MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

DECEMBER 2018 VOLUME 4 ISSUE 3

Published three times per year by Ordu University

ISSN 2149-7796

MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE (MBSJHS)

MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

The Middle Black Sea Journal of Health Science, which is international journal, is published by Ordu University Institute of Health Sciences on behalf of the Middle Black Sea Universities Collaboration Platform

e-ISSN 2149-7796

Middle Black Sea Journal of Health Science

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Web site: http://dergipark.gov.tr/mbsjohs

Sort of Publication: Periodically

Publication Date and Place: 27 / 12 / 2018, ORDU, TURKEY

Publishing Kind: Online

Indexing: *Turkey Citation Index, Index Copernicus, Rootindexing, Directory of Indexing and Impact Factor, Gooogle Scholar, Turk Medline*

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Conference Paper
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Thesis
Erakıncı G. Donörlerde parazitlere karşı oluşan antikorların aranması. İzmir: Ege Üniversitesi Sağlık Bilimleri Enstitüsü. 1997.

Article in Electronic Format
Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Structure

English title, author names and institutions.
Abstract (average 200-400 word)
Introduction
Methods
Results
Discussion and conclusion
References (most 30)
Whole text should not exceed 4500 words except for refences and abstract.

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Results
Discussion and conclusion
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The compilation text also including appropriate sub-headings,
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Conclusion
References (most 15)

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Topics related to the subject.
Conclusion
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300 words of text and original images about the subject

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MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

DECEMBER 2018 **VOLUME 4** **ISSUE 3**

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EDITORIAL

Four full years ...

We are in happiness as we achieve to perform our goal including publications from all areas of health sciences which is in our journal plans significantly.

Thank you very much to the referees, authors and the editorial board members from Turkey and different countries in our publishing family.

Again, in this issue, you can also access original articles on topics ranging from nephrology, orthopedy, gynecology, parasitology, emergine medicine and nursing to basic medical sciences, internal medicine and surgeon sciences.

Our aim is to continue our contribution to the science by new knowledges and more beautiful publications in the next issue.

See you soon…

PhD. Assoc. Prof. Dr. Ülkü KARAMAN

Editor

Middle Black Sea Journal of Health Science December 2018; 4(3):1-10

RESEARCH ARTICLE

Accidents Increase in Inadequate Housing Conditions: A Cross-Sectional Study from Turkey

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Received: 23 August 2018, Accepted 09 October 2018, Published online: 27 December 2018

Abstract

Objective: Housing is an important determinant of health, and substandard housing is a major public health issue. The aim of this study was to determine the prevalence of home accidents, and to evaluate the associations between housing conditions and home accidents in a health centre region in Ankara.

Methods: In this cross-sectional research, we collected data from a representative sample of 210 houses using two standard questionnaires, which was used in the WHO's Large Analysis and Review of European Housing and Health Status (LARES) project. In total, 528 people participated.

Home accidents were self-reported and questioned for the previous year. We developed a composite index to assess the overall housing conditions. Housing conditions were accepted "inadequate" if the score was below the median. We developed a logistic regression model to predict the housing-related factors in accidents.

Results: Of the participants, 60.4% were female, 89.0% had health insurance, and 56.1% were married. Fifty-eight point three percent of the respondents were living in inadequate housing conditions. The prevalence of home accidents during previous year was 21.2%. The first three most common accident types were falls, cuts, collision/striking and the mostly injured body parts were arm/upper limb, leg/lower limb, surface area of the body. Some of the items which have been involved in these accidents were construction features, kitchen equipment, and knives. The accidents were more common among females, people who reported fatigue, in kitchens with too little workplace and houses with noise problems($p<0.05$). The odds of home accidents were 1.8 times more (95% CI:1.1-2.8) among residents living in inadequate housing conditions. The logistic model showed that, accidents were 2.1 times more (95% CI: 1.1-4.2) in those living in houses where adaptations for physical constraints were lacking, and 1.9 times more among females (95% CI: 1.1-3.3).

Conclusion: Home accidents were common and related to housing conditions. We recommended that factors that can cause accidents in residential buildings be taken into consideration during the construction phase through cooperation of the municipality, the construction sector and the health personnel. This will enable everyone to benefit from these arrangements in the house.

Key words: Housing, home accidents, injuries, LARES, housing conditions, safety

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DOI: 10.19127/mbsjohs.454921

Note: Presented at 12th World Congress on Public Health: Making a Difference in Global Public Health, Education, Research, and Practice

Introduction

Housing is an important determinant of health, and substandard housing is a major public health issue. Housing definition therefore, needs to be comprehensive including the building, dwelling, and the immediate environment (Cobanoglu, 1996; Bonnefoy, 2007; Guler, 2008). The World Health Organization (WHO) defines healthy housing as the housing where health, hygiene, comfort and privacy, functional and adequate physical, social and spiritual conditions are provided (Cobanoglu, 1996; Bonnefoy, 2007; Guler, 2008; Jacobs, 2011). The United Nations Habitat Agenda defined adequate housing and shelter broadly. Housing is defined as meaning adequate privacy; adequate space; physical accessibility; structural stability and durability; adequate lighting, heating, and ventilation; adequate basic infrastructure, such as water supply, sanitation, and waste management facilities; suitable environmental quality and health-related factors; and adequate and accessible location with regard to work and basic facilities (Jacobs, 2011).

Housing is a complex construct that cannot be represented only by the physical structure of the home. The WHO approach to housing is, based on a four-layer model of housing, taking into consideration the physical structure of the dwelling as well as the meaning of home and the external dimension of the immediate housing environment, and the community with all neighbours (Bonnefoy, 2007).

Housing conditions and the built environment can significantly affect public health. Some of the housing related environmental health risks include; indoor or outdoor air pollution from cooking, heating and lighting, exposure to extreme heat or cold; disease vectors, damp and mould, design features, access to green spaces for physical activity, noise exposures, and use of unsafe construction materials and poor construction practices (Bonnefoy et al., 2003; Niemann and Maschke, 2004; Niemannet al., 2006; Bonnefoy, 2007; Veitch and Galasiu, 2012; WHO, 2018).

The home is also where accidents frequently occur. In the European Union, more than half of the 20 million home and leisure related accidents that occur each year take place in or around the home (Bonnefoy et al., 2003). Too many factors may pose a risk for accidents in dwellings like stairs, windows, electric installations, and heating devices. As these risks cannot be avoided fully, dwellings should be made as safe as possible through the necessary building and architectural designs. Guidelines were prepared for this purpose, in which the risks are defined, the hazards in the house are listed and the solutions are developed (WHO, 2001; Bonnefoy et al., 2003; CDC and US HUD, 2008; Guler, 2008).

Falls, poisoning and fires are the most frequent causes of accidental injuries and deaths that occur in residential areas (WHO, 2001; CDC and US HUD, 2008; Guler, 2008). Evidence shows that children and the elderly are the most affected, and low socioeconomic conditions are related with accidents (Last, 2008; WHO, 2001).

There are limited number of studies, which reveal the relationship among housing conditions and health impacts in Turkey. Studies are mainly focused on specific individual risk factors, or specific groups like elderly, community-dwellings and housing elements.

In this research, we aimed to determine the prevalence of the home accidents and the influencing factors that are related to housing conditions in a health center region in Ankara.

Methods

In this cross-sectional study, we collected data from a representative sample of 210 houses using two standard questionnaires, which was used in the WHO's Large Analysis and Review of European Housing and Health Survey (LARES) project. We got permission from WHO and applied two questionnaires; the first one was the face-to-face household questionnaire on the perception of residential conditions and the second was an individual self-administered health questionnaire. In total, 528 residents participated.

Accidents were self-reported and questioned for the previous year. We developed a composite index to assess the overall housing conditions. Housing conditions' composite index included number of rooms, number of children and adults sleeping in one room, desire for moving to another house, enough workspace in the kitchen, lighting, and accessibility of the house for handicapped people with wheel chair, walking aids or any other physical constraints, adaptability for the specific needs, renovation of the building or the house. These variables were scaled from zero to one point and the total score was calculated. We accepted housing conditions as "inadequate" if the total score was below the median. We calculated psychosocial benefit score of home (Kearns et al., 2000). The detailed information on description and evaluation of methods and approaches are described elsewhere (Bonnefoy et al., 2007).

We analyzed the data using SPSS 14, and Excel 2007. For descriptive analysis, we used percent distribution, mean, median, and standard deviation. We compared the groups by chi square test. We built a logistic regression model to predict the housing-related factors in accidents. In this model, after controlling for gender (female/male), age (0-9, 10-24, 25-49, 50-64, ≥65), labour (unemployed/employed), body mass index (BMI) (overweight/normal), socioeconomic level (low/high), education (secondary school or lower/higher education), health insurance (none/present), physical activity (no/yes) and renovation of the dwelling (no/yes); we explored housing-related factors (housing conditions, immediate environment, psychosocial benefit score of home, lighting, adaptations for the specific needs, dwelling easily accessible for handicapped people with wheelchair, walking aids like canes or any other physical constraints).

Results

Of the participants, 60.4% were female, 89.0% had health insurance, and 56.1% were married. A hundred and three were pre-school children, and 51 were still at primary school. Of the 425 teenagers and adults, 32.0% were primary school graduates, 29.6% high-school graduates, and 5.6% were illiterate. In the study, there were persons with disabilities in 11 dwellings (2.1%). Of the dwellings, 32% had adaptations for the specific needs like lift, broader doors, no doorsteps, specific installations, walk-in shower, and toilets with seats. These regulations were not made specifically for those persons. In the study, 38.5% of the participants stated that their sleep was disturbed by noise. Of the dwellings, 89% had sufficient daylight (data not shown).

Accidents occurred at 21 of 210 buildings (10%). Figure 1 shows the type of the accidents by age groups. Of the 25 accidents in the buildings, 16 were falls, three were burns, three were cuts or puncture wounds, two were collision or striking, and one was an elevator accident. Of these people, nine were children, nine were adults, and seven were elderly. Forty-three point eight percent of the falls were among elderly, 66.7% of burns were among children, and 66.7% of the cuts or punctures were among adults. Thirty-six percent of the injured were children, 36.0% were adults and 28% were elderly.

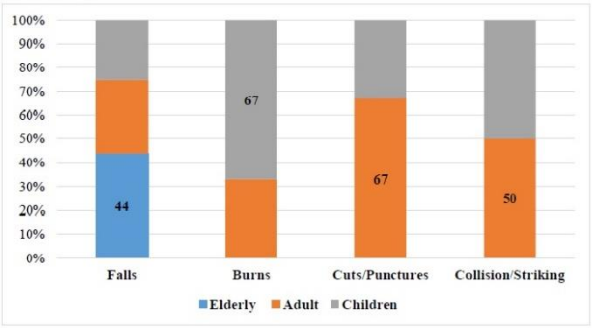

Figure 1. Type of accidents or injuries occurred in the building in the last 12 months by age groups

Table 1 shows the prevalence, type, and causes of the accidents residents reported in the dwellings, the items and the body parts involved. The home accident prevalence was 21.2% for the previous year. The most frequent injuries were falls (67.9%), cuts (35.7%), and collisions (27.7%). Most common injured body parts were arm or upper limb (67.9%), leg or lower limb (35.7%), surface areas (27.7%) and head (16.1%) respectively. The most frequently involved items in these accidents were construction materials (50.0%), kitchen equipment (47.3%), and knives and silverware (11.3%), respectively (Table 1).

Of the residents who reported at least one home accident in the previous year, 75.9% were female, and 24.1% were male. Females were more likely to report an accident (OR: 2.5, 95%CI: 1.5-3.9). Participants who reported insufficient work space in the kitchen were 1.9 times (95% CI: 1.0-3.5), and insufficient number of rooms were 1.7 times (95% CI: 1.1-2.6), participants who reported fatigue were 1.9 times (95% CI: 1.2-2.5) more likely to report an accident compared to the ones who did not (Table 2). Educational status, socio-economic level, renovation of the house was not related with the accidents (data not shown).

We evaluated the association between accidents and housing conditions by using the housing conditions composite index. We found that residents who reported inadequate housing conditions (58.3%) were 1.8 times (95% CI: 1.1-2.8) more likely to report home accidents (Table 3).

After adjusting gender, age, labour, BMI, socioeconomic level, education, health insurance, physical activity, and renovation of the dwelling; the likelihood of accidents in females was 1.9 times (95% CI: 1.1-3.3), in inadequate housing conditions was 1.9 times (95% CI: 1.1-3.3), and in houses with no adaptations for specific needs was 2.1 times more (95% CI: 1.1-4.2) (Table 4).

Table 1. Home accidents in the previous year

	n	%
Home accidents in the previous year (n=528)		
Yes	112	21.2
No	416	78.8
Type of injury (n=112*)		
Falls	61	54.5
Cuts	55	49.1
Collision	30	26.8
Burns	25	22.3
Choking	2	1.8
Electric accident	2	1.8
Gas intoxication	1	0.9
Injured body parts (n=112*)		
Arm/upper limb	76	67.9
Leg/lower limb	40	35.7
Surface area	31	27.7
Head	18	16.1
Lower trunk	7	6.3
Thorax/chest/upper back	4	3.6
Neck/throat	1	0.9
Items involved in the accident (n=112*)		
Construction features ((walls, floor, doors, windows, indoor stairs, lift)	56	50.0
Kitchen equipment	53	47.3
Knives and silverware	38	33.9
Furniture/furnishing (carpets, curtains, etc.)	24	21.4
Heating/cooling equipment	14	12.5
Washing/cleaning products, detergents, liquids etc.	5	4.5
Repairs materials	4	3.6
Toys	3	2.7
Electric equipment	2	1.8
Water/sanitary system	1	0.9
Gasses and fumes	1	0.9
Food items	1	0.9

*Participants could select more than one factor

Table 2. Accidents in dwellings by some housing characteristics

Characteristics (n=528)	Accidents in the dwelling				OR (95% CI)	p
	Yes (n=112)		No (n=416)			
	n	%	n	%		
Gender						
Female	85	75.9	234	56.3	**2.5 (1.5-3.9)**	**<0.001**
Male	27	24.1	182	43.8		
Age group						
≤5	8	7.1	28	6.7		0.247
6-64	100	89.3	354	85.1		
≥65	4	3.6	34	8.2		
Number of rooms						
Insufficient	56	50.0	155	37.3	**1.7 (1.1-2.6)**	**0.015**
Sufficient	56	50.0	261	62.7		
Enough workspace in kitchen						
No	16	14.3	34	8.2	**1.9 (1.0-3.5)**	**0.050**
Yes	96	85.7	382	91.8		
Satisfied with the size of the house						
No	57	50.9	164	39.4	**1.6 (1.1-2.4)**	**0.029**
Yes	55	49.1	252	60.6		
Sleep disturbed by noise						
Yes	49	43.8	154	37.0	1.3 (0.9-2.0)	0.194
No	63	56.3	262	63.0		
Residential natural lighting						
Insufficient	13	11.6	47	11.3	1.0 (0.5-2.0)	0.927
Sufficient	99	88.4	369	88.7		
Adaptations for specific needs						
No	96	85.7	274	65.9	**3.1 (1.8-5.5)**	**<0.001**
Yes	16	14.3	142	34.1		
Accessibility of the house for handicapped people						
No	78	69.6	186	44.7	**2.8 (1.8-4.3)**	**<0.001**
Yes	34	30.4	230	55.3		
Tiredness						
Yes	66	58.9	181	43.5	**1.9 (1.2-2.5)**	**0.004**
No	46	41.1	235	56.5		
Total	**112**	**21.2**	**416**	**78.8**		

Table 3. Odds of home accidents by housing conditions

Housing conditions	Accidents in the dwelling				OR (95% CI)	p
	Yes		No			
	n	%	n	%		
Inadequate	77	68.8	231	55.5	**1.8 (1.1-2.8)**	**0.012**
Adequate	35	31.3	185	44.5		
Total	**112**	**21.2**	**416**	**78.8**		

Table 4. Risk factors for home accidents

Risk factors	OR_{adj} (95% CI)	Wald test p
Female	**1.9 (1.1-3.3)**	**0,025**
Inadequate housing conditions	**1.9 (1.1-3.3)**	**0,028**
Low psychosocial benefit score of home	0.7 (0.4-1.1)	0,118
Bad immediate environment	1.6 (1.0-2.7)	0,099
Insufficient residential lighting	0.9 (0.4-2.2)	0,881
Insufficient space in kitchen	1.5 (0.7-3.1)	0,304
No adaptations for specific needs	**2.1 (1.1-4.2)**	**0,042**
Not easily accessible for handicapped people	1.7 (1.0-3.1)	0,056

Discussion

In recent years, housing conditions have been demonstrated to be one of the major environmental and social determinants of population health. World Health Organization/Europe tasked an international group to measure the health impacts of selected housing risk factors. The findings confirmed that housing is a significant public health issue and that policy-makers need to address it as a priority (WHO, 2011).

