in the patients. Moreover, ESR values were decreased with increasing duration of atorvastatin use that represents a relative decrease inflammation in patients under treatment.

The results of this study are similar to the findings of the studies conducted by other researchers.[17,26,27] Some studies performed on the duration of statins intake indicated a significant decrease in the serum levels of MDA as lipid peroxidation marker in patients treated with high-dose simvastatin for at least 6 months.[28] The results of the present study suggest that a daily dose of 20 mg/day atorvastatin taking for at least three months in patients with CAD has significant effects on improving the indices of oxidative stress compared to higher doses of the drug, along with the fewer side effects.

Among LDL-C particles, the sdLDLs are believed to be atherogenic since these particles are taken up more easily by arterial wall. They are highly susceptible to oxidation and have reduced affinity for LDL-C receptor and higher affinity for arterial proteoglycans.[5-7] In our study, we showed that sdLDL was slightly increased with long-time atorvastatin consumption in patients. The results of this study are similar to the results of other studies in terms of higher serum levels of sdLDL in patients with CAD treated with statins. These studies have shown no effect of statins in reducing serum levels of sdLDL.[29,30] However, despite the finding of the present study, other studies indicated that statins reduce sdLDL levels. Several studies conducted on the effect of statins on serum levels of sdLDL represent conflicting results on the decreased levels of sdLDL and increased size of LDL-C particles with statin therapy. Furthermore, the results of some studies suggest that the treatment with statins alters the size of LDL-C particles and, on the other hand, reduces the amount of sdLDL.[31,32] In this study, sdLDL cholesterol had a positive correlation with LDL-C, TG and TC and negative correlation with HDL-C. LDL-C, TG and TC are considered as major risk factors for coronary stenosis in atherosclerosis patients. Our findings are in agreement with previously reported outcomes demonstrating that patients with CAD had sdLDL particles and sdLDL was positively correlated to LDL-C, TG and cholesterol while was negatively correlated with HDL-C.[33]

Previous studies have shown that C-reactive protein (CRP), BMI, and metabolic syndrome can affect the level of sdLDL.[34,35] In the present study, there were no significant differences in CRP, BMI and metabolic syndrome among all groups. As increased BMI and metabolic syndrome are of factors effective in raising serum levels of sdLDL, and with respect to the fact that these variables have no significant differences in all the study groups, it

can be inferred that mild increase in serum sdLDL levels in the groups with increased duration of treatment with atorvastatin represents a negligible effect on the metabolism of sdLDL. The proposed possible mechanism may be that more than 90% of Apolipoprotein B (ApoB) is found in the LDL-C particles, and patients with sdLDL develop lower levels of ApoB.[36] Treatment with statin reduces sdLDL removal by the receptor, because statins increase LDL-C receptor activity, and other LDL particles are better ligands for LDL-C receptor than sdLDL. Therefore, a long-term treatment with statins may cause a slight increase in the levels of sdLDL.[37]

Conclusion

This study showed that atorvastatin has beneficial effects in reducing the elevated serum TBARS, as lipid peroxidation indicator, TC and LDL-C in coronary artery stenosis patients. Also, this study revealed that atorvastatin has beneficial effects in increasing the reduced serum level of FRAP as a total antioxidant capacity index in patients. Also, this study showed that sdLDL was negatively correlated with HDL-C and positively with LDL-C, TG and cholesterol. Hence, attenuation of total antioxidant capacity, TBARS, lipid profile and atherogenic index can decrease the risk of CAD and its complication such as inflammation, atherogenic process and myocardial infarction in coronary artery stenosis patients.

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Conflict of Interests

Authors have no conflict of interests.

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The relationship between shift work and Framingham risk score: A five-year prospective cohort study

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Original Article

Abstract

BACKGROUND: There is a small number of studies that considered the relationship between shift work (SW) and Framingham risk score (FRS). This study prospectively examined the association between SW and FRS among man workers based on the multilevel modeling approach.

METHODS: This five-year prospective cohort study was done among workers (using stratified random sampling) who work in Esfahan's Mobarakeh Steel Company (EMSC), Iran, from March 2011 to February 2015.

RESULTS: The study sample included 1626 man workers (mean age = 40.0 ± 6.2). Among these subjects, 652 (40.01%), 183 (11.3%) and 791 (48.6%) were day workers, weekly rotating shift workers and routinely rotating, respectively. After controlling unbalanced variables, there was no any significant association between SW and FRS.

CONCLUSION: The results of this prospective cohort study did not show a relationship between SW and FRS.

Keywords: Multilevel Analysis, Cohort Study, Night Shift Work, Iran

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Introduction

Shift work (SW) is an unusual working pattern in comparison to the workday. This work pattern is an integral part of the provision of services in many industrial, economic and service activities.[1] Although many studies have reported the relationship of SW to other diseases like type 2 diabetes,[2] overweight or obesity,[3] blood pressure[1] cholesterol and triglycerides,[4] total cholesterol as an indicator of lipid metabolism[5] and cardiovascular disease (CVD),[6] very limited evidence considered the correlation between SW and Framingham risk score (FRS). The FRS is a diagnostic tool that is widely used to estimate the risk of CVD in the next 10 years based on some variables such as age, sex, total cholesterol, high-density cholesterol (HDL), systolic blood pressure (SBP), history of smoking and history of diabetes.[7]

CVDs are one of the most important causes of death and inability in the human communities. Early identification of individuals at risk is the main objectives of public health in many societies.[8] A simple way for this subjects is Framingham algorithm.[9]

The association between SW and risk of CVDs based on the FRS was reported in a previous study.[10] Based on the findings of this study, the prevalence of CVD risk factors among night-shift workers is 67% higher than the workday.[10]

Furthermore, blood flow rate in the coronary arteries of woman nurses was considered in another survey. The results of this study demonstrated the increased risk of disordered coronary blood flow in night-shift nurses.[11] To our knowledge, a small number of studies considered the correlation between SW and FRS. Therefore, in this five-year prospective cohort study, we investigated the relationship between SW and FRS in Esfahan's Mobarakeh Steel Company (EMSC), Iran, from March 2011 to February 2015.

Materials and Methods

This five-year prospective cohort study was conducted in EMSC from March 2011 to February 2015. The protocol of this research was designed in accommodation with the platform of the Declaration of Helsinki and then approved by the Medical Ethics Committee of Tarbiat Modares University, Tehran, Iran (code number: 52D.3817).

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Individuals were contacted via phone and protocols of the study were thoroughly explained for each person. All subjects were willingly entered into the study and a written consent form signed by them.

