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VOLUME 10,ISSUE 4:

Detection of adulteration in the type and amount of meat in meat products: a systematic review

Abdolhossein Noroozi; Arefeh Erfani Jazi; Mohammad Hashemi

Volume 10, Issue 4 , December 2023, Pages 1-10

https://doi.org/10.22038/rcm.2023.66777.1409

Breast Cancer Survival Rate in Mashhad, Iran: A 10-year population-based study

Hamideh Ebrahimi Gore; Ali Taghizadeh; AmirAli Moodi Ghalibaf; Ali Shamshirian; Mohammad Reza Motie

Volume 10, Issue 4 , December 2023, Pages 11-17

https://doi.org/10.22038/rcm.2023.71633.1446

A Case Report of Renal Tubular Acidosis Type 1 without Glomerular Disease in an Adolescent with Pediatric-onset Systemic Lupus Erythematosus

Abdolreza Malek; Sepideh Seyedkaboli; Asma Batouri; Amir Muhammad khuban; Mahdieh Vahedi

Volume 10, Issue 4 , December 2023, Pages 18-20

https://doi.org/10.22038/rcm.2023.75435.1468

Effects of Abatacept in patients with rheumatoid arthritis and cancer risk

Saba Homapoor; Maryam Sahebari; Mandana Khodashahi

Volume 10, Issue 4 , December 2023, Pages 21-31

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Enhancing Growth in Epidermolysis Bullosa: Nutritional Supplements and Dietary Interventions for Children and Adolescents

Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Detection of Fraud in the Type and amount of Meat in Meat Products: A Systematic Review

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ABSTRACT

Introduction: The aim of this study was to identify methods for detecting composition and fraud in meat foods.
Methods: An extensive literature review was conducted in 2022 using the electronic databases: Web of Science, Scopus, SID, and PubMed. The search was limited to articles published in English from 1970 to 2022. Search terms used were "fraud", "meat products", "Iran," " authentleication," "detection," and "adulteration".
Results: Genetic-based molecular tests (PCR) and less use of histological and chemical tests were used to detect fraud and its type in meat products. PCR was used in 30 cases to identify the type of cheating in meat products such as sausages. Histological methods were used in 19 cases to detect type of violation.
Conclusion: Molecular methods for detecting food fraud are highly accurate; therefore, they have the highest detection rate.

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Introduction

Fraud in meat-contaminated foods has been a rising global problem in recent years. Cheat food is defined as the intentional incorporation or replacement of cheaper or inferior components in foods in order to improve their quality and lessen their environmental impact. Because of this, the health of the community has been linked directly to the authenticity of meat. Identifying meat species in various meat products, on the other hand, is particularly crucial in Islamic nations where people only consume halal meat. In the last few decades, PCR-based methods have been used to check the authenticity of meat from different raw, cooked, and cooked food products made from different animal species (1). World consumption of meat and animal carcasses is increasing these days. Because of how much money meat is worth, it is possible that illegal tissue could be used in processed meat. species of animal (2).

Authenticity and traceability of meat are big problems in our modern society. For example, there have been reports of horse meat being added to meat products that were not supposed to have it (3). This exemplifies the widespread demand of consumers for clear and accurate data on the food they consume. This is especially true for processed meat products, where you can't tell the different parts apart as easily by looking at them as you can with whole fresh meat (4). In fact, there

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are substantial underlying reasons behind this allegation. Nowadays, pricing and lifestyle, as well as religious or health considerations, might influence an individual's choice of feed products based on their composition. An excellent example of this is the Muslim community's growing need to certify the halalness of the meat they consume in an expanding global meat market (3).

Major food fraud and contamination occurrences occur with startling regularity and are known to be episodic, raising the question of when, not if, another large-scale food safety and integrity disaster will occur. Indeed, the issue of preserving food security is now widely acknowledged on a global scale. The growing size and complexity of food supply networks can make them much more prone to adulteration and contamination, as well as possibly dysfunctional (5). The deception of flesh products using undeclared or incorrectly stated animal species is a major concern around the world. There are several analytical tools for identifying meat types, but they are time consuming and require highly skilled workers (6). Fraudulent use of meat in processed foods is a serious subject because specific meat species are forbidden in various religions, including Islam and Judaism. Some meats may also be carriers of deadly diseases such as SARS, hepatitis, and anthrax. Furthermore, unintentional eating of some meat may result in an allergic reaction (7). In conclusion, the current study suggests that the real-time PCR-HRM method could be considered a reliable technique for detecting meat authenticity in processed products and distinguishing between halal and haram meat samples. However, some refinements are needed to improve the selectivity of these methods (8).

Materials and Method

Data sources

An extensive literature review was conducted in 2022 using the electronic databases Web of Science, Scopus, and Side, and PubMed. The search was limited to articles published in English from 1970 to 2022. Search terms used were "Fraud", "meat products", "Iran," " authentication," "detection," and "adulteration".