Home injuries are also a serious public health problem and rank fifth among the leading causes of death. Nearly 20 million home and leisure injuries requiring medical attention occur each year in the European Union. These injuries lead to hospital admissions and even deaths. Data from the WHO European Region showed that accidents account for the largest number of deaths among young people (WHO, 2007; WHO, 2011).

Human behaviour and dwelling design are the two causal factors that are relevant to home accidents. Residents' behaviour can contribute to home accidents by creating hazards like having loose carpets, leaving medicines and cleaning products easily accessible (WHO, 2007).

The other causal factor in home accidents is the quality of housing conditions. Many health problems are directly or indirectly related to the building itself, because of the construction materials that were used and the equipment installed, or the size or design of the dwellings (Bonnefoy, 2007). In our study, we found that nearly one out of five residents reported a home accident in one year. These accidents were more common among females, and the people who did not have enough kitchen space and who reported fatigue.

Results of LARES, a large cross-sectional study of housing and health in representative populations from eight European cities showed that accidents increase in houses that have crowded households and lack of kitchen workspace. The youngest, the oldest, female residents, and people with functional limitations experience relatively more accidents. Noise and less sleep was also related to more accidents (Bonnefoy et al., 2003; Ellaway et al., 2005; Bonnefoy, 2007; WHO, 2007).

Results of a community-based cross-sectional study of 796 households consisting of 4086 individuals residing in a semi-urban area conducted by Bhanderi and Choudhary showed that crowding was also an important factor for accidents. The incidence of domestic accidents was 1.7%, and the most common accident reported was falls. Occurrence of falls was found to be associated with age and overcrowding. Other accidents noted were burns, scalds, electrocution, injuries and accidental poisoning (Bhanderi and Choudhary, 2008). In our study, we also found that people who thought that they did not have enough rooms in the house and were not satisfied with the size of the house were more likely to suffer from accidents.

Braubach and Savelsberg looked at the households that lived in crowded conditions (less than one room per person), and found that the frequency of fall accidents in low-income households was much more and the rate of reporting a fall accident households was 16%. They concluded that inadequate housing conditions had a significant impact on the frequency of accidents and therefore housing conditions could be considered as one of the mechanisms through which social inequalities may translate into health inequalities (Braubach and Savelsberg, 2009).

Age is regularly identified as the major risk factor for the occurrence of home accidents (Bonnefoy, 2007; Braubach and Savelsberg, 2009; Braubach and Power, 2011). However, in our study, we did not find any significant associations between age and home accidents. These results might be due to the age characteristics of our study participants. We had few elderly people and few children under 5 years old. Bonnefoy et al. found that accidents were more frequent in females and in people under 5 years and over 80 years old when there was not enough kitchen space, poor lighting, few rooms (Bonnefoy et al., 2003; WHO, 2007; Bonnefoy, 2007).

Adequate daylight is one of the basic features of healthy homes (Krieger and Higgins, 2002; CDC and US HUD, 2008; Guler, 2008; DiGuiseppi et al., 2010]. It has been shown that the accident prevalence was higher in houses without enough sunlight (Guler, 2008; Brown and Jacobs, 2011). Brown and Jacobs found that people reporting inadequate natural light in their dwellings were 1.5 times (95% CI: 1.2-1.9) as likely to report a fall compared with those satisfied with their dwelling's light. After adjustment for major confounders, the likelihood of a fall increased to 2.5 (95% CI: 1.5-4.2) (Brown and Jacobs, 2011).

In our study, the lighting characteristics of the dwelling were evaluated by asking if the residents needed to turn on the lights when entering the house in daylight. The daytime lighting was accepted as the evaluation criteria. Most of the residents reported that there was enough daylight entering the house and it was not necessary to turn on the lights during

the day. We evaluated the features related to the lighting in the house only with the criterion of sunlight entry. The use of quantitative methods in determining lighting properties in homes will make it possible to evaluate this relationship more accurately.

Scientific evidence shows that the most frequent types of home accidents are falls and other ones are mechanical contact and collisions and cuts from materials such as glass. Falls account for 45% of all injuries in the home that require medical attention. Among persons 65 years and older, 60% of falls resulting in emergency department visits occurred at home (Bonnefoy, 2007; WHO, 2007a, 2007b; DiGuiseppiet al., 2010). Our results also showed that falls were the most common types of injuries and the most common causes of injury were building materials, kitchen utensils, knives and furniture, flooring materials. In LARES study, of the 13 housing factors listed as related to a fall, most were related to structural factors (48.6%) such as stairs or cracks in flooring, knives/silverware (22.5%), and furniture/furnishings (18.8%) (DiGuiseppi et al., 2010). Besides these structural factors, tiredness was also found to be a risk factor in home accidents. Bonnefoy explained this association by badly designed staircases, slippery floor materials and unfixed carpets, electrical installations, poor lighting, crowding and too little workspace, and noise exposure leading to tiredness and decreased attention (Bonnefoy, 2007). In our study, before adjustment for other housing related factors, we found that participants who reported fatigue were twice more likely to have an accident.

There are many features of dwellings that increase the risk and the severity of injuries. The injury outcomes may vary from minor cuts or bruises and broken bones to paralysis, long-term physical constraints and even death. They can also include burns and drowning or near drowning (WHO, 2011).

The overall evaluation of housing factors may be a better indicator as injuries occur as a result of complex interactions between individuals and the environment and can always be considered multi-factorial in nature. Unfortunately, it is difficult to quantify the individual effect of each of the housing conditions and to calculate the total effect for accident occurrence. In our study, we tried to evaluate housing conditions as a whole by scoring each factor and calculated a total score. However, it is difficult to reveal environmental health associations because environmental impact is multi-factorial and non-specificity of the effect, individual vulnerability, and late appearance of the environmental effects make it more difficult. Some indexes we created may not include some of the determinants and features that would affect these associations. We may not have been able to assess all the relevant factors that could affect this relationship. The health effects of each factor used in the composite index to evaluate the overall housing conditions may not be equal. For this reason, in further analyses, the factors that might be confounders like age, gender, education etc. were controlled and the possible risk factors for accidents were evaluated separately, as well.

Our results showed that residents in dwellings with no adaptations for specific needs was twice likely to report accidents. Howden-Chapman et al. summarized the results of the available research evidence in their systematic review. In order to establish clear guidance on maximising the health gains associated with accessible housing, MacLachlan et al. reviewed the scientific research and looked at whether residents with functional or cognitive impairments living in accessible home environments have better health and social outcomes than residents with functional or cognitive impairments living in conventional or unmodified home environments. They came to the decision that there was sufficient evidence to make a guideline recommendation concerning accessible housing. Home environments that lack accessibility modifications appropriate to the needs of their users were likely to result in people with physical impairments becoming disabled at home (Cho et al., 2016; Howden-Chapman et al., 2017).

Evci et al. conducted a study on 3277 people over 60 years of age living in Aydin province of Turkey. This cross-sectional study results showed that poor housing conditions, being female, living alone, having a chronic illness, physical and hearing disability, wearing eyeglasses, inactivity, use of assistive devices and more than four drugs were associated with having a home accident in the elderly (Evci et al., 2006). In our study, as we had few elderly participants we could not evaluate the risk factors for accidents in the elderly separately. However, our results on the most frequent type and cause of the accidents were similar with the elderly. Sahin and Erkal conducted a study in Kırıkkale Province and 175 elderly participated. They found that more than half of the elderly (59.4%) sustained home accidents in the previous year, and elderly who sustained fall accidents (70.2%) and those who

sustained accidents in the kitchen (31.7%) ranked in first place (Sahin and Erkal, 2016).

The characteristics of the houses located in the area where we conducted the survey were similar. Housing and health research should be conducted with a representative sample of Ankara, and then in other provinces. This could help to fulfil the gap in scientific evidence on housing health in our country and it will make it possible to make national and international comparisons.

Lyons et al in their systematic review in 2006, concluded that there was insufficient evidence to conclude that modifying the physical environment in the home will definitely reduce the injuries (with the exception of the provision and promotion of smoke alarm ownership, which was excluded from the review) as injuries occur as a result of complex interactions between individuals and the environment and can always be considered multi-factorial in nature. They stated that the quality and size of the studies were not sufficiently good or large to reach definitive conclusions (Lyons et al., 2006). DiGuiseppi et al, in their scientific review in 2010, aimed to inform decisions about which policies were likely to result in the best and most efficient use of resources to address structural deficiencies and to illuminate where further research was needed to allow informed policy decisions to be made. They concluded that interventions like installed, working smoke alarms; 4-sided isolation pool fencing; and hot water heaters that are preset by the manufacturer at a safe temperature were likely to significantly reduce residential morbidity and mortality if effectively implemented. They thought that addressing structural deficiencies to reduce unintentional injuries that occur in unsafe homes was likely to require concerted efforts from a broad range of agencies, organizations, and industries (DiGuiseppi et al., 2010).

Conclusion

In our study, we recommended that factors that can cause accidents in residential buildings be taken into consideration during the construction phase, which will enable everyone to benefit from these arrangements in the house. Permission to build houses is the responsibility of the municipalities. In the municipalities, it is necessary to employ trained personnel to make this task more conscious and in accordance with the health conditions. The formation of healthy housing and residential surroundings is possible through cooperation of the municipality, the construction sector and the health personnel. In the past years, in our country, although the licenses for housing have been supervised by physicians, this practice has been abolished. Re-enactment of this practice may contribute to healthy housing conditions. Healthy housing is possible through cooperation of the municipality, the construction sector and the health personnel. Increased awareness will also contribute to the establishment and implementation of adequate housing conditions.

It will be appropriate to use some environmental health indicators in future studies in order to standardize the studies that investigate housing health relationships, and to make comparisons between regions and over time.7

Acknowledgements: The authors thank Prof. Dr. Cagatay Guler, Prof. Dr. Banu Cakır who provided insight and expertise that greatly assisted the research, and thank WHO who gave permission to use the questionnaires of LARES Project.

Informed Consent: Oral and written informed consent was obtained from the participants.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - FT, SAV; Design - FT, SAV; Supervision- SAV; Materials - FT, SAV; Data Collection and/or Processing - FT; Analysis and/or Interpretation - FT, SAV; Literature Review - FT, SAV; Writing - FT, SAV; Critical Review - FT, SAV
Conflict of Interest: The authors declared no conflict of interest.
Financial Disclosure: The authors declared that this study has received no financial support.

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Middle Black Sea Journal of Health Science December 2018; 4(3):11-19

RESEARCH ARTICLE

Investigation of Cryptosporidium spp. in Immunosuppressive and Immunocompetent Cases with Diarrhea by Microscopic, Serological and Molecular Methods

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Received: 27 Semtember 2018, Accepted 26 November 2018, Published online: 27 December 2018

Abstract

Objective: In this study, our aim was to compare the diagnostic methods with each other and show the presence of Cryptosporidium oocysts by using molecular, serological and microscopic methods in stool samples which were collected from immunosuppressive and immunocompetent patients with diarrhea

Methods: Total 172 stool samples were collected from 80 immunosuppressive patients and 92 immunocompetent patients (between 0-94 years) with diarrhea. These stool samples were obtained from the different clinics of Ataturk University, Yakutiye Research Hospital between January 2014 and July 2014. Patient group composed of 49 persons between 0-14 years and 123 persons between 15-94 years. On the other hand, 141 patients were using tap water while 41 of them were using well water. Modified acid-fast staining, ELISA and DFA techniques were applied to detect the Cryptosporidium parasite positivity. Nested PCR method was performed to the samples which were detected positive with one of the above methods.

Results: The positivity was detected in 5.8%, 4.1% and 3.5% by ELISA, DFA technique and Modified acid-fast staining method, respectively. Cryptosporidium DNA was detected in only 1.2% by PCR method. The rates of positivity were 6.3% and 5.4% in immunosuppressive and immunocompetent patients, respectively. The positivity was detected in 10.2% and 4.1% in 0-14 age group and 15-94 age group patients, respectively. On the other hand, 4.3% and 12.9% positivity rates were detected in tap water and well water users respectively.

Conclusion: Our study pointed out that the investigation of Cryptosporidium oocysts as diarrhea agents in especially immunosuppressive patients, individuals in childhood and well water users may be useful. Because cryptosporidiosis is a common disease in children and immunosuppressive individuals. Additionally, we think that ELISA method can be preferred to other methods in terms of high sensitivity and ease of application.

Key words: Cryptosporidium, DFA, ELISA, immunosuppressive, immunocompetent, modified acid-fast staining, PCR

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DOI: 10.19127/mbsjohs.464833

Introduction

Susceptibility for parasitic infections increased along with elevated number of cancer patients, raising use of immunosuppressive agents, aging of the population, and as a consequence of malnutrition. Among them, cryptosporidiosis has become world-wide prevalent, which creates health problems both in immunosuppressive and immunocompetent individuals. Cryptosporidium spp. is a zoonotic protozoon, located intracellular and extracytoplasmic on the microvilli of digestive and respiratory epithelia of a broad spectrum of vertebrates, including human (Xiao et al., 2004). Infectious transmission is by contaminated food and water through fecal-oral route upon human-human and animal-human contacts (Fayer, 2004; Xiao et al., 2004). Currently, 26 different species of Cryptosporidium have been reported (Galvan et al., 2014). Severity and duration of the infections caused by Cryptosporidium spp. varies on the immune system and the age of the host (Alves et al., 2006; Fayer, 2010; Ekinci, 2012;). Causing agent was known to be associated with altered intestinal epithelial function, to affect intestinal epithelium and nervous system, and lead to microvillus dysfunction if parasitic infection is extensive (Kar, 2007).

Routine diagnosis of cryptosporidiosis is based on stool screening and direct inspection of the causative agent (Sears & Kirckpatrick, 2001). Oocysts are seen through such staining methods as Kinyoun's acid-fast, modified acid-fast, Giemsa, nigrosin, safranin methylene blue, and carbol-fuchsin (MacPherson and McQueen, 1993; Starling and Arrowood, 1993; Sears and Kirckpatrick, 2001). Antibodies developed in cryptosporidiosis could be detected via IFAT (indirect immunofluorescent antibody test), ELISA, and Western Blot assays. Monoclonal antibody-based DFA method is a valuable test for detection of surface antigens of the organisms isolated from the stool (Elgün, 2009). In addition, molecular research has provided important insights for the taxonomy and distinction of Cryptosporidium species (Xiao et al., 2002).

This study aimed to compare diagnostic values of molecular, serological, and microscopic methods to detect presence of Cryptosporidium parasite in stool samples that were collected from immunosuppressive and immunocompetent patients with diarrhea.,

Methods

Samples: A total of 172 diarrheic stool samples of 80 immunosuppressive and 92 immunocompetent patients from different clinics of Erzurum Yakutiye Research Hospital between January and July 2014 were included to the study. Immunosuppressive patients mostly consisted of patients with oncology department, patients with organ transplantation, patients with chronic renal failure, and patients with terminal age. The study was approved by the Ethics Committee for Clinical and Laboratory Trials of Ataturk University School of Medicine (Approval Date: 26.12.2013, Decision No: 18).