In this study, FRS and its components including SBP, cholesterol, and HDL were considered as a dependent variable, while SW was considered as an independent variable. Additionally, factors such as age, work experience, body mass index (BMI), smoking, and education status were considered as control variables. The FRS is a sex-specific method used to estimate the ten-year risk of CVD in individuals.

High score of FRS means the high probable risk of cardiovascular disease within a specified time course, generally ten to thirty years. FRS also shows who is the more prone to get the advantage of prevention.[12] To calculate this score, X_1, X_2, …, X_5 must initially be calculated according to the table 1, and then the FRS can be calculated using the following formula:

$$FRS = \sum_{i=1}^{5} X_i$$

The score ranges between -2 and 36. Higher FRS indicated the increased 10-year CVD risk of a person.

The work area of EMSC was arranged into strata and participants were randomly selected via stratified random sampling.

Inclusion criteria were willing to participate, official employment between March 2011 and February 2015 with at least two years of work experience in March 2011, and not taking antihypertensive and blood lipid-lowering drugs. Patients who met the following criteria were excluded from the study: retirement, death or dismissal (Figure 1). The optimal sample size, which contained 1971 cases, was calculated using the unequal t-test formula considering the effect size = 0.27 and dropout rate of 22% (α = 5%, β = 10%) based on a previous study.[1] After remaining in the sitting position for 5 minutes, the SBP of both arms was measured by three general practitioners using a calibrated portable or wall-mounted Baumanometer sphygmomanometer Kompak Model-260 mmHg (WA Baum, Copiague, NY). Laboratory variables were measured using calibrated instruments. In this study, regular smokers were people smoking at least one cigarette daily for at least one year. The scheduled of shift time is presented in Gholami Fesharaki et al.[1] study.

We used R software (version 3.2.1) and package "nlme" for analysis of data. Chi-square test was used to compare categorical variables, while analysis of variance (ANOVA) and Kruskal-Wallis tests were used to compare continuous variables. Intention-to-treat (ITT) analysis using multilevel modeling[1] was used for modeling correlated and longitudinal data and investigating the predictors of longitudinal changes in FRS after controlling for BMI, work experience, as well as educational status. The measurements for each individual were repeated 5 times, and each time interval measurement was one year. In this study, $P < 0.050$ was considered to be statistically significant.

Table 1. Scoring of age, smoking, cholesterol, high-density lipoprotein (HDL) and systolic blood pressure (SBP) for calculating Framingham risk score (FRS)

Age range	X1		X2		X3		X4	X5			
	Age		Smokers		Cholesterol (mg/dl) A: < 160, 190-199, 200-239, 240-279, ≥ 280		HDL (mg/dl) B: < 40, 40-49, 50-59, ≥ 280	SBP (mmHg) C: < 120, 120-129, 130-139, 140-279, ≥ 280			
	M	W	M	W	M	W	M or W	WT	WNT	MT	MNT
≤ 34	-7	-9	9	8	(0, 4, 7, 9, 11)	(0, 4, 8, 11, 13)	(-1, 0, 1, 2)	(0, 3, 4, 5, 6)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
35-39	-3	-4	9	8	(0, 4, 7, 9, 11)	(0, 4, 8, 11, 13)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
40-44	0	0	7	5	(0, 3, 5, 6, 8)	(0, 3, 6, 8, 10)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
45-49	3	3	7	5	(0, 3, 5, 6, 8)	(0, 3, 6, 8, 10)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
50-54	6	6	4	3	(0, 2, 3, 4, 5)	(0, 2, 5،4, 7)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
55-59	8	8	4	3	(0, 2, 3, 4, 5)	(0, 2, 5،4, 7)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
60-64	10	10	2	1	(0, 1, 1, 2, 3)	(0, 1, 3،2, 4)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
65-69	12	11	2	1	(0, 1, 1, 2, 3)	(0, 1, 3،2, 4)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
70-74	14	12	1	1	(0, 0, 0, 1, 1)	(0, 1, 1, 2, 2)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)
≥ 75	16	13	1	1	(0, 0, 0, 1, 1)	(0, 1, 1, 2, 2)	(-1, 0, 1, 2)	(0, 3, 4, 5)	(0, 1, 2, 3,4)	(0, 1, 2, 2, 3)	(0, 0, 1, 1, 2)

Data are shown as frequency

Framingham risk score (FRS) = X_1 + X_2 + X_3 + X_4 + X_5

HDL: High-density lipoprotein; SBP: Systolic blood pressure; M: Man; W: Woman; WT: Woman treated; MT: Man treated; WNT: Woman none treated; MNT: Man non treated

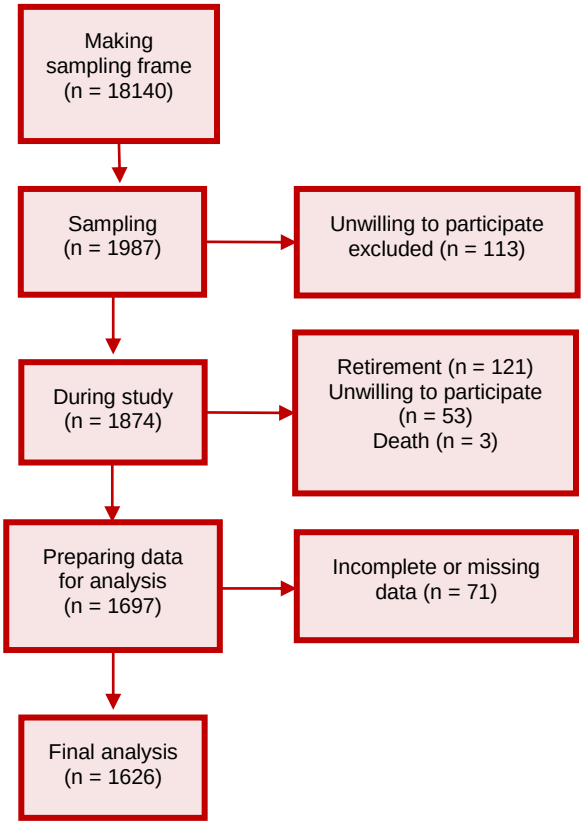

Figure 1. Cohort flow diagram

Results

This study was conducted on 1626 man workers of EMSC. Among these subjects, 652 (40.01%), 183 (11.3%) and 791 (48.6%) were day workers, weekly rotating shift workers and routinely rotating workers, respectively.

Demographical information of workers, presented according to the SW, can be seen in table 2. The mean of age ($P < 0.001$) and work experience ($P < 0.001$) and also the percentage of educational levels ($P < 0.001$) in day workers was significantly higher than routine and weekly rotating shifts.