In total, 1050 articles were found in Iran, of which 163 were useful. We had 65 duplicate articles and 48 articles were deleted for the following reasons.No access to full text (6), no percentage and type of fraud (2), livestock gene identification (9), article not original (6), fraud other than meat (7), test except target (9) Once preliminary results matching search terms were obtained, data was extracted in three steps: duplicate articles were identified and removed; remaining titles and abstracts were screened for eligibility against inclusion criteria; and full text articles were retrieved and assessed in terms of their study design and scientific approach. Then, all 50 articles that were found were reviewed critically and added to the overview as needed (Figure 1).

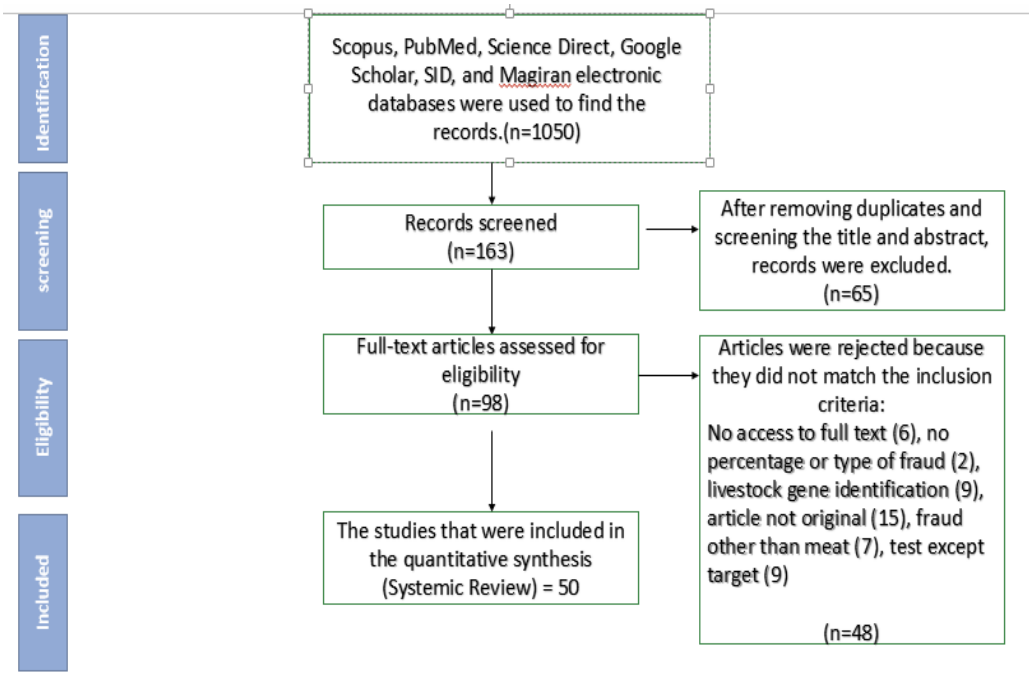

Figure 1: Article flow diagram

Results

The total number of samples are 1944. The following are meat products. In 21 articles on sausages, 3 articles on halal products, a kebab review in 12 articles, gelatin 2, fish, sandwiches, minced meat, 9, hamburger, 17, meat There have been two articles, pointing out that in some articles more than one meat product was reviewed.

Studies conducted in 1993, comprise two studies 2021 (1), 2016 (10), 2012 (2), 2011 (2), 2019 (3), 2020 (6), 2015 (2), 2013 (3), 2014 (5), 2018 (7), 2017 (4), 2009 (1) A study on meat fraud, type of meat and its products was collected.

50 articles collected from Tehran 21, Isfahan 4, Yazd 5, Tabriz 9, Shiraz and Khorasan each one, Khorramabad 1, Kermanshah 1, Urmia 1, North 2, Mashhad 1 and the rest have been done in the whole country.

In this study, looking at the collected articles from all over the country, we came to the interesting conclusion that the amount of unauthorized meat used in meat products is quite interesting. The use of poultry meat as an unauthorized additive and its permitted fat textures such as skin and fat in 21 articles It is mentioned that this issue indicates widespread fraud of this meat, the reason for which in Iran may be its lower price for producers. The use of ruminant meat as an unauthorized additive and its unauthorized tissues such as rumen and by-products are mentioned in (16) articles, in (3) only ruminant articles, in (8) beef mince articles, and in (6) articles. Mutton and goat meat are mentioned in the context of meat fraud. This indicates widespread fraud of this meat. which can have different reasons. The unauthorized use of horse meat and its unauthorized textures, all of which are prohibited, are mentioned in (7) articles that can be used in sausages and other products (Table 1).