Modified Acid-Fast (MAF) Staining: Stool samples of diarrheic patients were prepared by MAF staining method, and preparations were assessed under light microscope at 40x and 100x magnification (Garcia, Bruckner, Brewer, & Shimizu, 1983; Usluca, 2009).

ELISA Method: Cryptosporidium 2nd Generation ELISA kit (Diagnostic Automation Lot: Daln1082) was used to determine Cryptosporidium spp. antigens in patient samples. Stool samples at -200C and reactants stored at +40C were brought into room temperature. Reaction findings obtained by using blank, positive, and negative controls per test's manufacturer recommendations were read at 450-630 nm wavelength. Absorbance values indicating ≥0.15 or <15 optimal density (OD) on ELISA reader were considered as positive result or negative result, respectively.

DFA method: MERIFLUOR Cryptosporidium / Giardia (Made in USA) kit was used in this study. Results obtained by the use of positive and negative controls per test's manufacturer recommendations were assessed such that each well was screened completely at 100-200x magnification. Slides where fluorescence was observed were confirmed with further magnification. Any specimen with one or more apple-green fluorescence of characteristic oocyst morphology was regarded as positive for the presence of Cryptosporidium spp.

DNA extraction and Nested PCR amplification: Using i-genomic stool, DNA Extraction Mini Kit (lot no: 14210146) (Intron Biotechnology, Inc. South Korea) per manufacturer's protocol, DNA was obtained from stool specimens that stored at -800C without any added preservative. Primers that were reported to have successful results and targeted at SSU rRNA f of Cryptosporidium were used (Lihua Xiao et al., 1999; Yu, Lee, & Park, 2009). Nested PCR method was performed in this study. At the first step of PCR, rRNA (5'-TTC TAG AGC TAA TAC

ATG CG-3) and rRNA R (5'-CCC TAA TCC TTC GAA ACA GGA-3') primers were used to obtain 1325 bp PCR product. At the second step of PCR, nest rRNA F (5'-GGA AGG GTT GTA TTT ATT AGA TAA AG-3) and nest rRNA R (5'-AAG GAG TAA GGA ACA ACC TCC A-3') primers were used to obtain approximately 826 bp PCR product. Reaction volume was prepared to make a total volume of 50 µl. Pre-denaturation for 5 minutes at 940C and a final elongation for 10 minutes at 720C was performed at first and second step of PCR. During these two steps, amplification was performed by 35 turns for 50 seconds at 940C, for 30 seconds at 550C, and for 50 seconds at 720C through thermal cycle device. A positive and a negative control sample were used at every PCR test. Obtained PCR product was advanced in 1% agarose gel electrophoresis using 100 bp DNA marker, upon which DNA bands were visualized, and sizes of the bands were compared and recorded.

Statistical analysis: The association between variables such as patient groups, age groups, and source of used drinking water were determined through statistical analyses that were performed via SPSS software (version 17.0, SPSS Inc.). A p value below 0.05 was regarded as statistically significant according to Pearson's chi-square test results.

Results

Our study was performed with 172 patients, including immunosuppressive (n=80) and immunocompetent (n=92) subjects who applied to Research Hospital of Ataturk University Faculty of Medicine with the complaint of diarrhea. Forty-nine patients were at 0-14 age group and 123 patients were ≥15 years old (range: 0-94 years). While 141 subjects (82%) were using tap water, 31 subjects (18%) were using well water as the drinking source. In our study, six out of 172 specimens (3.5%) had oocysts that were though to belong to Cryptosporidium parasite by MAF staining method. In serological studies, 10 (5.8%) and 7 (4.1%) specimens elicited positive results in ELISA and DFA methods, respectively. These results were investigated via Nested PCR, where DNA's of Cryptosporidium spp. were confirmed in 2 (1.2%) specimens.

According to MAF staining, positivity was demonstrated in 3.8% and 3.3% of immunosuppressive and immunocompetent patients, respectively (n=6) (Figure 1).

Figure 1. Appearances of *Cryptosporidium* oocysts in MAF staining (100x)

This staining method showed positivity in 8.2% of the subjects below 15 years old and in 1.6% of the subjects ≥15 years old, where the difference was statistically significant. When patients were stratified according to source of drinking water, positivity was found in 2.1% and 9.7% of tap water and well water users, respectively (Table 1).

According to ELISA method, positivity was detected in 6.3% and 5.4% of immunosuppressive and immunocompetent subjects, respectively (n=10) (Figure 2). This method showed positivity in 10.2% of the patients below 15 years old and in 4.1% of the subjects ≥15 years old. Stratification by utilization of water showed that positivity of Cryptosporidium antigen was found in 4.3% and 12.9% of tap water and well water users, respectively (Table 1).

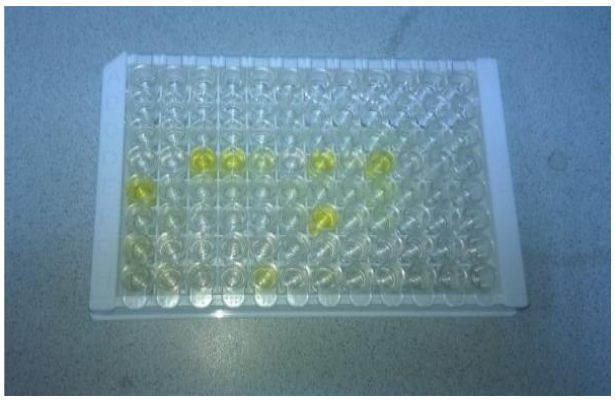

Figure 2. Appearance of preparations of 81 patients in ELISA plate

Positive results in DFA method (n=7) was found in 5.0% and 3.3% of immunosuppressive and immunocompetent patients, respectively (Figure 3). DFA method revealed positivity in 8.2% of the subjects below 15 years old and in 2.4% of the subjects ≥15 years old. Stratification by utilization of water showed that positivity of Cryptosporidium

antigen was detected in 2.8% and 9.7% of tap water and well water users, respectively (Table 1).

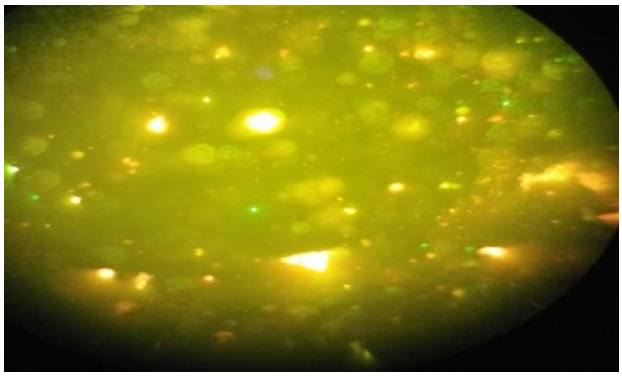

Figure 3. A specimen where a *Cryptosporidium* spp. oocyst was detected by DFA method (40x)

Among specimens stored at -800C, those stool specimens documented as positive by microscopic and serological methods were analyzed with nested PCR methods, and two of them showed Cryptosporidium spp. specific bands. In this method, positivity was demonstrated in 0.0% and 2.2% of immunosuppressive and immunocompetent subjects, respectively. This method showed positivity in 4.1% of the patients below 15 years old and in none of the subjects ≥15 years old. Positivity was found in 0.7% and 3.2% of tap water and well water users, respectively (Table 1).

Images of positive bands under UV system upon advancing of nested PCR-positive Cryptosporidium spp. specimens in 1% agarose gel were shown at Figure 4.

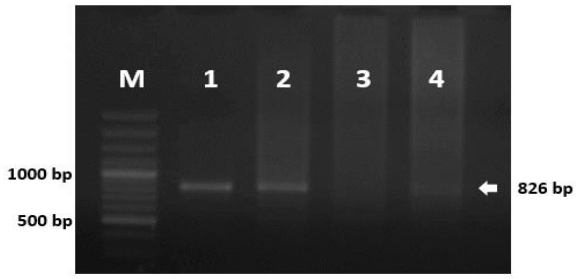

Figure 4. PCR image of cases in which *Cryptosporidium* spp. where detected. M: Marker (100 bp ladder) 1,2: positive result, 3: negative control, 4: positive control (826 bp).

Figure 4. PCR image of cases in which Cryptosporidium spp. where detected. M: Marker (100 bp ladder) 1,2: positive result, 3: negative control, 4: positive control (826 bp).Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of ELISA, MAF staining, and PCR methods were determined in reference to DFA method (Table 2).

Table 1. Distribution of *Cryptosporidium* spp. positivity detected by different methods in immunosuppressive and immunocompetent patients

Method / Patient group	MAF Staining N (%)	ELISA N (%)	DFA N (%)	NESTED PCR N (%)
Immunosuppressive	3 (3.8)	5 (6.3)	4 (5.0)	0(0.0)
Immunocompetent	3 (3.3)	5 (%5.4)	4 (%3.3)	2 (2.2)
P value	0.60	0.54	0.42	0.29
0-14 years old	4 (8.2)	5 (10.2)	4 (8.2)	2 (4.1)
≥15 years old	2 (1.6)	5 (4.1)	3 (2.4)	0 (0.0)
P value	0.05	0.12	0.10	0.08
Well water	3 (9.7)	4 (12.9)	3 (9.7)	1 (3.2)
Tap water	3 (2.1)	6 (4.3)	4 (2.8)	1 (0.7)
P value	0.07	0.08	0.11	0.33

Table 2. Diagnostic values of other methods in reference to DFA method

	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
ELISA	%100.0	%98.2	%70.0	%100.0
MAF Staining	%85.7	%100.0	%100.0	%99.4
PCR	%28.6	%100.0	%100.0	%97.1

Discussion

Increasing prevalence of some compromising diseases, e.g. malignancies, has uncovered immune system problems affecting every age groups. Being more common in these patient groups, many causative agents that are refractory to treatment and often overlooked during diagnostic tests have become increasingly observed among population, especially in these patient groups. One of these agents is the parasite, Cryptosporidium spp., which was well recognized in veterinary medicine by leading to fatal diarrhea cases in newborn calves with consequent economic losses, and it may now also cause fatal events in humans. In fact, it is of paramount importance that Cryptosporidium is the 3rd most common cause (7.2%) of nosocomial diarrhea (Ramratnam and Flanigan, 1997; Koturoglu et al., 2004; Eren, 2011). It is thought that Cryptosporidium spp. was responsible for 12% and 7% of diarrhea cases in developing and developed countries, respectively (Koturoglu et al., 2004; Alabdeen, 2014). The severity of human Cryptosporidium infections are correlate the host's immune status. İn immunocompetent people, the disease is self-limiting, while in immunosuppressed patients, especially those with T-cell deficiency, cryptosporidiosis is frequently chronic and severe with risks of extra-intestinal disease development (Brunet et al., 2016).

Kehl et al. of USA used two ELISA methods, DFA, and acid-fast staining methods do detect Cryptosporidium oocysts and found a positivity rate of 42.6% in 129 patients (Kehl et.al., 1995). Rosenblatt et al. detected the positivity by ELISA and IFA methods as 33.8% among 296 specimens obtained from patients applied to Mayo Clinics in the USA (Rosenblatt and Sloan, 1993). In Turkey, Elgun examined 154 diarrheal stool specimen by ELISA and MAF staining methods and found the positivity as 24.0% and 5.2%, respectively (Elgun, 2009). In our study, we evaluated 172 diarrheal specimens and found a positivity of 5.8% by ELISA method, 4.1% by DFA, 3.5% by MAF staining, and 1.2% by nested PCR.

In developing countries, cryptosporidiosis cases are more common at the age group of 1-4 years old (Xiao et al., 2001; Pereira et al., 2002; Gatei et al., 2006). While Fathy et al. (2014) reported positivity of 22.4% by PCR in 250 children from Egypt, Shah et al. from India found the positivity as 4.0% by Kinyoun's acid-fast staining and 27.4% by ELISA method from the specimens obtained from 175 diarrheic children (Bera et al., 2014). In Turkey, Yilmaz et al. (2008) reported presence of Cryptosporidium oocyst in 4.9% and 1.95% of specimens of 2000 children, documented by ELISA and staining, respectively. In our study, 49 diarrheal specimens from ≤14-year-old population showed a positivity of 10.2% by ELISA method, 8.2% by DFA, and 8.2% by MAF staining. On the other hand, 123 diarrheal specimens from ≥15-year-old population indicated a positivity of 4.1% by ELISA method, 2.4% by DFA, and 1.6% by MAF staining. Cryptosporidium was more prevalent during childhood in our study. This may be explained by the fact that contamination due to lack of hygiene in this age group may affect the prevalence.

Many studies investigated association of cryptosporidiosis with malignant diseases where the immune system was compromised. The prevalence of Cryptosporidium spp. in patients with malignancies was reported to vary between 1.3-1.7% (Tanyuksel et. al., 1995; Sreedharan et al., 1996). In their study of 111 cases with acute lymphocytic leukemia, chronic lymphocytic leukemia, anti-HIV positivity, or other immune deficiencies, Batero et al. (2003) reported Cryptosporidium as the most commonly encountered parasite. In an Indian study, Jayalakshmi et al. examined stool specimens of 89 diarrheic HIV patients with ELISA and MAF staining method and found 12.4% positivity in this group (Jayalakshmi et. al., 2008). Nwodo et al.

performed a study with stool specimens obtained from 35 HIV-positive diarrheic patients of South Africa and detected a positivity of 74.3% with sad-ELISA method (Omoruyi et. al., 2014). In Turkey, Eren et al. reported Cryptosporidium positivity as 7.4% in 254 immunosuppressed subjects and 3.6% in 55 healthy control subjects, as measured by ELISA in 2011 (Eren, 2011). In our study, while Cryptosporidium spp. positivity in immunosuppressive patients was found as 6.3%, 5.0% and 3.8% with ELISA, DFA, and MAF staining methods, this was 5.4% for ELISA, 3.3% for DFA, and 3.3% for MAF staining method in immunocompetent patients. Therefore, it may suggest that Cryptosporidium spp. should be included to the diagnostic work-up of diarrhea in immunocompromised patients.

There are conflictive findings regarding the association between source of drinking water and occurrence of the infection; while some research suggested an association to the use of unboiled tap water (Baumgartner et. al., 2000), others reported that use of tap water was not the main route of transmission for this infection (Xiao et al., 2001; Hunter et al., 2004; Usluca, 2009). In our study, percentage of Cryptosporidium positivity was higher in well water users than tap water users (Table 1). This is thought to be due to the fact that sources of drinking water in rural areas may be more prone to be contaminated by fecal wastes of both human and animal origin.

Microscopic examination is the most common method in the assessment of stool specimens for Cryptosporidium (Morgan et al., 1998). Modified Kinyoun's acid-fast method is regarded as useful due to several factors such as easy applicability, low cost, persistency, and detailed visualization of inner structures of oocysts. ELISA kits are recommended to detect and monitor Cryptosporidium infection in epidemiological and prospective studies (Moss et. al., 1998). IFAT test was reported to have near 100% sensitivity, being capable of detection even 100 oocysts in a 1 ml fluid (Clark, 1999). Nevertheless, the test also had some disadvantages, such that it may be influenced by oocyst concentration techniques or the composition of the feces (Leng et.al., 1996). More importantly, tests that are based on the demonstration of antigen-antibody formation possess the risk of cross-reaction with other microorganisms to some extent (Fayer et. al., 2000).