According to the shift schedule, trends in SBP, HDL, fasting blood sugar (FBS), cholesterol and FRS from 2011 to 2015 are presented in table 3 and figure 2. We found decreasing trend for cholesterol and FBS levels from 2011 to 2015, while an increasing trend was observed for SBP and FRS. Finally, significant fluctuations were found in HDL values. These trends were similar according to the day and shift workers.

Table 4 shows the mean changes of FRS and its constituent variables according to the SW. The non-significant difference was found in shift schedule during the time. Moreover, the relationship of SW to FRS and constituent variables by controlling the baseline and confounder variables is demonstrated in table 5. There was no significant relationship between shift schedule and FRS, SBP, HDL, FBS and cholesterol, after controlling the baseline and confounder variables.

Discussion

Our results have revealed that changes in FRS and other factors were not significant during the period of 5-year study. Therefore, we conclude that the observed difference in results of multilevel modeling is not because of the SW effect, but this difference is related to the baseline.

Although few number of researches have examined the relationship between SW and FRS, these results have not been consistent with our findings. Our data were inconsistent with the study of Pimenta et al.[10] and Kubo et al.[11] that showed a significant relationship between FRS and SW. None of the FRS sub-items showed any significant change in the SW.

Table 2. Demographical characteristics of workers according to the shift Schedule

Variable	Shift schedule			Total	P*
	Routine rotating shift workers	Weekly rotating shift workers	Day workers		
Sex (Man)	791 (100)	183 (100)	652 (100)	1626 (100)	P > 0.9999
Smoke (Yes)	122 (15.4)	24 (13.1)	94 (14.4)	240 (14.7)	0.694
Education (upper diploma)	42 (5.5)	12 (6.8)	208 (33.1)	262 (16.1)	< 0.001
Age (year)	39.3 ± 5.9	40.2 ± 5.9	40.7 ± 6.5	40.0 ± 6.2	< 0.001
Work experience (year)	7.0 ± 8.2	5.3 ± 7.5	8.3 ± 8.7	7.4 ± 8.4	< 0.001
BMI (kg/m2)	26.2 ± 3.3	25.7 ± 3.4	26.0 ± 3.5	26.0 ± 3.4	0.268

Data are shown as number (%) or mean ± standard deviation (SD); * Chi-square or analysis of variance (ANOVA) or Kruskal-Wallis tests
BMI: Body mass index

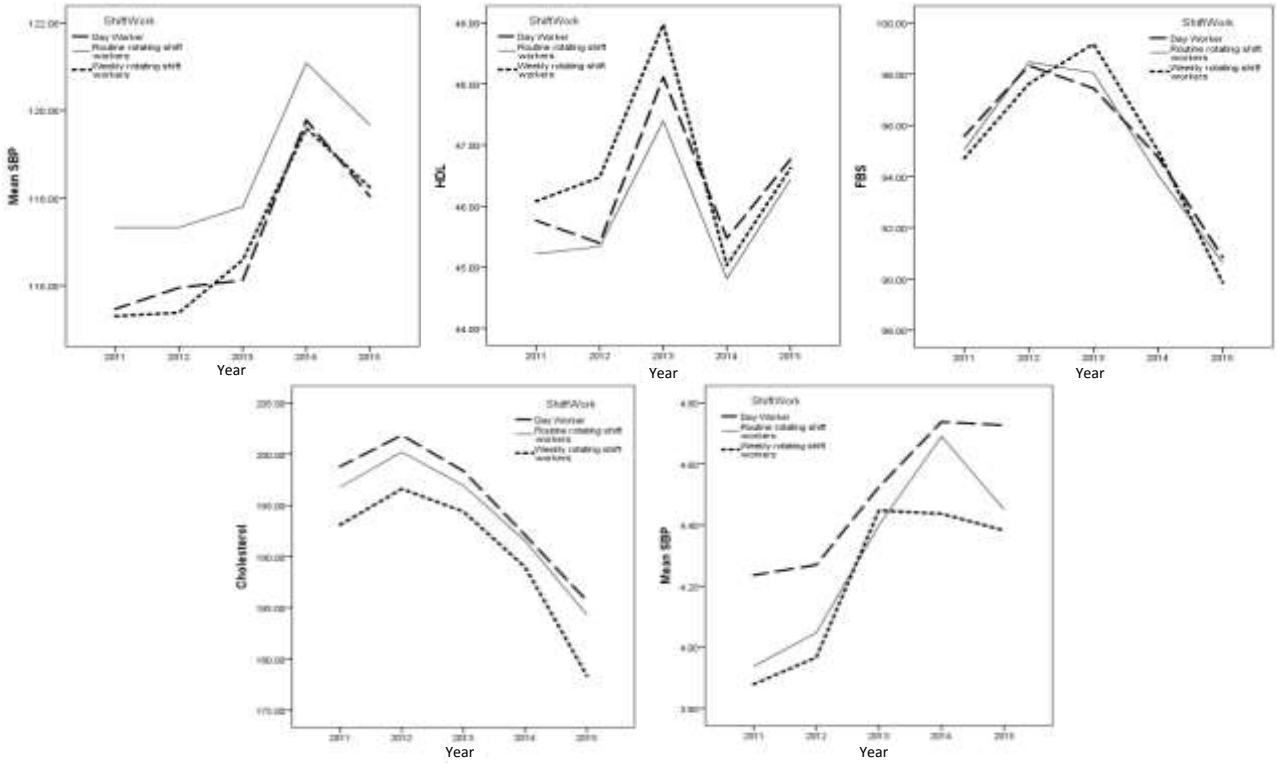

Figure 2. Trend plots of systolic blood pressure (SBP), high-density lipoprotein (HDL), fasting blood sugar (FBS), cholesterol and Framingham risk score (FRS) from 2011 to 2015

Such result has been supported in the previous studies like Gholami Fesharaki et al.,[1] Murata et al.,[13] Hublin et al.,[14] Yadegarfar and McNamee,[15] Virkkunen et al.,[16] Sfreddo et al.,[17] Puttonen et al.,[18] and it is not compatible with some other studies[19-26] regarding the blood pressure and it is consistent[4,27,28] and inconsistent[29,30] with other studies regarding the lipid profile.