Table 1. Methods for detecting food fraud

Journal Year	Location	Fraud	Percentage	Product	Detection Method	Sample Number	Reference
1993	Four factories	Unauthorized texture	7–30%	Hamburger meat	Histology	120	(9)
2021	Markets in Tehran	Mislabeling of cattle, sheep, chicken, turkey, and wild pig	Pos	Raw and cooked mincemeat samples	Real-time PCR	Five species (cattle, sheep, chicken, turkey)	(8)
2018	Markets in Tehran	Chicken and red meat	Pos	Hamburger meat	Simplex and Duplex PCR	10	(2)
2019	Markets, in north-east Iran	Unauthorized tissues	Muscle fiber (100%), fat tissue (100%) and plant material (97.70%).	Sausage	Histological	20	(3)
2016	Yazd	Avian skin and adipose tissue	5-20% avian skin	Mincemeat	Histological	15	(10)
2017	Iran	Bovine, buffalo and porcine	Beef frankfurters (71%) Hamburger meat (85%)	Beef frankfurters and Hamburger meat	PCR	-	(11)
2018	Markets in Tehran	Chicken and red meat	Sausage (60%)	Sausage	Multiplex PCR	114	(12)
2018	Tabriz	Donkey meat	Pos	Mincemeat and bovine	PCR	98	(13)
2009	Factories in the north and south	Chicken and red meat	1%	Fishmeal	(QC-PCR)	30 commercial samples of fishmeal	(14)
2016	Restaurants in Tabriz	Unauthorized tissues	41.4%	Kebabs	Histological and chemical	44	(6)
2018	Markets in Tehran	Unauthorized tissues	54.76%	Hamburger meat	Histological	42	(15)
2020	Markets in Tehran	Mislabeling	67%	Premade kebabs contain 70 and 90% red meat	FTIR	36	(16)
2018	Markets in Tehran	Chicken and red meat	Sausage (60%)	Sausage	Multiplex PCR	114	(12)
2018	Tabriz	Donkey meat	Pos	Mincemeat and bovine	PCR	98	(13)

Table 1. Continue

2009	Factories in the north and south	Chicken and red meat	1%	Fishmeal	(QC-PCR)	30 commercial samples of fishmeal	(14)
2016	Restaurants in Tabriz	Unauthorized tissues	41.4%	Kebabs	Histological and chemical	44	(6)
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2020	Markets in Tehran	Mislabeling	67%	Premade kebabs contain 70 and 90% red meat	FTIR	36	(16)
2012	Factories in Iran	Poultry and ruminants	9-25%	Fishmeal	PCR	124	(17)
Control 2014	Restaurants and supermarkets	Chicken and red meat	6-100%	Raw Hamburger meat	PCR	300	(18)
2019	Factories in Iran	Ruminant, poultry, and pork	80% of sausage samples and 90% of cold cut	Sausages, cold cuts and ground meat	PCR	Each -10 samples,	(19)
2016	Tehran, Tabriz, and Isfahan	Horse, donkey, pig, and other ruminants	50.50,60.40% ,% 70.30%	Halal meat	PCR	35 samples	(20)
2020	Supermarkets in Iran	Mislabeling of bovine and chicken	25 cases	Supermarket hamburger meat	PCR-RFLP	31 samples	(21)
2020	Tabriz	Mixing poultry meat	41.38%	Processed, semi-processed products	PCR	58 samples	(22)
2012	Mashhad and Tehran	Sheep, cattle and goat	24 cases	Iranian commercial meat products	PCR-RFLP	30 samples of oil in mincemeat, kebabs, beef burgers and canned meat	(23)
2016	Tabriz	Bone, cartilage and lung tissues	9.1-18.2%	Ground meat used for kebabs	Histological and chemical	33 samples	(24)
2015	Restaurants and supermarkets	Unauthorized tissues	15 cases	Two types of red meat kebabs, sausages, handmade hamburger meat	Morphological	20 samples	(25)
2014	Different companies and food markets	Beef, sheep, pork, chicken, donkey, and horse	58.7%	Hamburger meat, sausages, frankfurters, cold cuts	PCR	224 meat products	(26)
2014	Various food factories	Mislabeling of bovine and chicken	50%	Sausages	PCR	10 sample sausages	(27)
2016	Markets in Tehran	Mislabeling of chicken	Pos	Hamburger meat	Multiplex PCR	10 samples of specified brands	(28)
2018	Tehran	Bovine and chicken	43%	Hamburger meat (60-90%)	PCR	10 samples of specified brands of hamburger meat	(2)
	Tehran	Unauthorized texture	Pos	Sausages	Spectrophotometric	60 sample sausages	(29)
2020	Markets in Tehran	Amount meat		Industrial kebabs and sausages	Histological	5(3)	(30)
2018	Markets in Urmia	Unauthorized tissues	Transparent bone and cartilage frequency 41.7% and 54.2%	Sausages	Histological	24	(31)