PCR as a molecular method allows for differentiation between species by some methods like sequence analysis or RFLP. Despite being a rapid, reliable, and sensitive method, it has some limitations leading to emergence of false positive results due to detection of non-viable microorganisms or laboratory contamination (Fayer et al., 2000). It was also reported that PCR method might elicit less positive results compared to microscopic examination in case that the number of oocysts in the stool specimen is very few and they are not evenly distributed within the specimen (Amar et. al., 2004; Magi et. al., 2006; Usluca, 2009). Moreover, microscopic examination and PCR might give differing results since some oocysts may be damaged before DNA extraction during the latter method (Amar et al., 2004; Magi et al., 2006). In addition, it is reported that nested PCR technique is 4-5 times more sensitive than Simple PCR technique (Kato et. al., 2003).

Compared to demonstration of genomic DNA of oocysts in 2 specimens by nested PCR, we detected oocysts in 10 of specimens by ELISA, in 7 of specimens by DFA, and in 6 of specimens by MAF staining method. In general, specimens detected to have Cryptosporidium were observed to have few oocysts. This might be explained by lesser quantity and uneven distribution of oocysts in the stool. Lower detection of positivity in PCR may also originate from potential damage to oocysts prior to DNA extraction.

In their study of 138 patients below 12 years old, Sirrisena et al. confirmed PCR-positivity of only one of the eight specimens detected as positive by modified Ziehl-Neelsen stain (Sirisena et. al., 2014). In our present study of 172 patients, we found six positive specimens by MAF staining and could confirm only two of them by PCR.

When we considered DFA method as the gold standard, ELISA method had 100% sensitivity and 98.2% specificity, MAF staining method had 85.7% sensitivity and 100% specificity, and PCR method had 28.6% and 100% specificity.

Conclusion

Our study has shown that diagnostic work-up for Cryptosporidium oocysts will be useful in the investigation of diarrhea that is especially seen in immunosuppressive people, or during childhood, or in those using well water as the source of drinking water. Since it has high sensitivity, allows for assessment of multiple specimens, gives rapid results, and provides easy applicability, ELISA method may be preferred over other methods in facilities where great numbers of specimens should be tested.

Ethics Committee Approval: The study was approved by the Ethics Committee for Clinical and Laboratory Trials of Ataturk University Medical Faculty (Approval Date: 26.12.2013, Decision No: 18).
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – AY and HU; Design – AY and HU; Supervision– HU; Materials - AY and OA; Data Collection and/or Processing – AY, OA, HA and EG; Analysis and/or Interpretation – AY, OA and EG; Literature Review - AY; Writing - AY; Critical Review – AY and HU.
Conflict of Interest: No conflict of interest was declared by the author.
Financial Disclosure: The author declared that this study has received financial support from Atatürk University Scientific Research Project Unit (2013/237)

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Middle Black Sea Journal of Health Science December 2018; 4(3):20-25

RESEARCH ARTICLE

Maternal Vitamin D Deficiency and Insufficiency - Prevelance and Effective Factors

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Received: 26 October 2018, Accepted 09 December 2018, Published online: 27 December 2018

Abstract

Objective: We aimed to reveal the prevalences of vitamin D deficiency and severe deficiency in pregnant women who applied for routine examination in our study.

Methods: Between January 2015 and January 2018, 635 healthy pregnant women who applied to our clinic for the first trimester routine pregnancy examination were included in the study. The age, place of residence, season in which the material was taken, 25 - OH vitamin D levels were reached. Vitamin D level under 10 ng/ml was accepted as severe deficiency, 10 - 30 ng/ml insufficiency and 30 - 100 ng/ml normal.

Results: Vitamin D deficiency was detected in 58.1 % and severe deficiency was found in 36.9 %, while vitamin D levels were normal in only 5 % of the patients. Vitamin D levels were significantly lower in the 35 years old group (95.8 % - 90.9 %) (p = 0.03). There was no statistical correlation between the place of residence and vitamin D (94.6 % - 95.7 %) (p = 0.529). We found abnormal vitamin D results in winter and autumn season (98 % - 99.4 % versus 89.4 % - 93.3 %) (p = 0,000).

Conclusion: We observed that vitamin D deficiency and severe deficiency, which have serious maternal and neonatal effects, are quite common in our society (95 %). We are in the belief that how much decrease in negative maternal and perinatal outcomes have been observed with the 'Vitamin D Supplement Programme in Pregnancy' hold by T.C. Ministry of Health, via using 1200 IU vitamin D perday from 12 week of pregnancy until postpartum 6th month, by cross-sectional studies and if the first 6 year results are in positive direction, deficiency must be replaced according to age, season, and the geographic region with more effective methods.

Key words: Vitamin D, pregnancy, prevalence

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DOI: 10.19127/mbsjohs.474947

Introduction

Vitamin D is one of the fat-soluble vitamin and is first named in 1920. It is nowadays known as a steroid hormone, not a classical vitamin because it can be synthesized endogenously in the appropriate biological medium, Although it is known that the most important effect is on calcium-phosphorus metabolism and bone mineralization, it is also known to be associated with cancer (such as breast, ovarian, endometrium, gastrointestinal tumors, lymphoma), cardiovascular diseases, metabolic syndrome (hypertension and diabetes), infectious diseases, autoimmune diseases (multiple sclerosis, arthritis), musculoskeletal diseases (osteoarthritis), which are associated with many health problems

(Barrett and McElduff, 2010; Iyidir and Altınova 2012).

Vitamin D is known to be synthesized in the skin by the effects of ultraviolet (UV) rays in 90 - 95 %. Dietary intake of fatty fish species, fish oil and vitamin D-containing preparations have little effect on vitamin D levels in the body (Fidan et al., 2014). Ergocalciferol (vitamin D2) is derived from plant-derived ergosterol, and 7 - dehydrocholesterol (provitamin D3) is cholecalciferol (vitamin D3). Due to its biomolecular structure, the biological activity of vitamin D2 is 3 to 10 times lower than that of vitamin D3. Vitamin D, formed by epidermis with UV effect, binds to vitamin D binding peptide (DBP) and transported to the liver. In the liver by 25 alpha hydroxylation is converted to 25 - OH vitamin D (calcidiol). Although 25 - OH vitamin D is not biologically active, it is very important to show the level of vitamin D in the body thanks to its long half - life of 2 - 3 weeks. The 25 - OH vitamin D is converted to 1,25 - OH vitamin D (calcitriol), a biologically active form, by passing through the circulation and undergoing 1 alpha hydroxylation in the kidney under parathormone (PTH) control. 1,25 - OH vitamin D has a half - life of 4 - 6 hours and can not be used for measurement. Parathormone, hypocalcemia/phosphatemia, increases active metabolite conversion (Bikle, 2007; Meer et al., 2011; Mallah et al., 2011).

In order for fetal bone mineralization to take place in a healthy way during pregnancy, comprehensive adaptations to vitamin D and hence calcium metabolism occur. Approximately 25 - 30 grams of calcium passes through the fetus during pregnancy. Mother does that calcium balance which in favor of fetüs by increasing calcium absorption from the mouth (the most important effect), by increasing the kidney calcium uptake and by increasing calcium mobilization from the bone. In pregnancy placenta, amnion, umbilical cord, decidua, breast and fetal parathyroid secrete PTH-related peptide (PTHrP) and the amount increases as pregnancy progress. Renal calcitol synthase increases with estrogen, prolactin, human chorionic somatotropin in addition to PTHRP (Kovacs and Kronenberg, 1997; Kent et al., 1991; Kovacs and Kronenberg, 2006).

Maternal vitamin D has been shown to be effective in fetal bone development as well as in dental, neuronal and fetal growth. In addition, normal vitamin D levels have been shown to reduce the incidence of diabetes (Pludowski et al., 2013).

We have seen in recent years that vitamin D deficiency has been studied in more detail in terms of causing severe maternal and neonatal problems, and in many health policies, strategies to prevent deficiency of maternal vitamin D (Kiely and Hemmingway, 2017).

We aimed to emphasize the importance of determining the prevalence of maternal vitamin D deficiency and its treatment

Methods

Ordu Provincial Health Directorate and Ordu University Medical Faculty Training and Research Hospital Clinical Practice Ethics Committee approvals were obtained (Date:26/04/2018, Number: 2018-87). Between January 2015 and January 2018, 635 healthy pregnant women who applied to the obstetrics clinics for the first trimester routine pregnancy examination were included in the study. Those who have chronic disease and / or metabolic disease, who use drugs effective on bone metabolism, are excluded from the study. Patients' age, place of residence, 25 - OH vitamin D levels obtained using Architect System, and the season in which the material was received were obtained from our hospital registry system.

25 - OH vitamin D level was < 10 ng/ml, severe deficiency, 10 - 30 ng/ml insufficiency and 30 - 100 ng/ml were normal. Patients were divided according to age groups as under and over 35; according to residence site as rural and urban; and to the season in which materials obtained as spring, summer, autumn and winter.

Statistical analysis of the data was performed using the SPSS 20 program. Student's t-test was used to compare numerical data, chi-square and Fisher Exact test were used to compare continous data. A P value < 0.05 was considered statistically significant.

Results

The mean age of pregnancies was 28.9 $\pm$ 5.34 (18 - 40). 66.8 % of the patients were urban, and 33.2 % were living in the rural area.

25 - OH vitamin D insufficiency and severe deficiency were found to be 58.1 % (369/635) and 36.9 % (234/635) respectively, while only 5 % (32/635) of the patients was normal for vit D levels. Vitamin D insufficiency, severe deficiency and normal prevelances is showen in Table 1.

Table 1: Vitamin D insufficiency, severe deficiency and normal prevelances.

Insufficiency	369/635	58.1 %
Severe Deficiency	234/635	36.9 %
Normal	32/635	5 %

Patients were divided into two groups as < 35 years and > 35 years according to age groups. The abnormal 25 - OH vitamin D results were found to be 95.8 % in the < 35 age group and > 90.9 % in the 35 years' age group. 25 - OH vitamin D level was significantly lower in the < 35 years' age group (p = 0.03). Abnormal vitamin D ratio -age related is showen in Table 2.

Table 2: Abnormal vitamin D ratio - age related

Age group	Abnormal patient number	Abnormal patient ratio
<35	503	95.8 %
>35	**100**	**90.9 %**

The rate of abnormal 25 - OH vitamin D values in urban and rural population was found to be close to each other (94.6 % - 95.7 %). There was no statistical correlation between the place of life and 25 - OH vitamin D values (p = 0.33). Abnormal vitamin D ratio - residence related is showen in Table 3.

Table 3: Abnormal vitamin D ratio - residence related

Place of residence	Abnormal patient number	Abnormal patient ratio
Urban	401	94.6 %
Rural	**202**	**95.7 %**

The abnormal 25 - OH vitamin D results were ranked according to the seasons as follows; Summer 89.4 % - Spring 93.3 % - Winter 98 % - Autumn 99.4 %. We found that abnormal 25 - OH vitamin D results were more common in winter and autumn (p = 0,000). Abnormal vitamin D ratio - season related is showen in Table 4.

Table 4: Abnormal vitamin D ratio - season related

Season	Abnormal patient number	Abnormal patient ratio
Summer	147	89.4 %
Spring	**154**	**93.3 %**
Winter	**144**	**98 %**
Autumn	**158**	**99.4 %**

Discussion

Maternal vitamin D deficiency; composses the risk of vitamin D deficiency in neonates and also risk for infantile rickets. It is also emphasized that pregnancy is a critical period and fetal effects continue all the life time when the effects of vitamin D on fetal bone and neuronal development is taken into account. Complications such as low birth weight, skeletal problems, neonatal hypocalcemia, immunodeficiency, and type 1 diabetes in the newborn can occur in the newborn while poor results such as maternal sub/infertility, preclampsia, gestational diabetes and increased cesarean rate occur due to lack of vitamin D in the mother.

Vitamin D measurement is biochemically challenging. Different results can be obtained with different methods of analysis, with more than 10 %. Immunoassay (radio, manual, automated) and LC-MS / MS (liquid chromatography-tandem mass spectrometry) are the most widely used methods. It is possible to perform healthier prevalence studies by standardizing the methods used worldwide (Ferrari et al., 2017).

We have taken the thresholds for our study according to a report published by the Ministry of Health in 2011. According to this report, 25 - OH D levels of 30 ng / ml are considered as threshold values in adults, which are not considered to cause parathyroid hormone elevation. Values between 10 and 30 ng / ml are considered to be inadequate and values below 10 ng / ml are considered to be severe.

The average maternal vitamin D concentration ranged from 5.2 to 52 ng / mL (13 to 130 nmol / L) according to a review, which included studies from 1959 to 2014. Globally, vitamin D deficiency is present in 54% of cases (threshold value of 20 ng / mL) and severe vitamin D deficiency is in 18 % (threshold value of 10 ng / mL) (Rajneeta et al., 2016).

In the study of USA, Australia, Middle East and South Asia, where the threshold values were 30 ng / ml for failure and 20 ng / ml for severe deficiency, the prevalence of vitamin D severe deficiency was 26-98%, while the prevalence of insufficiency was 66-100% (Daphna, 2011). These data show how much the thresholds affect the prevalence.

The results of a meta-analysis of 121 studies conducted between 1962 and 2009 were published by the World Health Organization in six geographical regions (Asia, Europe, Africa, Latin America, North America and Australia). According to the report, the prevalence of vitamin D deficiency

in the world (at a cut-off value of 30 ng / mL) ranges from 2 to 97 %. Regionally, it is found that the severe deficiency is a more important problem in Asia (18 - 48 %) and the Middle East (9 - 80 %) (Mithal et al., 2009).

As many studies exist in Turkey related to vit D levels containing children, adolescent, postmenapausal patients, we found no studies demonstrating the prevalence D deficiency.

In all studies, it is possible to reach the conclusion that vitamin D deficiency is more common in some regions but it is a worldwide health problem and affects the pregnant / newborn population more frequently (Mithal et al., 2009; Daphna, 2011; Rajneeta et al., 2016).

In our study, vitamin D insufficiency was found in 58.1 % and severe deficiency was found in 36.9 %. Normal vitamin D levels were detected in only 5 % of the patients. These data show that our threshold value and the proportion of studies conducted in our region are close to each other, and maternal vitamin D deficiency is a very important public health problem.

Geographical factors are very effective in vitamin D metabolism. Many factors affect the efficiency of the sun's rays, such as cloud density, ozone layer density, air pollution level, altitude, time / duration of sun exposure, and social clothing style. In the North American study, it has been shown that 5 to 15 minutes of sunshine everyotherday, face and arms exposed to the sun are important to meet the need for vitamin D (Hollick, 1996). On the one hand, modern business life, urbanization and industrialization are obstacles to the duration and quality of exposure to the sun. On the other hand, the conservative society that condemns women to their homes and the low socioeconomic level also affect the production of vitamin D in a poor way (Cidem et al., 2013).

In our study, the incidence of abnormal vitamin D in the urban and rural population was found to be close to each other (94.6 % - 95.7 %). There was no statistically significant correlation between the place of life and vitamin D levels ($p = 0.529$). So that points out the importance of other contribiutor factors like personal factors.

Seasonal differences with geographical factors are also important. In the north and south of 33 degrees' latitude, it is shown that there is no vitamin D synthesis due to the oblique angle of incidence of sun rays in winter. The closer to the equator region, the seasonal difference disappear (Wacker and Holick, 2013). As in many studies, a recently published Chinese study has also shown that vitamin D deficiency is more frequent in winter and autumn (Yuan-Hua et al., 2018). In our study, we also tried to reveal the correlation between vitamin D and seasons. The incidence of vitamin D deficiency was as follows in the seasons; summer 89.4 % - spring 93.3 % - winter 98 % - autumn 99.4 %. We have also found that vitamin D deficiency is seen higher in winter and autumn, as in other studies. ($p = 0, 000$)

Depending on personal factors such as dark skin pigmentation, advanced age (> 70), malabsorption (such as cystic fibrosis, Crohn's disease, gluten enteropathy), liver / kidney disease and topical sun cream (especially > 30 factors), vitamin D levels differ (Dror and Allen, 2010). We investigated the effect of age on vitamin D levels in our study. Vitamin D deficiency was found to be 95.8 % in the < 35 age group and > 90.9% in the 35 years' age group. Thus, in the < 35 years' age group, we found significantly more vitamin D deficiency ($p = 0.03$).