Table 3. Trends in systolic blood pressure (SBP), high-density lipoprotein (HDL), fasting blood sugar (FBS), cholesterol and Framingham risk score (FRS) from 2011 to 2015 according to the shift schedule

Variable	Shift schedule	Time duration					P†
		2011	2012	2013	2014	2015	
SBP (mmHg)	DW	115.5 ± 10.5	116.0 ± 12.0	116.1 ± 12.2	119.8 ± 12.9	118.0 ± 11.9	< 0.001
	RRS	117.3 ± 12.2	117.3 ± 11.8	117.8 ± 12.4	121.1 ± 13.0	119.7 ± 12.8	< 0.001
	WRS	115.3 ± 10.4	115.4 ± 10.4	116.6 ± 11.3	119.6 ± 11.5	118.2 ± 12.7	< 0.001
	P*	0.004	0.026	0.033	0.101	0.037	
HDL (mg/dl)	DW	45.8 ± 7.9	45.4 ± 9.2	48.1 ± 9.6	45.5 ± 9.6	46.8 ± 9.5	< 0.001
	RRS	45.2 ± 7.3	45.3 ± 8.6	47.4 ± 10	44.8 ± 9.4	46.4 ± 8.8	< 0.001
	WRS	46.1 ± 7.1	46.5 ± 7.9	49.0 ± 8.4	45.0 ± 8.5	46.6 ± 10.4	< 0.001
	P*	0.213	0.210	0.070	0.418	0.794	
FBS (mg/dl)	DW	95.6 ± 19.2	98.3 ± 21	97.5 ± 18.1	94.8 ± 20.5	90.9 ± 21.0	< 0.001
	RRS	95.1 ± 18.0	98.5 ± 17.4	98.1 ± 17.4	94.1 ± 20.9	90.6 ± 25.2	< 0.001
	WRS	94.7 ± 17.5	97.6 ± 21.4	99.2 ± 15.6	95.0 ± 21.3	89.9 ± 18.8	< 0.001
	P*	0.798	0.883	0.436	0.758	0.829	0.798
Cholesterol (mg/dl)	DW	198.8 ± 35.9	201.9 ± 36.3	198.3 ± 37.9	192.1 ± 36.8	185.7 ± 36.3	< 0.001
	RRS	196.8 ± 35.0	200.2 ± 35.7	196.9 ± 37.3	191.5 ± 37.8	184.3 ± 37.3	< 0.001
	WRS	193.1 ± 31.5	196.6 ± 33.7	194.4 ± 35.0	189.0 ± 36.5	178.4 ± 33.1	< 0.001
	P*	0.109	0.186	0.407	0.590	0.035	
FRS	DW	4.2 ± 2.4	4.3 ± 2.4	4.5 ± 2.9	4.7 ± 2.7	4.7 ± 2.8	< 0.001
	RRS	3.9 ± 2.4	4.1 ± 2.3	4.4 ± 2.8	4.7 ± 2.9	4.5 ± 2.5	< 0.001
	WRS	3.9 ± 2.2	4.0 ± 1.9	4.5 ± 3.2	4.4 ± 2.7	4.4 ± 2.8	0.001
	P*	0.038	0.109	0.708	0.409	0.111	

Data are shown as mean ± standard deviation (SD); * Analysis of variance (ANOVA) or Kruskal-Wallis tests; † Multilevel modeling
SBP: Systolic blood pressure; HDL: High-density lipoprotein; FBS: Fasting blood sugar; FRS: Framingham risk score; DW: Day worker; RRS: Routine rotating shift workers; WRS: Weekly rotating shift workers

Table 4. The comparison of Framingham risks score and its constituent variables changes during the study time

Variable	Shift schedule						P*
	Routine rotating shift workers		Weekly rotating shift workers		Day workers		
	Mean	Median (Q1:Q3)	Mean	Median (Q1:Q3)	Mean	Median (Q1:Q3)	
SBP (mmHg)	0.59	0 (-10:10)	0.73	0 (-10:10)	0.64	0 (-10:10)	0.847
HDL (mg/dl)	0.31	0 (-4:5)	0.14	0 (-5:5)	0.25	0 (-5:5)	0.772
FBS (mg/dl)	-1.11	-1 (-9:6)	-1.21	-1 (-8:6)	-1.20	-1 (-8:6)	0.598
Cholesterol (mg/dl)	-3.07	-2 (-20:14)	-3.68	-4 (-20:14)	-3.28	-3 (-20:15)	0.834
FRS	0.13	0 (-1:1)	0.13	0 (-1:1)	0.12	0 (-1:1)	0.759

* Kruskal-Wallis test

For variable Y, at first $D_1 = Y_{2012} - Y_{2011}, D_2 = Y_{2013} - Y_{2012}, D_3 = Y_{2014} - Y_{2013}, D_4 = Y_{2015} - Y_{2014}$ was calculated, then the variable change was calculated using Change $Y = \bar{D}$

SBP: Systolic blood pressure; HDL: High-density lipoprotein; FBS: Fasting blood sugar; FRS: Framingham risk score; Q1: First quartile; Q3: Third quartile

The lack of association between FRS and SW might be due to the fact that younger and healthier people are usually recruited as shift workers because of low education, while weaker and older individuals are hired as day workers because of high education. Additionally, most of the day workers have administrative jobs, therefore less active. It, in turns, leads to weight gain (a risk factor of blood pressure elevation). Gholami Fesharaki et al.[31] found a significant increase in BMI (around 0.78 kg/m^2) among day workers compared to weekly rotating shift workers.

The other reason can be related to "stopping hypertension in EMSC" (SHIMSCO) plan for controlling of hypertension in EMSC.[32] SHIMSCO is one of the workplace intervention projects to control hypertension of EMSC workers, where workers received an educational schedule containing healthy lifestyle and self-care suggestions for hypertension management.

Conclusion

Using powerful statistical modeling method for data analysis, sufficient sample size, homogeneity of the study population, and calculation of lipid profile and blood pressure in the clinic by 3 physicians are the strengths of this prospective cohort study. Nevertheless, lack of proper evaluation of the family history of blood pressure, information on previous work experiences, sleep, incomes, stress, and job satisfaction were considered as weaknesses of this research.

Table 5. Multilevel modeling for assessing the effect of shift work (SW) on systolic blood pressure (SBP), high-density lipoprotein (HDL), fasting blood sugar (FBS), cholesterol and Framingham risk score (FRS) by controlling baseline and confounder variables

Response	Weekly rotating shift/day worker			Routine rotating shift/day worker			P‡	ICC (%)
	β	SE	P*	β	SE	P†		
SBP (mmHg)	-0.143	0.696	0.838	0.664	0.447	0.138	0.273	30
HDL (mg/dl)	0.217	0.484	0.653	0.084	0.315	0.789	0.899	36
FBS (mg/dl)	0.876	0.985	0.374	0.235	0.641	0.714	0.673	31
Cholesterol (mg/dl)	-2.374	1.863	0.202	-1.631	1.211	0.178	0.288	39
FRS	0.018	0.129	0.887	-0.039	0.083	0.634	0.839	38

* For weekly rotating shift compared to day worker; † For routine rotating shift compared to day worker; ‡ Simultaneous P for weekly rotating and rotating shift compared to day worker

Result controlled for education, age, work experience, baseline body mass index (BMI), baseline SBP (just For SBP), and baseline FRS (just For FRS)

SBP: Systolic blood pressure; HDL: High-density lipoprotein; FBS: Fasting blood sugar; FRS: Framingham risk score; SE: Standard error; ICC: Interclass correlation

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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Isolated double-orifice mitral valve in a young girl

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Case Report

Abstract

BACKGROUND: Double-orifice mitral valve (DOMV) is an extremely rare cardiac malformation. It has been found to be accompanied by congenital anomalies (CAs), however, it can be detected as an isolated anomaly. The clinical findings of a DOMV are variable and depend predominantly on the associated cardiac abnormalities, particularly atrioventricular septal defects or mitral valve (MV) regurgitation and/or stenosis.

CASE REPORT: In this regard, we describe an isolated DOMV in an 18-year-old young girl who complained of a short-term nonspecific chest pain. She underwent transthoracic and transesophageal echocardiographic examinations. The examinations revealed a DOMV without any valvular or structural malformations. Other examinations were unremarkable. The patient did need neither medical nor surgical interventions.

CONCLUSION: The isolated cases of DOMV do not need therapy and might be only followed up using echocardiographic examinations. However, a careful echocardiographic examination for detection of concomitant structural malformations would be of great importance in the management of such cases.

Keywords: Echocardiography, Heart Valve Disease, Mitral Valve, Congenital Heart Defects

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Introduction

Double-orifice mitral valve (DOMV) is an extremely rare congenital anomaly (CA) diagnosed by the detection of two orifices in the left mitral valve (MV) area, as separated chordal attachment to the papillary muscle. This cannot be attributable to the endocardial cushions and abnormal development of the MV originating from the primitive left ventricle.[1] The DOMV is usually associated with CAs, however, it is also detected as an isolated malformation.[2,3]

The majority of cases are detected by 2-dimensional transthoracic echocardiography (2D TTE),[2] however, transesophageal echocardiography (TEE) also provides more detailed analysis of both the structures and functions of the MV.[4,5] This report describes an isolated DOMV identified using both 2D TTE and TEE imaging modalities.

Case Report

An 18-year-old girl patient was referred to the outpatient clinic of Rajaie Cardiovascular Medical and Research Center, Tehran, Iran, complaining of an atypical chest pain during the past one month. Her physical examination was unremarkable and her pulse rate was regular, with a blood pressure of 110/70 mmHg. She had a normal heart auscultation with no abnormal sounds. Moreover, the electrocardiography and chest roentgenography tests revealed no abnormalities.

She also underwent a routine echocardiographic examination. The 2D TTE revealed 2 separate orifices in the MV location in parasternal short-axis view (PSAV) during diastole with a posteromedial and anterolateral orifice (Figure 1). The sizes of the 4 cardiac chambers were normal. There were neither heart valve diseases, including regurgitation

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or stenosis, nor regional wall motion abnormalities.

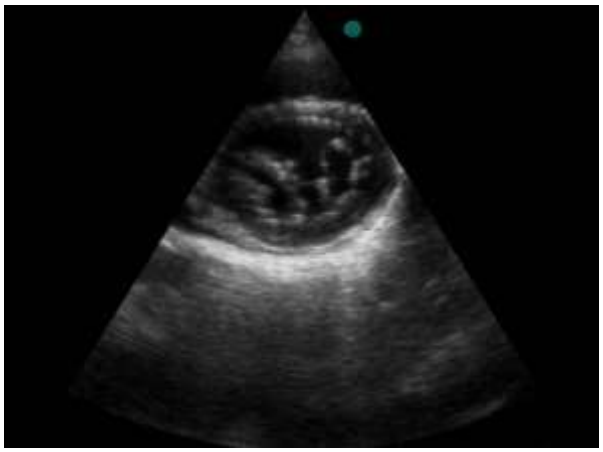

Figure 1. Short-axis transthoracic view showing complete bridge type of double-orifice mitral valve (DOMV)

The left and right ventricular ejection fractions were normal. Furthermore, no pericardial effusion was detected. No CAs were also diagnosed. A TEE-based examination was also performed in order to evaluate the MV comprehensively to provide more precise information regarding this lesion. In addition, the TEE-based examination revealed two orifices in the MV area with separate leaflets (Figure 2). The color Doppler imaging (CDI) also showed two different blood flows thorough different valves (Figure 2 and video 1). No other abnormalities were identified. The findings were consistent with a diagnosis of DOMV without any cardiac malformations. Therefore, the patient was discharged without any specific treatment.

Discussion

In this report, we described a rare form of DOMV in a young girl who complained of a short-term nonspecific chest pain. We did not find any valvular involvements in this case except an isolated DOMV which did not require neither medical nor surgical interventions.