The review published in 2014 does not fully support routine vitamin D supplementation in pregnancy. It is argued that routine vitamin D monitoring in pregnancy is more accurate in providing support to patients with low levels (Weinert and Silveiro, 2015). In studies dealing with dietary regulation other than vitamin D support, it has been shown to be useful to question dietary habits and to take note of body mass index during early prenatal referral (Milman et al., 2016).

In addition, many organizations recommend routine vitamin D supplements without regard to vitamin D levels. The American College of Obstetricians and Gynecologists (ACOG), the Endocrine Society, and the Institute of Medicine (IOM) recommend 600 IU / day vitamin D for pregnancy (Institute of Medicine, 2011; Holick et al., 2011; ACOG Committee on Obstetric Practice, 2011). In addition, the Nordic Council of Ministers (NORDEN) and the Scientific Advisory Committee on Nutrition (SACN) offer lower dose (400 IU / day) support (Nordic Council of Ministers, 2012; Scientific Advisory Committee on Nutrition, 2016). T. C. On May 2011, the Ministry of Health announced 1200 IU / day vitamin D from the 12th week of pregnancy to the 6th month of postpartum in the 'Gebelere D Vitamine Support Program' report (Republic of Turkey Ministry of Health, 2011). In addition, IOM has been reported to be acceptable for vitamin D, with nontoxic dose of 4000 IU / day (Institute of Medicine, 2011). According to a newly published metaanalysis that examines the preterm

birth risk and newborn outcomes of vitamin supplementation, vitamin D levels < 30 ng /mL have an 11 % increase in the risk of preterm labor. Vitamin D supplementation can reduce the risk of preterm labor and associated perinatal mortality (Amegah et al., 2017).

Because of the different measurement techniques used to measure vitamin D levels, it is difficult to define the threshold value for optimal bone health. For this reason, there is a need for work done with standard reference methods adapted to the whole world that can accurately and reliably measure 25 - OH D levels. Different definitions of deficiency and insufficiency in the literautre also makes it difficult to compare the vitamin D deficiency and insufficiency (Ruddersa and Camargo, 2015).

Conclusion

In our study, we observed that vit D deficiency which having maternal effects such as preclampsia, preterm labor, abortus, early osteoporosis and neonatal effects like rickets, tetany, hypocalcemic convulsions, congenital cataracts, and severe shortage, is in a high percentage in our society (95 %). Considering that this high prevalence is related to limited sunlight exposure and dietary factors, we suggest that dietary supplementation and vitamin D supplementation should be appropriate. In addition, the importance of sunbathing and patient education about dietary content can also contribute to treatment.

We concluded that in which ratio vit D treatment decreases perinatal and maternal adverse outcomes is to be evaluated with cross-sectional studies and if the results of that 6 years are positive, it would be improved by more effective methods by taking into consideration the age group, season and geographical region.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University Medical Faculty.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept-D.D.K.; Design-D.D.K.; Supervision- D.D.K.; Funding- D.D.K.; Materials- D.D.K.; Data Collection/Data Process-D.D.K.; Analyze or Comment- D.D.K.; Literature Scanning- D.D.K.; Writer of Paper- D.D.K.; Critical Review- D.D.K.
Conflict of Interest: No conflict of interest was declared by the author.
Financial Disclosure: The author declared that this study hasn't received no financial support.

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Middle Black Sea Journal of Health Science December 2018; 4(3):26- 33

RESEARCH ARTICLE

Factors Affecting Bone Mineral Density in Hemodialysis Patients

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Received: 17 December 2018 Accepted: 20 December 2018, Published online: 27 December 2018

Abstract

Objective: Disorders of mineral metabolism and bone density are common complications in chronic kidney disease and are an important cause of morbidity. Recently used definition is chronic kidney disease-mineral and bone disorder (CKD-MBD). The aim of our study was to evaluate the correlation between bone mineral density and influencing factors in patients with end-stage renal failure undergoing hemodialysis.

Material and Methods: In our study, cases were evaluated by being divided into 3 groups depending on bone mineral density (BMD). Our study included 124 cases and was designed as a cross-sectional observational study. The demographic data of the cases were recorded separately for each case. Routine biochemical analyses were studied.

Results: The median vit D value of the patients with osteoporosis participated in the study was 14.44 mg/dl in the osteopenic group and the median value of the patients without osteoporosis was 20.14 mg/dl. The lowest and highest vit D values of the patients with osteoporosis were 3 mg/dl and 34.77 mg/dl, respectively. There was a statistically significant difference between all 3 groups for the age variable (p=0.002). There was a statistically significant difference between all 3 groups for the BMI variable (p=0.011). For 3 groups divided according to BMD measurements, statistically significant results were found in the PTH, Ferritin, Hgb, CRP, ALP, Albumin, e-GFR, hip and lumbar BMD values, respectively (p<0.001, p=0.001, p=0.004, p=0.001, p=0.003,p=0.005, p=0.001, p<0.001, p<0.001).

Conclusion: In conclusion, our study revealed that the most important risk factor associated with osteoporosis in patients undergoing hemodialysis was PTH elevation and low vitamin D levels. For this purpose, BMD measurements and biochemical parameters of CKD patients undergoing hemodialysis should be studied in appropriate periods by adhering to the guidelines. Vit D replacement should not be neglected in order to avoid osteoporosis and to treat the detected cases.

Key words: Chronic kidney injury, hemodialysis, bone mineral density, vitamin D

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DOI: 10.19127/mbsjohs.498264

Introduction

Disorders of mineral metabolism and bone density are common complications in chronic kidney disease and are an important condition worsening the quality of life and morbidity. There is evidence that these disorders in mineral and bone metabolism are associated with increased risk for cardiovascular calcification, morbidity and mortality (Block and Cunningham 2006). The term used to define abnormalities in bone morphology developing in CKD is renal osteodystrophy (Freemont and Malluche, 2005). Recently used definition of chronic kidney disease-mineral and bone disorder (CKD-

MBD) is evaluated as a systemic disease characterized by biochemical abnormalities (calcium, phosphate, parathyroid hormone (PTH) and vitamin D), abnormalities in bone turnover and extraskeletal calcification. Secondary hyperparathyroidism is an important condition predicating biochemical abnormalities for CKD-MBD (Quarles and Berkoben, 2018). Routine measurement of serum levels of calcium, phosphate and PTH is the most practical way to monitor secondary hyperparathyroidism (Ketteler et al., 2017).

Dual-energy x-ray absorptiometry (DXA) of the spine, hip and forearm is the only technique for the diagnosis of osteoporosis in the absence of a fragility fracture and is the best technique to monitor changes in BMD over time for a number of reasons (Cosman et al.,2014). The World Health Organization (WHO) classification is still widely used to determine bone density. The WHO has defined the diagnostic thresholds for low bone mass and osteoporosis according to the T-score of BMD measurements compared to the young adult reference population (Report of WHO study Group,1994).

Since osteoporosis is an important cause of mortality and morbidity in CKD, it is important to determine the risk factors. Appropriate renal replacement therapy should be determined and osteoporosis should be avoided in patients who are at risk in this respect. These patients should also be examined and treated properly for osteoporosis. Protecting these patients from osteoporosis is very important.

The aim of our study was to evaluate the correlation between bone mineral density and influencing factors in patients with end-stage renal failure undergoing hemodialysis. In addition, it was aimed to investigate the prevalence of osteoporosis in CKD patients in our region.

Methods

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of the Ordu University, Faculty of Medicine (Date:29.11.2018 Decision Number: 2018/238). Volunteer consent was obtained from all patients participated in our study.

Our study included 124 cases and was designed as a cross-sectional observational study. The demographic data of the cases (age, BMI, gender) were recorded separately for each case. Hemogram was studied with a Cell-dyn ruby device. Routine biochemical analyses (serum BUN, creatinine, albumin, potassium, calcium, CRP, iron, uric acid) were studied with a Cobas c501 module, hormones (folate, vitamin B12, vitamin D, ferritin, PTH) with a Roche-cobas e 601 device. Bone densitometry was measured with a hologic SQ-15882 device using the dual-energy x-ray absorptiometry (DXA) technique.

Body mass indexes (BMI) of all cases were calculated with the formula of BMI=weight (kg)/height $(m)^2$. The cases were divided into 3 groups according to the BMD criteria of the world health organization. T score above -1 was considered as normal, T score of (-1) to (2.5) was considered as osteopenia and T score below -2.5 was considered as osteoporosis. The healthy participants did not have any known disease and were admitted to our outpatient clinic for a check-up. There were no biochemical abnormalities in their laboratory tests and no hematuria and proteinuria in their urinalyses. The estimated glomerular rate (e-GFR) of healthy subjects was calculated using the MDRD equation. All of them had an e-GFR of >60ml/min/1.73m2. The patients receiving hemodialysis treatment were undergoing hemodialysis 3 days a week for at least 3 months.

Our exclusion criteria were as follows: patients with active infection/inflammation, patients with malignancy, peritoneal dialysis patients, pregnant women and patients who did not want to participate in the study.

Statistical Analysis:

The data were analyzed using the IBM SPSS v.23. The normality assessment of the data was analyzed by the Shapiro Wilk test. The Kruskal-Wallis test was used for the comparison of non-normally distributed data between 3 groups. Values showing normal distribution were analyzed by the one-way analysis of variance (ANOVA). The analysis results were presented as mean ± standard deviation for data showing quantitative and normal distribution, and as median (min-max) for data not showing normal distribution. The significance level was accepted as $p<0.05$.

Results

One-hundred and twenty-four patients were included in our study, 70 (56.5%) were female and 54 (43.5%) were male. Of the cases, 35 (28.22%) were normal, 51 (41.12%) were osteopenic and 38 (30.64%) were osteoporotic according to the BMD measurement results. Of the patients undergoing hemodialysis, 26 (45.6%) had osteoporosis, 21 (36.8%) had osteopenia and 10 (17.5%) had normal

BMD. The mean age of the patients was 58.84 and there was no statistically significant difference between the mean ages of the groups. For each of the 3 groups, statistically significant results were found in the PTH, Ferritin, Hgb, CRP, ALP, Albumin, e-GFR, hip and lumbar BMD values. No statistically significant results were found in the Ca, P, uric acid, folic acid and B12 values for each of the 3 groups. The descriptive statistical values of the cases are presented in Table 1.

Table 2 shows the gender distribution of all cases (HD and healthy volunteer patients).

There was no significant difference between the healthy participants and HD group in terms of gender (p=0.06)

The descriptive statistical values of the 3 groups according to the T score (normal, osteopenic and osteoporotic) are presented in Table 3.

The median vit D value of the patients with osteoporosis participated in the study was 14.44 mg/dl in the osteopenic group and the median value of the patients without osteoporosis was 20.14 mg/dl. The lowest and highest 25 OH vit D values of the patients with osteoporosis were 3 mg/dl and 34.77 mg/dl, respectively. There was a statistically significant difference between all 3 groups for the age variable (p=0.002). There was a statistically significant difference between all 3 groups for the BMI variable (p=0.011). There was a statistically significant difference between all 3 groups for the total hip BMD variable (p<0.001). Likewise, there was a statistically significant difference between all 3 groups for the total lumbar BMD variable (p<0.001). Again, there was a statistically significant difference between all 3 groups for the creatine, e-GFR, albumin, ALP variables (p values: p = 0.006, p = 0.001, p = 0.005, p = 0.003, respectively). Likewise, there was a statistically significant difference between all 3 groups for the PTH and ferritin variables (p values: p<0.001, p=0.001, respectively). Again similarly, there was a statistically significant difference between all 3 groups for the CRP, Hgb variables (p values: p=0.001, p=0.004, respectively). There was no statistically significant difference for the Ca, P and uric acid variables (p values: p=0.322,p=0.332,p=0.274, respectively).

The correlation between the BMD measurements and variables according to the Spearman's rank correlation analysis is presented in Table 4.

Table 1: The descriptive statistical values of all cases

	Mean ± std	**Min-Max**	**P value**
Total Hip BMD	0.865±0.186	0.446-1.354	p<0.001*
Total Lumbar BMD	0.975±0.175	0.383-1.406	p<0.001*
e-GFR (ml/min/1.73 $m^{2)}$	48.95±41.223	4-146	p=0.001*
Albumin (g/dl)	4.272±0.465	2.7-5.3	p=0.005*
ALP	98.78±63.11	31-442	p=0.003*
Ca	9.534±0.72	7.9-11.5	p=0.234* ^{NS}
P	3.915±1.252	0.6-10.5	p=0.332* ^{NS}
CRP	1.248±2.872	0-24.05	p=0.001*
Uric acid	5.541±1.478	1.7-10.2	p=0.274* ^{NS}
Hgb (g/dl)	12.224±1.858	7.6-19.4	p=0.004*
Folic acid	7.39±5.04	0.6-20	p=0.619* ^{NS}
B12	4.36±357.228	96.57-2000	p=0.389*^{NS}
Ferritin	5.623±602.882	6.4-2000	p=0.001*
PTH	1.489±188.83	2.85-1045	p<0.001*
Vit D	17.086±8.851	3-41.35	p=0.002*
Age	58.84±13.318	22-84	p=0.402*^{NS}
BMI	29.972±6.495	17.08-52.21	p=0.001*

(*): Kruskal-Wallis test NS: Non-Significance

Table 2: The gender distribution of the cases

Gender	**Healthy Participant**	**Hemodialysis**	**Total**
Female	43 (61.4%)	27 (38.6%)	70
Male	24 (44.4%)	30 (555.6%)	54
Total	67 (54%)	57 (46%)	124

Table 3: The descriptive statistical values of the 3 groups according to the T score

	Mean ± std	Min-Max	P value
Total Hip BMD			
Normal	1.050±0.110	0.81-1.246	p<0.001[µ]
Osteopenia	0.858±0.108	0.643-1.119	
Osteoporosis	0.699±0.138	0.446-0.999	
Total Lumbar BMD			
Normal	1.115±0.966	0.933-1.307	p<0.001 [µ]
Osteopenia	0.953±0.94	0.787-1.139	
Osteoporosis	0.811±0.158	0.383-1.134	
Ferritin			
Normal	386.307±508.398	6.4-1433	p=0.001 [µ]
Osteopenia	552.423±606.130	8.18-2000	
Osteoporosis	890.621±641.525	7.08-2000	
PTH			
Normal	99±103.747	27.28-475.4	p<0.001 [µ]
Osteopenia	143.393±195.957	2.85-961	
Osteoporosis	212.583±217.785	26.38-1045	
Vit D			
Normal	19.491±7.75	4.68-34.84	p=0.002 [µ]
Osteopenia	17.556±10.083	3-41.35	
Osteoporosis	14.316±7.278	3-34.77	
BMI			
Normal	31.023±6.410	20.35-52.21	p=0.011 [µ]
Osteopenia	30.517±6.417	20.70-45.52	
Osteoporosis	27.513±6.685	17.08-42.5	

Table 4: The correlation between the BMD measurements and variables according to the Spearman's rank correlation analysis

	r	p
Age	0.25	P=0.005*
BMI	-0.254	P=0.005*
Creatinine	0.274	P=0.002*
e-GFR	-0.33	P<0.001***
Albumin	-0.287	P=0.001**
ALP	0.295	P=0.001**
Ca	-0.134	P=0.144[NS]
P	0.037	P=0.688[NS]
CRP	0.318	P<0.001***
Uric acid	-0.147	P=0.108[NS]
Hgb	-0.284	P=0.002[*]
Folate	-0.81	P=0.387[NS]
B12	-0.093	P=0.318[NS]
Ferritin	0.319	P<0.001***
PTH	0.331	P<0.001***
Vit D	-0.326	P<0.001***

(*): According to the Sperman's rank correlation analysis p<0.05
(**): According to the Sperman's rank correlation analysis p=0.001
(***): According to the Sperman's rank correlation analysis p<0.001
NS: Non-significance

When the correlation between the variables were analyzed according to the Sperman's rank correlation analysis and BMD measurement results, there were significant correlations between many parameters. There was a positive statistically significant correlation between the age and BMD results. In other words, as age increased, the risk of osteoporosis increased (r=0.25, p=0.005). As creatinine clearance decreased, the risk of osteoporosis increased (r=-0.33, p<0.001). Likewise, as the albumin value decreased, the risk of osteoporosis increased (r=-0.287, p=0.001). As the level of alkaline phosphatase increased, the rate of osteoporosis increased (r=0.295, p=0.001). There was a positive correlation between the CRP and ferritin levels and osteoporosis risk (r=0.318, p<0.001, r=0.319, p<0.001). In terms of the risk of developing osteoporosis, we found a positive correlation with PTH and a negative moderately significant correlation with vit D level (r=0.331, p<0.001, r=-0.326, p<0.001). It can be said that the strongest risk factor for the development of renal osteodystrophy was PTH elevation and decreased vit D level in our study.