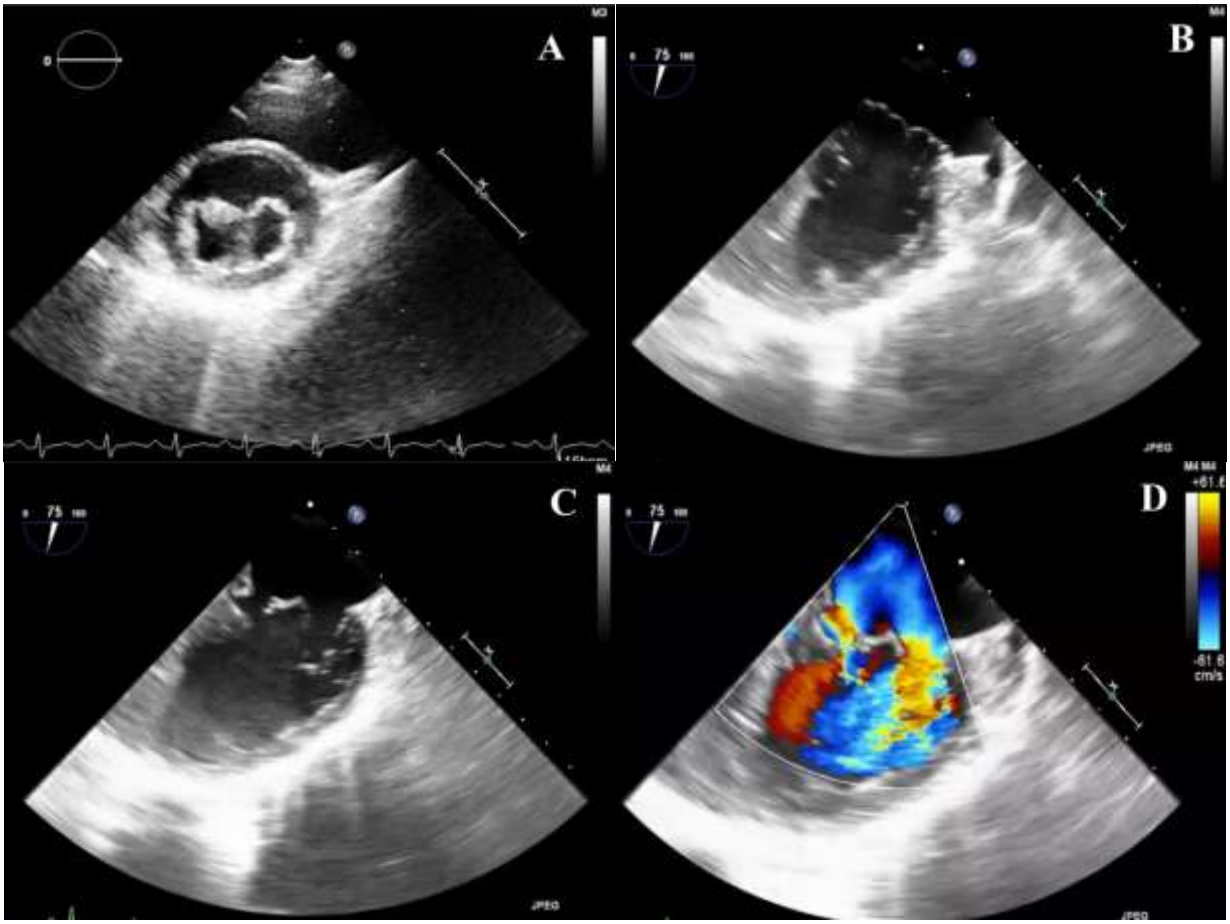

Figure 2. Transesophageal echocardiographic (TEE) images showing (A) double-orifice mitral valve (DOMV), two anatomical mitral valve (MV) leaflets in (B) systolic and (C) diastolic state, and (D) Doppler echocardiography of concomitant flows through two valves

DOMV is a rare CA which may be either an isolated malformation or concomitant with other CAs like endocardial cushion defect, bicuspid aortic valve, coarctation of aorta (CoA or CoAo), and patent ductus arteriosus (PDA).[6] The most common associated anomaly is partial atrioventricular septal defect. Occasionally, some other anomalies were rarely reported, particularly right-to-left shunting anomalies.[7] Moreover, the presence of DOMV associated with other syndromes has also been reported, including down syndrome,[8,9] Kabuki syndrome,[10] and Cornelia de Lange syndrome.[11] According to the coexistence of these syndromes and DOMV, we should consider other extra cardiac anomalies in such cases, including limb anomalies, facial defects, gastrointestinal defects, and neurological manifestations. In addition, in the present case, there was neither intra cardiac nor extra cardiac anomalies.

The exact incidence of DOMV is still unknown; however, in two retrospective studies on the evaluation of the echocardiographic findings of two referral centers, the incidence of DOMV has been found to be approximately 0.04% and 0.01% among children[12] and adult individuals, respectively.[4] The clinical findings of the DOMV are variable and depend predominantly on the associated cardiac abnormalities. Patients with an isolated DOMV usually have mild symptoms including chest pain and palpitation similar to the present case and are found incidentally at echocardiographic assessment for nonspecific symptoms.[2,13]

Trowitzsch et al.[14] described 3 various types of DOMV according to 2D TTE examination: hole type, accounting for approximately 85% of such cases detected as a small accessory orifice placed at either the anterolateral or posteromedial commissure, detectable at the mid-leaflet level view; complete bridge type, accounting for about 15% of DOMVs, with a central bridge of fibrous connecting the 2 leaflets associated with normal papillary muscles of each orifice; and incomplete bridge type, including a partial connection at the leaflet edge. The MV can operate normally among approximately 50% of patients with DOMV.[15] The presence of separated leaflet chordae for both valve openings distinguishes DOMV from acquired lesions, including perforation, fusion of MV leaflets by inflammation, perforated aneurysm of a leaflet, trauma, and iatrogenic.[12]

Echocardiography is the method of choice for detecting and monitoring the progression of DOMV. Other concomitant congenital heart defects are detected during echocardiographic examination. PSAV in TTE is the best view for showing the numbers of MV leaflets and orifices. Long-axis and off-axis views can be used to define the chordal attachments to their respective papillary muscles. Pulse wave, continuous wave, and color Doppler technologies can also be implemented to evaluate the functional status of structural anomalies.[2,4] Three-dimensional echocardiography has also been used for more comprehensive evaluation in suspected cases.[5] This modality may be helpful to detect the anomalous relationship between the papillary muscles and mitral chordae among patients with the DOMV diagnosis.

The management of DOMV depends on the severity of involvement and concomitant anomalies. All patients with significant mitral stenosis and/or insufficiency need medical management to relieve pulmonary congestion and to stabilize them before any required interventions. Surgical approaches are implemented in cases with severe MV stenosis and/or regurgitation or among patients with concomitant cardiac anomalies, particularly septal defects. The type of surgical interventions depends on the detailed anatomical and functional characteristics of the MV abnormality. The MV repair procedure is treatment of choice unless the valve is markedly abnormal or malfunctioned where the valve replacement is needed.[13] Other asymptomatic cases, particularly isolated ones, need neither medical therapies nor surgical correction and may be followed up using echocardiographic examinations. Based on the experience obtained in the present case, we emphasize the importance of careful echocardiographic examination to detect any valvular or structural malformations in such cases to determine the best treatment.

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None.

Conflict of Interests

Authors have no conflict of interests.

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The evaluation of left ventricular diastolic dysfunction in patients with non-hemorrhagic stroke and atrial fibrillation

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Short Communication

Abstract

BACKGROUND: Atrial fibrillation (AF) is the most common tachyarrhythmia and an important risk factor for thromboembolic stroke. CHA_2DS_2-VASc score was introduced for assessment of embolic events and as criteria for starting anticoagulants. This study was performed to evaluate the left ventricular diastolic dysfunction (LVDD) in patients with non-hemorrhagic stroke and AF.

METHODS: This cross-sectional study consisted of 76 consecutive patients with suspected non-hemorrhagic stroke referred to the Cardiology Department of Alzahra and Ayatollah Kashani hospitals in Isfahan, Iran, during 2015-2016. Demographic, anthropometric and clinical characteristics were evaluated for all patients at baseline. CHA_2DS_2-VASc score was calculated for all. All eligible patients underwent transthoracic echocardiogram (TTE) and LVDD was measured in the patients.

RESULTS: The mean age of the patients was 64.64 ± 5.95 years and 28 subjects (36.8%) were women. The most common underlying disease in the patients was hypertension (HTN) (65.8%). Median (range) CHA_2DS_2-VASc score was 4 (1-7). Four patients (5.3%) had paroxysmal AF and 16 cases (21.1%) had LVDD. Analysis showed that LVDD in patients with non-hemorrhagic stroke and coexisting AF was not associated with CHA_2DS_2-VASc score (r = 0.151, P = 0.192).

CONCLUSION: LVDD is not associated with CHA_2DS_2-VASc score in patients with non-hemorrhagic stroke and coexisting AF.

Keywords: Left Ventricular Dysfunction, Stroke, Atrial Fibrillation

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Introduction

Atrial fibrillation (AF), as the most common tachyarrhythmia, affects more than 5% of adults aged 65 years. This arrhythmia is progressive and its prevalence rises with increasing age.[1] AF is associated with some conditions and cardiovascular diseases in 80% of patients.[2] AF, as compared with sinus rhythm, is associated with a higher incidence of death, stroke, and coronary events.[3] Non-valvular AF is the most common cause of stroke due to cardiac embolism so that the risk of stroke in patients with AF is 3 to 7 times more than patients without AF.[4] In patients with known risk factors for stroke, anticoagulant therapy has a particular importance.[5,6]

So far, various scoring systems have been developed to determine the risk of stroke in patients with AF, but none of them are considered the echocardiographic variables. Recently, CHA_2DS_2-VASc score was introduced for assessment of embolic events and as criteria for starting anticoagulants. The main components of this scoring system include congestive heart failure or left ventricular systolic dysfunction, hypertension (HTN), older age, diabetes mellitus (DM), prior stroke or transient ischemic attack (TIA) or thromboembolism, vascular disease and being woman, but left ventricular diastolic dysfunction (LVDD) are not placed in the criteria.[7,8] AF in patients with LVDD is caused stasis and stroke by increasing left ventricular and left atrial end-diastolic pressure.[9,10]

Regarding the predictive role of above factors for hemorrhagic stroke and LVDD, these two variables may be related.[11] Due to lack of sufficient studies in the world, this study was performed to assess the relationship between LVDD and non-hemorrhagic stroke in patients with atrial

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fibrillation. According to the absence of LVDD in CHA_2DS_2-VASc score (despite encompassing systolic dysfunction) and the recent establishment of new LVDD assessment criteria in patients with AF and due to lack of sufficient studies in the world, this article had been designed to evaluate the importance of LVDD in the occurrence of thromboembolic events.

Materials and Methods

The cross-sectional study consisted of 76 consecutive patients with suspected non-hemorrhagic stroke referred to the cardiology department of Alzahra and Ayatollah Kashani hospitals in Isfahan, Iran, between April 2015 and March 2016. Demographic characteristics of the patients such as age and sex were recorded in the checklist. Anthropometric and clinical evaluations were performed for all patients at baseline. The patients' height and body weight were measured, and their body mass index (BMI) was calculated as follows: weight in kilograms divided by the square of the height in meters (kg/m^2). A complete medical history and physical examination were performed for all patients. A history of smoking, diabetes mellitus, hypertension and vascular disease was taken. In a 2-year period, patients with suspected stroke were under initial assessment including history, physical examination and imaging by the resident or specialist of neurology. After confirming the diagnosis of non-hemorrhagic stroke, the eligible patients with coexisting AF in the electrocardiogram (ECG) were selected for the study.

The study was approved by the Human Subjects Committee at Isfahan University and conformed to the ethical guidelines of the 2013 Declaration of Helsinki.[12] Written informed consent was obtained from all participants before enrollment.

Inclusion criteria were all patients with non-hemorrhagic stroke and coexisting AF and having consent to participate in the study. Exclusion criteria were all patients with heart valve stenosis, moderate or severe valve regurgitation, left ventricular ejection fraction (LVEF) < 45%, heart valve surgery, pacemaker insertion, New York Heart Association (NYHA) class IV for congestive heart failure (CHF), other arrhythmias such as ventricular tachycardia (VT) or supraventricular tachycardia (SVT) and lack of consent to participate in the study.

Regarding obtained data from the patients with non-hemorrhagic stroke, CHA_2DS_2-VASc score was calculated for all of them and they received necessary neurologic treatment and care in the course of hospitalization (if CHA_2DS_2-VASc score ≥ 2, anticoagulant was required). Simultaneously, the patients underwent transthoracic echocardiogram (TTE) by Samsung Medison and GE Vivid 3 echocardiography machines to assess LVDD. To evaluate LVEF of the patients, three echocardiographic methods were used including M-mode fractional shortening, Simpson's method and Eyeball estimation in several consecutive turns. If LVEF ≥ 45% was confirmed and the patients did not have the exclusion criteria, they were enrolled and LVDD evaluation was performed as following steps: 1- Measurement of left ventricle inflow velocities including E velocity, deceleration slope (DT), a velocity and duration, 2- Measurement of left atrial inflow including peak systolic velocity, peak diastolic velocity, atrial velocity peak and duration, 3- Measurement of mitral annulus velocity by Doppler tissue imaging including early myocardial velocity (E') and atrial myocardial velocity (A'). Due to the lack of A velocity and atrial myocardial velocity (A') in patients with AF, American Society of Echocardiography (ASE) guidelines 2016 [13] were used to assess LVDD as follows: 1- Peak acceleration rate of mitral E velocity (≥ 1,900 cm/s^2), 2- Isovolumetric relaxation time (IVRT) (≤ 65 ms), 3- Deceleration time (DT) of pulmonary venous diastolic velocity (≤ 220 ms), 4- E/Vp ratio (≥ 1.4), 5- Septal E/e' ratio (≥ 11). Two above items including DT and E/Vp ratio were not measured in this study due to technical problems and needing to transesophageal echocardiography (TEE).

All statistical analysis was performed using SPSS software (version 24, IBM Corporation, Armonk, NY, USA). The qualitative variables were described with number and percentage. Quantitative variables were described using mean ± standard deviation (SD), median (range) and interquartile range (IQR). To analyze the correlation between LVDD and CHA_2DS_2-VASc score, Spearman correlation was used. Chi-square test was used to evaluate the difference between categorical variables in two groups. $P < 0.050$ was considered statistically significant at 95% confidence interval (95% CI).

Results

A total of 76 patients with non-hemorrhagic stroke and coexisting AF completed the study. The mean age of the patients was 64.64 ± 5.95 years (range = 54-78). Baseline characteristics are shown in table 1. The results of echocardiographic

evaluations of the patients are shown in table 2. These results are only a status report and the control group does not exist.