Discussion

Basically, the bone cells have a less vitality in CKD patients than in normal individuals due to increased uremic toxins and insulin resistance. Uremic osteoporosis describes qualitative bone loss in normal bone structure due to uremic toxins. As renal failure progresses, metabolic acidosis and hyponatremia also cause bone loss (Posa et al., 2016). Disorders of the mineral metabolism in regard to Ca, P, Mg, PTH and vit D metabolism are common in chronic kidney disease. These abnormalities also affect the skeleton as much as other factors related to the uremic condition. Traditionally, the diseases associated with these are considered as bone lesions and the bone-related findings are defined as renal osteodystrophy. However, recent evidence suggests that mineral disorders also play an important role in the pathogenesis of non-skeletal calcifications and result in vascular calcification and cardiovascular complications and mortality. It is defined as mineral and bone disorders in chronic kidney disease (CKD-MBD: chronic kidney disease-mineral and bone disorder) to describe this broad pathological spectrum including both bone and other findings. CKD-MBD is associated with extensive biochemical and clinical disorders (Tominaga et al., 2007; Defechereux and Meurisse, 2009). Vascular calcification, bone abnormalities develop due to the effect of these. This extensive clinical syndrome leads to cardiovascular disease, fracture, decrease in quality of life and mortality, as a result of these underlying pathological developments (Aksu et al., 2005).

CKD-related bone mineral disorders are a major health problem. The prevalence of osteoporosis varies depending on CKD stages. Fractures in early-stage CKD patients (stage 1 - 3a CKD) are more similar to traditional osteoporosis. However, most patients with stage 4 or 5 CKD have a certain degree of low bone mineral density (BMD) and/or a certain level of CKD-MBD. Osteoporosis, which is one of the causes of CKD-MBD, has been found in 53% of patients with CKD (Festuccia et al., 2017). In our study, osteoporosis was found in 26 (45.6%) of the patients under hemodialysis, similar to the rates in the literature.

In the study by Onat SS et al., (2013) there was a statistically significant difference between the age groups and femur and lumbar T score, and as the age progressed, the T score worsened. In the study by Aslan A et al., (2013) a negative correlation was found between age and BMD. In the study of global osteoporotic fracture burden by Johnel and Kanis et al., (2006) it was shown that hip fracture peaked between the ages of 75 and 79 in males and females, but the highest age range was 50-59 years for all fractures, and that the rate of hip fracture decreased after the age of 80. In our study, there was a positive significant correlation between age and BMD results. As age increased, bone mineral density decreased; in other words, as age increased, the risk of osteoporosis increased. Our study is consistent with the study by Onat S and Aslan A et al. from this aspect. Our study is not consistent with the study by Johnel and Kanis et al., (2006) in terms of the risk of hip fracture due to osteoporosis after the age of 80, and we are of the opinion that this is due to the fact that only BMD measurement was made in our study, whereas the study by Johnel O et al. was at the same time about the determination of fractures.

Vitamin D is a hormone required for normal bone formation and mineralization, and plays a critical role in bone biology (Posa et al ., 2016). In secondary hyperparathyroidism, vit D also plays an important role with other changes in mineral metabolism. Vit D deficiency is common at advanced stages of CKD, and BMD is positively correlated with serum 25 (OH) D concentration (Elder et al., 2006). When Heike A et al. investigated the correlation between vitamin D and BMD in various races and age groups, they found a positive correlation between vit D and BMD in all races and age groups, especially in the white race (Bischoff-Ferrari et al., 2004). Collins et al. (1998) found a similar positive correlation between hip BMD and vit D, Fradinger and Zanchetta (2001) between femur BMD and vit D. In numerous studies, there was a positive correlation between vitamin D and BMD. In our study, we also found a significant decrease in the bone densitometers of the patients with low vitamin D levels.

In terms of impaired calcium and phosphate balance in CKD, hypersecretion of parathyroid hormone is initially beneficial for the body, but as the process progresses, elevated PTH levels lead to increased osteoclastic activity and bone resorption. Mandiroglu S et al. found a negative correlation between intact PTH (iPTH) and BMD in hemodialysis patients (Mandiroglu et al., 2013). Likewise, Hutchison et al. found that iPTH values were significantly higher in hemodialysis patients with severe osteitis fibrosa (Hutchison et al., 1993). In our study, we also found that PTH levels were

significantly higher in osteopenia and osteoporosis groups compared to the patients with normal BMD measurements. Compared with other studies, we found that PTH was high in both osteoporosis and osteoporosis group in our study. The most important factor in terms of the risk of developing osteoporosis was found to be PTH elevation and low vitamin D levels in our study. This is due to the fact that our Black Sea region is less sunny and very cloudy and rainy throughout the year.

In the study by Oh et al., (2017) it was shown that low Hb levels or anemia findings did not imply BMD loss, but patients with anemia as a phenotype of various diseases other than typical iron deficiency anemia were more likely to have BMD loss. Therefore, BMD assessment may be significant in patients with underlying disease and anemia findings. In a study by Huang et al., (2009) the factors affecting osteoporosis in patients undergoing hemodialysis were investigated. According to the results of this study, body weight, body mass index, gender and postmenopausal or amenorrheic status were found to be important for osteoporosis. However, it was found that the hemoglobin level and age were not very effective. In our study, there was a negative correlation between hemoglobin and BMD in hemodialysis patients without iron deficiency anemia but with chronic disease anemia due to CKD. Our study was consistent with the literature. Unlike the study by Huang et al. (2009) Hgb level and age were also found to be significant risk factors for osteoporosis in our study.

In the study by Edwards et al. (2008) there was a significant correlation between low albumin levels and the risk of developing osteoporosis in CKD with hip fracture developed secondary to osteoporosis. Likewise, in our study, we found a significant correlation between low albumin levels and the risk of developing osteoporosis. We are of the opinion that the nutritional limitations of hemodialysis patients lead to decreased albumin levels. Our results are consistent with the literature findings.

In a study by Huang et al. (2015) investigating the correlation between Mg level and osteoporosis in CKD patients, there was a correlation between decreased Mg levels and osteoporosis. The authors also analyzed ALP levels. They found a correlation between elevated ALP level and osteoporosis development. In our study, we also found a significant correlation between ALP elevation and osteoporosis development. In this respect, our results are similar to the study results of Huang et al (2015).

Conclusion

In conclusion, we found that in our study, low vit D levels, as well as elevated PTH levels most important risk factor contributing to osteoporosis in patients with end-stage renal failure undergoing hemodialysis. It is important to determine the risk factors since osteoporosis is an important cause of mortality and morbidity in patients with end-stage renal disease. For this purpose, BMD measurements and biochemical parameters of CKD patients undergoing hemodialysis should be studied in appropriate periods by adhering to the guidelines. Vit D replacement should not be neglected in order to avoid osteoporosis and to treat the detected cases.

Patient Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University Medical Faculty. (Date: 29.11.2018 Decision Number: 2018/238).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept-A.K.; Design-A.K., E.C Supervision- A.K., E.C.; Funding-None Materials-A.K.; Data Collection/Data Process-A.K.; Analyze or Comment-E.C.; Literature Scanning-A.K.E.C; Writer of Paper- A. K, E.C Critical Review-E.C.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study hasn't received no financial support.

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Middle Black Sea Journal of Health Science December 2018; 4(3):34-38

CASE REPORT

Severe Pseudotumor Formation on an Asymptomatic Well Functioning Metal-On-Metal Total Hip Arthroplasty - A Case Report and Follow - Up

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Received: 09 October 2018 Accepted: 11 October 2018, Published online: 27 December 2018

Abstract
A 72-year-old patient who had metal-on-metal (MMO) total hip arthroplasty (THA) 7 years ago has developed a gluteal mass and pain. Plain radiographs showed a well-fixed THA on the left side with an increased acetabular inclination. Needle aspiration of the mass showed "milky stained" highly viscous liquid. After the approval of the patient, a revision total hip arthroplasty had been performed. There were severe necrosis and granulomatous type reactions all around the hip and the components. After extensive debridement, all the components were removed and a long-stemmed revision total hip arthroplasty with polyethylene insert was performed. We think that patients with MMO THA must be carefully reviewed after the surgery even if they are asymptomatic.
Key words: Metal-on-metal ,pseudotumor, ALVAL, total hip arthroplasty

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DOI: 10.19127/mbsjohs.468546

Introduction

Although metal-on-metal (MMO) total hip arthroplasty had become popular since the last decade, there is limited studies showing the long-term results. MMO is a choice in total hip arthroplasty with elimination of polyethylene wear debris and the possibility of using larger femoral head for better stability and range of motion. A variety of soft tissue reactions caused by metal-on-metal bearing surfaces had been described in earlier studies including ALVAL (aseptic lymphocyte-dominated vasculitis associated lesion) (Willert et al., 2001), pseudotumor (Pandit el al., 2008) and metallosis (Neumann et al., 2010). Herein, a case with a gluteal mass and pain associated with a MMO total hip arthroplasty was reported. Because this complication is rare, arthroplasty surgeons should be aware of this entity and how to diagnose and treat this condition.

Case

A 72-year-old male patient admitted to our outpatient clinic with a complaint of left groin and gluteal mass. He had a metal-on-metal total hip arthroplasty seven years ago in a different institution due to coxarthrosis on the left hip. After six years without any complaints some swelling had begun to occur slowly on his left buttock. Because the patient had minimal pain without any restrictions of daily activities patient didn't administered to any doctor. But the gluteal swelling had become noticeably large and severe pain especially at night had begun to disturb the patient.

On physical examination, there was a large gluteal and anterolateral groin mass without any erythema or skin lesion except the posterolateral surgical incision. The patient was able to walk without crutches with a mild limp on his left side. With palpation the mass was semi-solid and fluctuation was notable. There was no local temperature difference over the mass. Plain radiographs showed a well fixed uncemented total hip arthroplasty with an increased inclination of the acetabular cup and a large diameter femoral head (Figure 1).

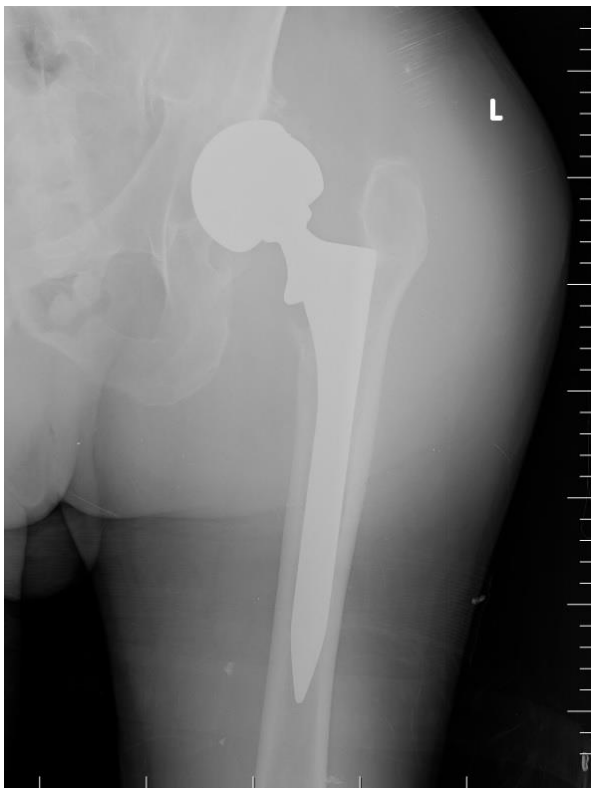

Figure 1. Total Hip Arthroplasty with a large diameter femoral head and an increased acetabular inclination

There was no evidence of loosening or implant failure of the components on the radiographs. WBC and CRP levels were normal but sedimentation was high. Needle aspiration of the mass showed "milky stained" highly viscous liquid. Microscopic examination and cultures including tuberculosis were negative. There was a large posterolateral cystic mass on the CT scan of the pelvis but no sign of osteolysis or aseptic loosening of the components (Figure 2).

Figure 2. CT of the hip showed a large gluteal mass around the prosthetic components

Left hip of the patient was functioning so well that he had hesitations about the necessity of the revision surgery. After detailed information given to the patient we performed a hybrid revision total hip arthroplasty to left hip through posterolateral incision. Massive "milky stained" fluid was drained after the incision of the fascia lata. There were severe necrosis and granulomatous type reactions all around the components, trochanteric region, gluteal muscles, pericapsular tissues and short external rotators (Figure 3).

Figure 3: Severe pseudotumor and necrosis around the components and hip

Multiple pathologic specimens were taken from involved areas. After intense debridement of necrotic tissues and irrigation acetabular component which is placed with increased inclination was removed. There were no osteolysis or bone deficiency around the acetabulum. After reaming and medialization of the acetabulum an uncemented acetabular component was inserted. There was no femoral component loosening but after reaming and medialization of the acetabular component, we decided to revise the femoral component. A cemented long femoral stem was placed with a bone cement with antibiotic. A 36 mm femoral head and a polyethylene insert was used. After the reduction of the hip the stability was good (Figure 4).

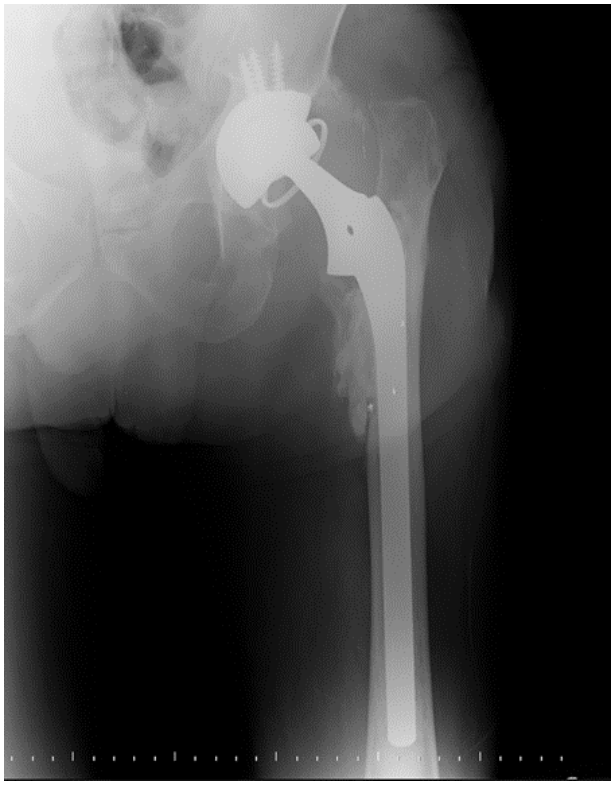

Figure 4: Revision arthroplasty with a longer stem and a polyethylene insert

Following intense debridement of necrotic tissues and irrigation incision was closed. Ten days after the surgery patient has suffered a posterolateral hip dislocation. Closed reduction under general anesthesia was successful but stability seemed to be poor, therefore we decided to replace the acetabular component. The acetabular component was replaced with a locked acetabular component. Weight bearing with a cane was allowed immediately on the first day of the surgery. Patient was reviewed at 5 months at the outpatient clinic. He was walking without crutches with a mild limp without pain on daily activities. Also, there were no evidence of gluteal mass or swelling and patient satisfaction was fairly enough.