Table 1. Baseline characteristics of patients (n = 76) with non-hemorrhagic stroke and coexisting atrial fibrillation (AF)

Characteristic	Statistics
Age > 75 (year)	4 (5.7)
Sex (woman)	28 (36.8)
Smoker	12 (15.8)
DM	14 (18.4)
HTN	50 (65.8)
Vascular disease	13 (17.1)
CHA_2DS_2-VASc	4.0 (1-7)
BMI (kg/m^2)	25.83 ± 1.77

Data are shown as number (%) or mean ± standard deviation (SD) or median (range)
BMI: Body mass index; DM: Diabetes mellitus; HTN: Hypertension

Analysis showed that LVDD in patients with non-hemorrhagic stroke and coexisting AF was not correlated with CHA_2DS_2-VASc score (r = 0.151, P = 0.192).

Table 2. Echocardiographic parameters in the patients (n = 76) with non-hemorrhagic stroke and coexisting atrial fibrillation (AF)

Parameter	Statistics
Heart rate (bpm)	74.51 ± 6.16
LVEF (%)	55.00 ± 4.40
E (cm/s)	1.32 ± 0.43
Septal E/e' (ratio)	9.78 ± 1.16
IVRT (ms)	77.17 ± 10.76
LVDD	16 (21.1)
Paroxysmal AF	4 (5.3)

Data are shown as n (%) or mean ± standard deviation (SD)
AF: Atrial fibrillation; LVEF: Left ventricular ejection fraction; E: Peak acceleration of mitral E wave; IVRT: Isovolumetric relaxation time; LVDD: Left ventricular diastolic dysfunction

The assessment of variables in patients with CHA_2DS_2-VASc score ≥ 2 showed that there was a significant difference between the patients with and without LVDD it in terms of sex (P = 0.037), smoking (P = 0.032), DM (P = 0.001) and vascular disease (P = 0.020), but It was not significantly different regarding age (P = 0.655), BMI (P = 0.274) and HTN (P = 0.255) (Table 3). In addition, in terms of echocardiographic parameters, there was a significant difference between two subgroups of LVDD (with or without) in terms of E index (P < 0.001), septal E/e' ratio (P < 0.001) and IVRT (P < 0.001). However, it was not significantly different regarding paroxysmal AF (P = 0.345), heart rate (P = 0.860) and LVEF (P = 0.145).

Discussion

In this study, patients with non-hemorrhagic stroke with coexisting AF were evaluated in relation to LVDD and its association with CHA_2DS_2-VASc score. About one-fifth of the patients had LVDD. A limited number of patients were over 75-year old (about 5%), and about one-third were women. Most patients (over 70%) were overweight or obese. The most common underlying disease in the patients was HTN (about two-thirds) and less than one-fifth had DM or vascular disease, or were smokers. All of the patients had a CHA_2DS_2-VASc score ≥ 2, and so they needed anticoagulant therapy. None of the patients had bradycardia or tachycardia, and none of them had LVEF less than 50%. Few patients had paroxysmal AF (about 5%). Echocardiographic parameters such as peak acceleration of mitral E wave, septal E/e' ratio and IVRT were abnormal. Being woman, smoking, DM and vascular diseases had significant effects on LVDD, but over 75-year age, overweight or obesity and HTN did not significantly affect LVDD.

Table 3. Distribution of left ventricular diastolic dysfunction (LVDD) in baseline clinical characteristics of the subjects with CHA_2DS_2-VASc score ≥ 2

Characteristic	Diastolic Dysfunction		P
	Yes	No	
	n = 16	n = 60	
Sex (man)	6 (37.5)	42 (70.0)	0.037
Smoke	0 (0.0)	18 (30.0)	0.032
HTN	13 (81.2)	41 (68.3)	0.255
DM	8 (50.0)	6 (10.0)	0.001
Vascular	7 (43.7)	8 (13.3)	0.020
Paroxysmal AF	0 (0.0)	4 (6.7)	0.345

Data are shown as number (%)
HTN: Hypertension; DM: Diabetes mellitus; AF: Atrial fibrillation

In addition, echocardiographic parameters such as E index, E/e' ratio and IVRT had significant relationships with LVDD, while heart rate, LVEF and paroxysmal AF (no persistent) had no significant relations with LVDD.

AF is the most commonly associated illness in hospitalized patients, which is the cause of nearly 25% of stroke cases in the 80-89 age groups.[14] In patients with persistent AF or prolonged paroxysmal AF that have risk factors for stroke, anticoagulant therapy with warfarin is recommended, with the goal of maintaining the international normalized ratio (INR) between 2 and 3.[4,5,15] Aspirin is used in a small number of patients who are not at risk or when warfarin is contraindicated. However, aspirin is less effective in preventing thromboembolism than is warfarin. Despite the easier treatment, the strength of aspirin in preventing thromboembolism is much less than warfarin and should only be considered in low-risk patients. The risk of stroke in these patients is high (1% per year), but due to the high prevalence of this arrhythmia, prevention of these events in patients is important. Warfarin reduces this chance by about 75%, but aspirin has about one-third of warfarin effects on stroke prevention.[4,5] Various studies have been done on the classification of AF patients to determine the high or low risk of thromboembolic events, such as stroke. The CHA_2DS_2-VASc score is one of the scoring and risk assessment systems, that has been much considered in recent years. The American College of Chest Physicians recommends treatment with warfarin in patients with CHA_2DS_2-VASc score ≥ 2.[4,5,14]

Few studies have been conducted to evaluate the presence of LVDD and estimate CHA_2DS_2-VASc score to predict the non-hemorrhagic stroke.[16,17] The only available study in this field is a study by Kosiuk et al. to investigate the association between LVDD and stroke in patients with AF.[18] Their results showed that 37% of stroke patients had LVDD. Also, the CHA_2DS_2-VASc score was higher in patients with LVDD. There was a statistically significant relationship between CHA_2DS_2-VASc score and LVDD ($r = 0.392$, $P = 0.001$). In addition, LVDD compared to CHA_2DS_2-VASc score was a stronger predictor of stroke in AF patients. However, in the present study, the frequency of LVDD in patients with non-hemorrhagic stroke and coexisting AF was approximately 20% that was about half of the rate reported in the study of Kosiuk et al.[18]

Two studies can be different. In Kosiuk et al.[18] they worked on patients with strokes of different origin, including strokes with and without AF, as well as cryptogenic stroke. The authors analyzed the complex relationships among LVDD, cerebral ischemic events, and AF. But in the present study, only patients with non-hemorrhagic stroke were investigated.

Conclusion

The results of this study showed that LVDD in patients with non-hemorrhagic stroke and coexisting AF is not associated with CHA_2DS_2-VASc score.

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Conflict of Interests

Authors have no conflict of interests.

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