Discussion

Metal-on-metal hip arthroplasties had become popular in past years for potential solution of polyethylene wear problems. Use of large diameter femoral heads in this type of articulations provides increased stability and range of motion. But nowadays, after various reports of periarticular soft tissue masses, increased plasma metal ion levels and early failure rates, there is a great debate about the

future of metal-on-metal bearing surfaces (Hallows et al., 2011; Smith et al. ,2012; Almousa et al., 2013).

A variety of soft tissue reactions caused by metal-on-metal bearing surfaces had been described earlier including ALVAL (Willert et al., 2001), pseudotumor (Pandit el al., 2008) and metallosis (Neumann et al., 2010). ALVAL is a histological diagnose characterized with infiltration of lymphocytes around pericapsular tissue while pseudotumors generally appears as periarticular soft tissue masses (Willert et al., 2001; Pandit el al., 2008). These periarticular soft tissue masses so called "pseudotumors" may be solid granulomatous or destructive cystic lesions of non-neoplastic origin (Daniel et al., 2012).

The prevalance of asymptomatic pseudotumors after metal-on-metal resurfacing arthroplasties had been reported by Kwon et al. was 4% with the use of ultrasound as the initial imaging modality (Kwon et al., 2011). According to a recent meta-analysis study, incidence of pseudotumor/ALVAL ranged from 0% to 6.5% of hips with a mean follow-up ranging from 1.7 to 12.3 years across the studies (Wiley et al., 2013). But with the use of MARS-MRI (metal artifact reduction sequence magnetic rasonance imaging) the prevalence of pseudotumors could be find as high as 60.9% or 61% according to some studies (Hart et al., 2009; Sutphen et al., 2015).

There is also debate about the pathogenesis of pseudotumors. Some studies show that pseudotumors are adverse tissue reactions to metal wear debris and raised metal ion levels (Pandit et al., 2008). Bosker et al. reported that patients with elevated serum metal ion levels had four times increased risk of developing a pseudotumor (Bosker et al., 2012). But some studies demonstrated that pseudotumors may be seen in patients with asymptomatic MOM arthroplasties (Kwon et al., 2011) and also with low serum metal ion levels (Matthies et al., 2012; Sutphen et al., 2015). Also, a recent study reported that there is no correlation between the presence of pseudotumor and major risk factors including metal ion levels, femoral head size, femoral offset, head neck taper length, acetabular component inclination angle, patient sex or age, body mass index, WOMAC or UCLA activity score (Bayley et al., 2015).

Our patient had an asymptomatic well-functioning hip for almost 6 years after the surgery. A gradual swelling occurred on his buttock and groin last year without any limitation of function and pain. This seems to be correlating with various reports reporting that pseudotumors can be found in asymptomatic MOM hip arthroplasties. (Hart et al., 2012; Almousa et al., 2013). Increased inclination of the acetabular component may be associated with severity of the pseudo tumor due to increase of metal wear debris. But as we mentioned before some studies reported that there is no correlation between acetabular component inclination and pseudotumor formation (Bayley et al., 2015).

Conclusion

It is important to investigate metal-on-metal arthroplasty patients whether they are symptomatic or not. Also, further studies needed to discover the factors associated with pseudotumors and pathogenesis of adverse tissue reactions.

Patient.Approval: Approval was received for this study from the patient.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – H.Ö.; Design- H.Ö.; Supervision- H.Ö., S.A.; Funding- None.; Materials- H.Ö.; Data Collection/Data Process- H.Ö.; Analyze or Comment- H.Ö.; H.Ö., Literature Scanning- H.Ö; H.Ö.- Writer of Paper-HÖ.; Critical Review-H.Ö .
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The author declared that this study hasn't received no financial support.

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Middle Black Sea Journal of Health Science December 2018; 4(3):39-42

CASE REPORT

A Rare Cause of Carbon Monoxide Intoxication: Hookah

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Received: 05 November 2018, Accepted: 26 November 2018, Published online: 27 December 2018

Abstract
Carbon monoxide is a gas formed by partial combustion of carbon containing fuels and leads to intoxication with various non-specific clinical findings. Although hookah is a common way of tobacco consumption among people living in the Middle East, Asia and Africa, it has become popular in European countries and United States of America (USA). In our country, hookah smoking is especially common among young people and more common in cafes. In our case report, two cases with carbon monoxide (CO) poisoning findings after smoking hookah. In both cases, non-specific clinical findings were observed after hookah smoking and blood carboxyhemoglobin fraction (FCOHb) levels were higher than normal values in laboratory parameters.
Key words: Carbon monoxide, hookah, intoxication

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DOI: 10.19127/mbsjohs.478663

Introduction

Carbon monoxide (CO) is a colorless, tasteless, odorless gas formed by partial combustion of carbon containing fuels and it has a 200 times greater binding affinity to hemoglobin than oxygen (Kesner et al., 2012). CO intoxication is one of the most common causes of morbidity and mortality in the world, which can be prevented and treated (Sircar et al., 2015). CO intoxication findings are seen in vital organs with high oxygen dependence such as central nervous system and cardiovascular system (Karaca et al., 2013). Suicidal or fire exposure related intoxications are more commonly reported in developed countries, whereas in our country, poisonings due to stoves, water heaters and combi boiler use are more common (Metin et al, 2011). The use of barbecues and hookah smoking in small areas with insufficient ventilation are rare causes of CO intoxication. Hookah is a common way of tobacco consumption among people living in the Middle East, Asia and Africa. Nowadays, it is becoming

more popular in European countries and United States of America. In our country, hookah smoking is more common among young people (Kocak et al., 2017). In our case report, we aimed to discuss two cases of CO intoxication with different clinical findings after hookah smoking and to draw attention to the risks of hookah smoking.

Case 1

A 37-year-old male was brought to the emergency department with presyncope. His general status was good, oriented and cooperative. Glasgow Coma Score (GCS) was 15, blood pressure was 120/80 mmHg, heart rate was 80/min (min) and respiratory rate was 22/min. In his detailed anamnesis, he described nausea, dizziness and faintness after smoking hookah in an outdoor café for about 1 hour. In his background story, there is no features other than smoking. He did not describe any illicit substance intake. Detailed physical examination revealed no abnormal findings. His 12-lead ECG was normal. Blood hemogram and biochemical values were normal. Cranial imaging of the patient did not show any abnormal findings. In the arterial blood gas analysis of the patient, carboxyhemoglobin fraction (FCOHb) was measured as 32.3% (0.5-1.5%). The patient was diagnosed as CO intoxication and normobaric oxygen (O2) therapy (at a rate of 10 lt/min) was started with a reservoir mask. After 12 hours of oxygen therapy, the FCOHb levels regressed to normal values and no impairment was observed in the patient's clinic. The patient was completely recovered and discharged from the emergency department.

Case 2

A 25-year-old male patient was admitted to the emergency department with headache, discomfort, numbness in the hands, nausea, chest pain and tachycardia. GCS was 15, blood pressure was 120/70 mmHg, heart rate was 118 / min and respiratory rate was 25/min. In the detailed anamnesis of the patient, he described hookah smoking in a café for about 40 minutes. The patient described that his complaints had begun after smoking hookah. His 12-lead ECG revealed sinus tachycardia. FCOHb was measured as 28.5% (0.5-1.5%) in the arterial blood gas. Other laboratory parameters were normal. The patient was diagnosed as CO intoxication and treated with normobaric O2 (at a rate of 10 lt/min). After 8 hours, FCOHb levels regressed to normal values and the patient whose clinical findings were stable discharged with full recovery. After 8 hours, FCOHb levels regressed to normal values and the patient whose clinical findings were stable discharged with full recovery.

Discussion

Although hookah is a common way of tobacco consumption among people living in the Middle East, Asia and Africa, it has become popular in European countries and United States of America (USA). Especially among the young-adults, the use in cafes has become widespread (Kocak et al., 2017). Hookah consists of various shapes, sizes, materials and colors. A typical hookah contains the following sections (Figure 1) (Karaca et al., 2013);

Figüre 1: Hookah

1. A pit head located at the top, where the tobacco is placed and usually burned with embers.
2. A jug or bottle of water to filter smoke.
3. The long body of the hookah that connects the head to the bowl and carries the smoke into the water through a tubular line.
4. Tube (hookah tube) that takes the smoke from the bottle and delivers it to the mouth

When a person inhales from hookah tube, the smoke draws from the reservoir and then passes through the water in the bottle and reaches the smoker. The water in the hookah, cools the smoke and filters some of the tar and some particles in the smoke (Ozkan et al., 2013). Water in the hookah, filters only a small part of the harmful substances. Longer duration of hookah smoking and deep

inhalation with less irritant effect of water moistened smoke causes more CO is exposed than cigarette (Shihadeh and Saleh, 2005). Also, due to the coal used to burn the hookah tobacco, the CO concentration increases (Knishkowy and Amitai, 2005). In addition, hookah is often smoked in indoor areas and this leads to CO level increase in area due to accumulation of smoke, and the O2 level decreases in the opposite direction. This increases the likelihood of CO intoxication for people who smoke hookah.

The clinical signs and symptoms of CO intoxication may be non-specific. The best method for detecting intoxication is clinical suspect. Although nonspecific symptoms such as fatigue, nausea, vomiting, headache, and dizziness are observed, loss of consciousness, seizures, cardiac arrhythmias, myocardial ischemia and even death may develop. The severity of toxicity is related to the current chronic diseases, advanced age and CO exposure time (Von Rappard et al., 2014; Ozkan et al., 2013).

After evaluating vital functions, the basis of treatment of CO intoxication cases is O2 support. In order to eliminate CO from circulation, normobaric or hyperbaric oxygen therapy can be used. While the half-life of CO in room air is 4-5 hours, it may decrease to 60 minutes with normobaric oxygen therapy and to 20 minutes with hyperbaric oxygen therapy. Treatment continues until patients become asymptomatic and until the level of blood FCOHb is lower than 10% (Kao and Nanagas, 2004; Yurtseven et al., 2015).

Conclusion

As seen in our cases, CO intoxication can be encountered in patients presenting with nonspecific symptoms after hookah smoking. Physicians should keep CO intoxication in mind in the differential diagnosis of patients presenting with nonspecific symptoms to the emergency room and focus on detailed anamnesis; and should not forget that CO intoxication due to hookah use can be seen.

Patient.Approval: Approval was received for this study from the patient.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - G.A, M.M.Y, S.Y., Design - G.A, M.M.Y, S.Y.; Supervision - M.I., A.A.; Materials - G.A, M.M.Y, S.Y.; Data Collection and/or Processing - G.A, M.M.Y, S.Y.; Analysis and/or Interpretation - G.A, M.M.Y, S.Y., M.I.; Literature Review - G.A, M.M.Y; Writing - G.A, M.M.Y, M.I., A.A.; Critical Review - A.A., O.B.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The author declared that this study hasn't received no financial support.

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Middle Black Sea Journal of Health Science December 2018; 4(3):43-46

LETTER to the EDITOR

Tabanid Infestation of Cattle and Its Implications for Public Health

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Received: 03 September 2018, Accepted 26 November 2018, Published online: 27 December 2018

Abstract
Female tabanids are important for public health and veterinary. As bloodsucking pests and vectors of disease agents they give harm to humans. They serve as vectors of such agents as *Dermatobia hominis, Loa loa*, Tularemi, *Bacillus anthrasis, Trypanosoma evansi, Coxiella burunetti, Dirofilaria repens*. Cattlemen living here raise their cattle on vineyards and orchards, olive groves, almond groves or uncultivated lands. These animals are tied with a halter and a long strap fixed to the ground. The aim of this study was to investigate tabanids infestation in Datça, Muğla. The study was conducted from April 2018 in Datça district. In this paper the Holstein hybrid cattle, old male cattle, tabanids sucking its blood as well as the diseases caused by tabanids are the subject of the study. The skin integrity on the lateral aspect of distal metacarpus in the left hind limb of the cattle was impaired. Hundreds of tabanids lacerated the skin, and sucked blood, causing hemorrhage in the epidermis. This study revealed that tabanids constitute a threat to animal and public health. We highlight the importance of effective prevention and control measures during periods in which these flies are active. More comprehensive epidemiological studies should be undertaken and national control programs are required to keep the tabanids infestation under control.
Key words: Cattle, public health, tabanids

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Main text and Results

Tabanids are ectoparasites which are widely seen in Turkey just as in other parts of the world. They are colloquially known as moth, warble fly or horsefly. In Turkey there are 173 tabanid species of tabanus genius (Girişkin, 2017).

Female tabanids are important for public health and veterinary. As bloodsucking pests and vectors of disease agents they give harm to humans. They serve as vectors of such agents as *Dermatobia hominis, Loa loa, Tularemi, Bacillus anthracis, Trypanosoma evansi, Coxiella burunetti, Dirofilaria repens* (Taylor and Smith, 1989).

With their piercing-sucking mouthparts tabanids impair the skin integrity of hosts, thereby causing pain. Having anticlotting enzymes in their salivary glands, tabanids feed on the blood of hosts, which wells up. Being large, robust and agile these flies have a body length between 5 and 30 mm. Their

body parts are head, thorax and abdomen (Bernard, 2003).

Tabanids can be found all over the world except for Antarctica. Their natural habitats are the bush, meadows, forests, scrubs, ponds, lakes and swamp. They are usually found in warm areas. They cannot fly when it is below 13°C, windy, stormy, rainy and dark. They are active in the daytime (Yücel, 2015).

To complete their life cycle tabanids may require one year while an average lifespan of an adult is one to one and a half month. As for mating the female of the species needs a blood meal before depositing her egg mass. Following the mating process, they lay their eggs at the hottest time of the day on wet sites or vegetation that stands over water. The larval stage usually lasts from 6 to 11 months, although it shows variation across species. Until the arrival of warmer seasons larvae stay inactive 5 to 10 cm. below the soil surface. Larvae mature and pupate when spring arrives. The pupal stage can last one month depending on climate conditions. After that period pupae become adults (Wall and Shearer, 1997).

Tabanids are given as vectors of blood-dwelling pathogens of several human diseases (e.g., tularemia, anaplasmosis, filariosis, anthrax, Lyme disease), and they induce allergic reactions in the host when sucking blood (Hornok et al., 2007).

This study deals with tabanids as nuisance pests of cattle as well as their importance for public health.

Methods

The study area is Datca, a district of Mugla Province in the Aegean region. Datca peninsula is at the meeting point of the Aegean and the Mediterranean, lying approximately between 36.60° -36.75° N. latitude and 27.40° - 28°E.longitude. Its shoreline length is 235 km. It has 52 bays of different sizes. The peninsula, which is between Gökova and Hisarönü bays, has a Mediterranean climate, characterized by rainy winters and dry summers. The locals depend mostly upon tourism for their livelihood (Anonymous, 2018).

This study was carried out in Datca in April 2018. Cattlemen living here raise their cattle on vineyards and orchards, olive groves, almond groves or uncultivated lands. These animals are tied with a halter and a long strap fixed to the ground. In the pastures they consume the required amount of dry matter on a daily basis. In this paper the Holstein hybrid cattle, an old male cattle, tabanids sucking its blood as well as the diseases caused by tabanids are the subject of the study.

Results

It was observed that hundreds of flies were flying around the cattle. Cattle's restlessness and not grazing were noted. At the clinical examination of the animal, a heavy infestation with tabanids was detected especially in it slower-extremity. The skin integrity on the lateral aspect of distal metacarpus in the left hind limb of the cattle was impaired. Hundreds of tabanids lacerated the skin, and sucked blood, causing hemorrhage in the epidermis (Fig.1).

Figure 1. Tabanids feeding on a cattle's leg. Previous feeding lesions can also be seen.

Discussion and Conclusion

Tabanids are mechanical and/or biological vectors of 27 infections, posing a real risk to human and animal health (Table 1).

The study was conducted in Datça, Mugla Province, which has major tourist destinations. In 2007 alone Mugla Province received 2.089.503 million tourists, 7 percent of the average number of international tourists to Turkey (Anonymous, 2018). With its 235 km. shoreline and 52 bays Datça has a great significance for Mugla Province. Datca receives a huge influx of tourists especially between May and October. As cattle raising takes place near the seaside, a heavy infestation with tabanids affects public health in the town as well. Since many tourists from different countries come to this holiday destination, it is also probable that people

whosuffer from Filaria, Loiasis and Q fever travel to here.

In general tabanids suck blood every 5 minutes and change hosts very frequently. As a result, they play a significant role in the transmission of infectious agents (Hornok et al., 2007). We argue that this may pose a great risk to the health of local residents as well as tourists in Datca.

Table 1. Tabanids of infections that infect humans and other animals

No	Disease agent	Vectoring
1	*Besnoita besnoiti*	mechanical
2	*Trypanosoma evansi* (Surra)	mechanical
3	*Trypanosoma vivax*	mechanical
4	*Trypanosoma congolense*	mechanical
5	*Trypanosoma equinum*	mechanical
6	*Haemoproteus metchnikovi*	mechanical +biological
7	*Loiasis*	biological
8	*Elaeophora schneideri*	biological
9	*Dirofilaria repens*	biological
10	*Dirofilaria. roemeri*	biological
11	*Clostiridium perfringens*	mechanical
12	*Fusobacterium necrophorum*	mechanical
13	*Coxiella burnetii (*Q fever)	mechanical
14	*Anaplasma marginale*	mechanical
15	*Francisella tularensis* (Tularemi)	mechanical
16	*Bacillus anthracis* (Anthrax)	mechanical
17	*Borrelia burgdorferi*	mechanical
18	*Influenza*	mechanical
19	*Bovine Viral Diarrhoea*	mechanical
20	*Equine Infectious Anemia*	mechanical
21	*Enzootic bovine leucosis*	mechanical
22	*Rinderpest*	mechanical
23	*Tick-Borne Encephalitis*	mechanical
24	*West Nile Virus Infection*	mechanical
25	*Swine Fever*	mechanical
26	*California encephalitis*	mechanical
27	*Dermatobia hominis*	mechanical

Because of considerable irritation caused by tabanids, bovine animals cannot graze sufficiently and lose weight. According to the findings of a study conducted during a grazing season in the USA, a cattle suffered from a weight loss of 90 kg. because of serious irritation caused by tabanids (Davis 1979). Furthermore, in another study it was reported that tabanids led to a decrease in the milk production of dairy cattle (Lehane 2005). The present study showed that being irritated by tabanids, most of the animals cannot eat enough feed.

A study found the amount of blood which tabanids suck at every bite is 0.168 ml (Tashiro and Schwardt, 1953). The present study reported attacks by tabanids on the cattle for bloodsucking. This resulted in open wounds on the skin, which in turn attracted myiatic flies as well as made the cattle susceptible to severe infection with secondary bacterial agents.

In conclusion, tabanids constitute a threat to animal and public health. We highlight the importance of effective prevention and control measures during periods in which these flies are active. More comprehensive epidemiological studies should be undertaken and national control programs are requiredto keep the tabanids infestation under control.

Acknowledgements

We would like to thank Associate Professor A. Onur Girisgin (Uludag University, Faculty of Veterinary Medicine, Department of Parasitology) for his invaluable help in this study on tabanids.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A; Design- M.A; Supervision- M.A; Funding- M.A.; Materials- M.A.; Data Collection/Data Process- M.A; Analyze or Comment- M.A.; Literature Scanning- M.A.; Writer of Paper- M.A.; Critical Review- M.A.

Conflict of Interest: No conflict of interest was declared by the author.

Financial Disclosure: The author declared that this study hasn't received no financial support.

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Middle Black Sea Journal of Health Science December 2017; 4(3):47-52

REVIEW

The Use of Prospective Meta-Analysis

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Received: 14 December 2018, Accepted 20 December 2018, Published online: 27 December 2018

Abstract

Meta-analysis is a powerful statistical instrument to summarize the knowledge in a research field, and to estimate overall measures of effects based on reported or unreported results. In performing meta-analysis is generally used the results of the reported trials. Knowing the results before the meta-analysis may affect the description of the review query, the entry and exclusion specifications because the researchers may also be prejudiced in selecting favor of reports supportive for their own thoughts. With these problems the retrospective meta-analysis is becoming a controversial tool in terms of "bias". The prospective meta-analysis can cope with these problems of the retrospective meta-analysis. A prospective meta-analysis is a type of next-generation systematic reviews where studies are investigated to be eligible before reporting their original studies. Prospective Meta-Analysis is unaware of the results of all studies because of the prospective identification and application of selection criteria for trials. In this paper, it is aimed to give information about Prospective Meta-Analysis, and to promote the use.

Key words: Meta-Analysis, Prospective Meta-Analysis, Randomized controlled trial, Publication bias, Researcher bias

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Introduction

The combining the results of studies on the same subject is continuing for long time as a scientific tradition (Berlin and Colditz, 1999). The utilization of meta-analysis is becoming increasingly prevalent for combine methodological and formally similar studies.

Meta-analysis, a notion coined by Glass (1976), is purposed to ensure the statistical investigation of a wide compilation of analysis outcomes from independent researches for the aim of combination the outcomes. Meta-analysis, or research synthesis, or research combination is a scientific method of achieving this goal through certain statistical methods, and really it has a long and former history (Hartung et al., 2008).

Meta-analysis, which is a complicated statistical method containing the generation of data obtained from related researches to determine impact size or result, has more and more noticed and influence evidence-based medicine, particularly in the area of health sciences. Through the arrival and unfulfilled

needs of the evidence-based medicine, the initial registerable article of a meta-analysis that addresses the efficiency of the typhoid vaccine in 1904, the quality and number of meta-analyses published in relative to health sciences have increased significantly (Mak et al., 2010).

Upon the emergence of evidence-based medicine approach in the recent two decades, meta-analysis has turned into a widespread study instrument to compound inputs from different studies and process them collectively. Meta-analysis ensures crucial data for creating clinical outlines and generating health policy suggestions (Turok et al., 2011; Tu and Faggion, 2012). Conventional meta-analysis, however, suffers from the heterogeneousness of the including studies of where tests a distinctive interference and evaluates a particular main result. While attempting to include all relevant data, other traps associated with meta-analysis contain discriminative reporting biases and reluctance of investigators' project data (Turok et al., 2011).

Assembling the results of many randomized studies in a meta-analysis ensures a strong and systematic tool to reliably predict modest but valuable developments in therapy. However, traps in the meta-analysis can further be run across because of the strength of this approach in determining small biases. Some of the traps in meta-analysis contain:

- the selection bias of patients;
- the selection bias of studies;
- the bias owing to the post hoc choice of study queries, suitability criteria, result description, or subgroups (Simes, 1995).

Bias in Meta-Analysis

Meta-Analysis is a statistical method used by researchers to quantify methodologically similar studies. In other words, Meta-analysis is a quantitative systematic review used "to combine the results of a number of different reports into one report to create a single, more precise estimate of an effect" (Ferrer, 1998). With the Meta-Analysis developed as an alternative to traditional literature review, new conclusions are generally made from the results of the previous research. Before applying the meta-analysis, the researchers decide which studies and which statistical methods to use. In this decision phase, they learn the results of previous studies, and this may cause the selection to be made in favor of the own ideas. This potential case arises the problem of bias in meta-analysis (Eysenck, 1994; Egger, 1998).

Typical retrospective meta-analysis looks like investigative rather than corroborative study. Decisions regarding researches to be subsumed, statistical investigations, and moderating elements are generated after the researchers recognize the results of the researches. These retrospective decisions have high probability for bias. For ensure confirmatory proof, methodological decisions in meta-analysis must be carried out prospectively, before the outcomes of the studies are known and ideally before studies are made (Watt and Kennedy, 2017). Multi-center randomized controlled trials and meta-analysis are the gold standard for evidenced-based medicine. However, multi-center trials are expensive and relatively uncommon in the literature. Recently, the use of meta-analysis is popular, because it combines inputs from many trials and processes them in the aggregate. Yet, selective reporting biases when trying to incorporate all thematic input (Turok et al., 2011).

In the meta-analysis, bias is a very important issue. The potential sources of publication bias are "researcher bias", "selection bias of subjects and trials" and "bias due to post hoc selection of study questions", "eligibility criteria", "outcome definitions or subgroups" (Alderson et al., 2004; Askie et al., 2011). Publication bias may cause critical results, particularly in the areas of epidemiology and medicine (Weiss and Wagner, 2011). Dickersin (2005) notes that there are some studies that is influenced by meta-analyses that suffer from publication biases in the even the treatment of life-threatening diseases (Weiss and Wagner, 2011).

Prospective Meta-Analysis

A meta-analysis may be performed by retrospective or prospective. While, a retrospective meta-analysis is performed on data extracted from the literature, a prospective meta-analysis (PMA) is performed on the actual raw data from the various studies. "Patient-level data" that have been directly and individually collected during a clinical trial as individual will only be available in a prospective analysis (Herson, 2009). This chance of using patient-level data is one of the advantages of PMA.

PMA analysis plans are unaware of the results of all studies. This prevent possibly biased, data-dependent emphasis on specific subgroups or specific endpoints. Most widely, PMA has been

practiced to randomized studies, but it is a method that could be practiced to the PMA of observational studies as well (Berlin and Ghersi, 2004). They have joint properties with both aggregate meta-analyses

and including individual patient data. PMA may help to tackle some of the known troubles of retrospective meta analyses;

- indicating hypotheses that are unaware of the results of individual studies;

- to ensure that the study election criteria are implemented prospectively;

- to ensure that a priori declaration of planned analysis, including subgroup analyses, to be generated before the outcomes of individual studies are known. This prevent probable challenges in explication relevant to the data-dependent emphasis on specific subgroups (Ghersi et al., 2011).

PMA is a meta-analysis where studies (generally randomized controlled trials) are defined, evaluated and specified to be suitable before the results of any of the studies become known. This is distinct from a systematic review because the included studies are usually determined after completion and reporting of results (Ghersi et al., 2011; Anonymous 2, 2018; Anonymous 2, 2018)

Retrospective meta-analysis rises the statistical strength and ensure more exact predictions of the therapy influence by incrementing sample size. While meta-analysis use widespread in medical literature, PMA is a comparatively new methodology. In PMA, the certain interferences as well as the primary and secondary results are stated before data from certain studies are published (Turok et al., 2011). PMAs enable the identification of hypotheses before the results of individual studies, and provide a prospective execution of study selection criteria; and perform the pre-definitions of the intended analyses. PMAs are generally undertaken by a common group and generally collect and analysis individual patient data (Ghersi et al., 2011).

Watt and Kennedy (2017) reported that there are three options for PMA:

1. The most apparent choice is to pre-register the meta-analysis plan and include in the meta-analysis only researches handled after the plan was registered. The meta-analysis plan would determine the statistical investigations and the criteria for detecting which studies are included. Next studies that abide by the inclusion/exclusion criteria would be included in the meta-analysis. Unhappily, that choice is probably to keep remarkable retrospective deciding.

2. A more powerful alternative for PMA is to pre-determine the protocols of studies included as part of the meta-analysis plan. In multicenter studies, all researchers generally need to adopt the same protocols.

3. It is recommended that a registration-based PMA in which the decision to include or exclude a specific research study prospectively based on preliminary records for the research.

PMA ensures statistical strength to study substantial queries about rare cases and subgroups. The skill to examine subgroups is not specific to PMA, but a pre-description of subgroups in a PMA submits the benefit of preventing the bias in the post hoc description of prevalently used subgroups (Rothstein et al., 2005).

A PMA must be planned meticulously and should be registered a protocol such as that sustained by the Cochrane Collaboration from the outset (Reade et al, 2010; Anonymous 8, 2018). There are several PMAs maintained over decades, with new trials adding to the enlarging data compile (Porgue and Yusuf, 1998). If the meta-analysis is finished prematurely, there is little instance to conduct the reactions of researchers in trials still underway (Reade et al., 2010).

A PMA should have a openly obtainable guideline. Berlin and Ghersi, (2004) and Ghersi et al. (2011) note that the contents of this protocol can be summarized as in Table 1.

An Example for the Use of PMA

Askie et al. (2018) performed a study to compare the influences of varying target intervals for oxygen saturation as determined via pulse oximetry (SpO2) on major morbidity or death. A prospectively planned meta-analysis named as Neonatal Oxygenation Prospective Meta-analysis (NeOProM) composed of independent participators' input for five clinical trials (Anonymous 1-6, 2018) These researches were assessed as suitable to include in the meta-analysis before the outcomes of any of the trials given (Ghersi et al. 2011). 4965 newborns were included to this study. The study protocol was published in 2011 and registered on "ClinicalTrials.gov". The statistical analysis plan was finalized in 2015. In this PMA results of independent participators' input from highly preterm newborns, no significant difference was detected in a comparison between

lower SpO2 target interval and higher SpO2 target interval on the early combined result of major disability or death at an adjusted age of 18 to 24 months. The lower SpO2 target interval was related with an elevated threat of necrotizing enterocolitis and death, however a lower risk of retinopathy during premature therapy. All in all, the use of PMA provided in this study that the researchers can comply to take advantage of the identical tool to define a specific result, and to determine the results at the identical check-points in the trials.

Table 1. Contents of a PMA as stepwise (Berlin and Ghersi, 2004)

Objectives

- Specify the particular hypotheses.

Methods

Criteria according to the studies for this review

- Suitability criteria for trial designation (e.g. needs for randomization).
- Suitability criteria for the target population.
- Suitability criteria for each comparator and intervention.
- Result information: determination of definitions, primary and secondary endpoints, timing, measurement instruments.
- Details of subgroups.

Search methods for identification of studies

- Describe efforts made to identify ongoing trials.

Data collection and analysis

· *Trial details:*

- List details of trials specified to include.
- An expression outlining if, at the time of submission for registration of the PMA, any trial results were known; Trials should be included only if their results were unknown at the time they were identified and added to the PMA
- Whether a signed agreement to cooperate has been obtained from the appropriate reference of each trial (e.g. the principal researcher or sponsor).·

Analysis Plan:

- Sample size and power calculation, subgroup analyses etc.

Management and Coordination:

- Details of management structure and committees.
- Data management (data to be collected, format required, quality assurance procedures, etc).
- Responsibility for statistical analyses.

Publication Policy:

- Policy of authorship (e.g. publication in 'group' name).
- Writing Committee (membership, responsibilities).
- Policy of manuscript (e.g. shared to all collaborators for interpretation).

Conclusion

In this paper, it is aimed to give information about PMA, a type of next-generation systematic reviews, and to promote the use.

In recent years, the use of meta-analysis has become increasingly popular, as it has ability to combining the results of different studies in one study. However, publication bias is a serious problem in meta-analysis, which can affect statistical power. Thus, PMA with some distinct advantages should be preferred rather than retrospective meta-analysis of published data.

As a result, PMA can used in the medical research to establish causative links between treatment and results. Planning a PMA of collected data from associated researches and the usage of real data will help a more accurately prediction of the influences of treatment methods in health sciences.

Peer-review: Externally peer-reviewed.
Author Contributions: Concept- T. Y., Design- T. Y., Supervision- T. Y., Literature Review- T. Y., Writing- T. Y., Critical Review- T. Y.
Conflict of Interest: No conflict of interest was declared by the author.
Financial Disclosure: The author declared that this study hasn't received no financial support.

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