2019	Markets in Tehran	Unauthorized tissues(chicken)	Pos	Sausages (30 & 90%), industrial kebabs (70%)	Histological	5	(32)
2016	Markets in Yazd	Unauthorized tissues (destruction and viscera)	50%	Mince meat	Histological	20.3	(1)
2017	Tehran, Isfahan, Tabriz	Horse, donkey	(17%)	Sausages, mincemeat, hamburger meat	PCR	35	(33)
2014	Tehran	Chicken and meat	Pos	Sausages55%))	PCR method	10	(34)
2017	Kermanshah	Unauthorized tissues	Muscle or skeleton was not observed in 96.2% and 30.8% of adipose tissue samples. Organ or heart was found in 19.2% of the samples. Mature cartilage and bone were found in 96.2% of the samples. In 57.6% of samples, immature employment were found.	Sausage	Histological	720	(34)
2020	Yazd	Quantitative detection of meat	Low	Sausages (30%, 50%, 70%, 90%), kebabs (70)%	Histological	5	(30)
2020	Factory in Tabriz,	Donkey meat adulteration	Varied	Sausages	PCR	3	(39)
2013		Detection and quantification of chicken	5-90%	Sausages	PCR	4	(40)
2019	Yazd	Unauthorized tissues (chicken skin and bone)	5-20%	Kebab (70%) and cold cuts 90% & 30%	Histological	5	(32)
2014	Yazd	Unauthorized tissues (avian skin and adipose tissues) of chicken	5-20%	Mince meat	Histological	5	(10)
2016	Tehran	Unauthorized Tissues/ chicken	5-20%	Mince Meat	Histological	10	(41)
2017	Isfahan, Tabriz, and Tehran	Horse (11%), pork and donkey (6%)	17%	Sausages, kebabs, and hamburger meat	PCR	35	(42)
2013	Iran	Bovine, ovine, and caprine	100%, 50%, 10%, 5%, 1%, 0.5%, 0.1%	Pure and binary mixtures and heat processed meats	PCR	-	(35)
2016	Markets	Chicken paste in meat products	Sausages 84%, hamburger meat 26%-(10-50%)	Sausages, hamburger meat	PCR	150	(36)
2011	Khorramabad factory	Collagen	0.02-0.13 g/100	Sausages	Histological	30	(37)
2018	Tehran	Unauthorized tissues	57.48%	Handmade hamburger meat	Histological	35	(15)
2016	Various restaurants	Unauthorized tissues	-	Red meat sandwich products	Applying light, histochemical and scanning histological methods	105	(7)

2016	Isfahan, Tabriz, and Tehran	Horses, donkeys, pigs, cows, sheep	17%	Hamburger meat, sausages, mincemeat, kebabs	histological methods	35	(38)
Journal Year	Location	Fraud	Percentage	Products	Detection Method		
2015	Super-markets in Tehran	Pork	0.1%	Gelatin-	PCR	16	(43)
2013	Super-markets in Tehran	Incorrect labeling	11%	Fish species	PCR	3 Alaska Pollack samples	(44)
2020	North Khorasan province	Beef, lamb, pork, chicken, donkey, and horse	Beef (100%), lamb and chicken (83%), and horse (10%)	Kebab	TaqMan real-time PCR	150	(45)
2011	Tabriz, Iran	Pork		Halal meat products	PCR	20	(4)
2016	Shiraz	Chicken and red meat mislabeling	10-50%	Sausages	PCR	100	(5)

The study of donkey meat added to meat products has been done in 9 articles. Also, pork and its products were collected in 8 studied articles. In the continuation of the study, it was found that the use of unauthorized tissues such as skin and livestock in 18 The article has been in the country. Among them, the use of buffalo meat in meat products has been studied in 1 article. Also, collagen 1 and hair 1 and the least amount of tissue added to meat have been studied. The use of incorrect labeling and labeling has been studied in six articles. Among these violations were the use of buffalo meat, as well as collagen and hair, which were added to fewer meat products (Figure 2).

But in the meantime, the use of poultry waste as well as other meat waste accounted for the highest rate of meat fraud, with added to meat products that should not have been added.

Genetic-based molecular tests (PCR) and less use of histological and chemical tests were used to detect fraud and its type in meat products. PCR was used in 30 cases to identify the type of cheating in meat products such as sausages. And histological methods were used in 19 cases to detect the type of violation, in one case using histological and chemical methods to detect counterfeiting of meat products (Figure 3 and 4).

Discussion

Regarding the economic value of meat, use of unauthorized animal tissue is not impossible in meat products. Meat fraud generated a huge outrage amongst customers in 2013 in Europe due to the horsemeat scandal. (4). In this regard,

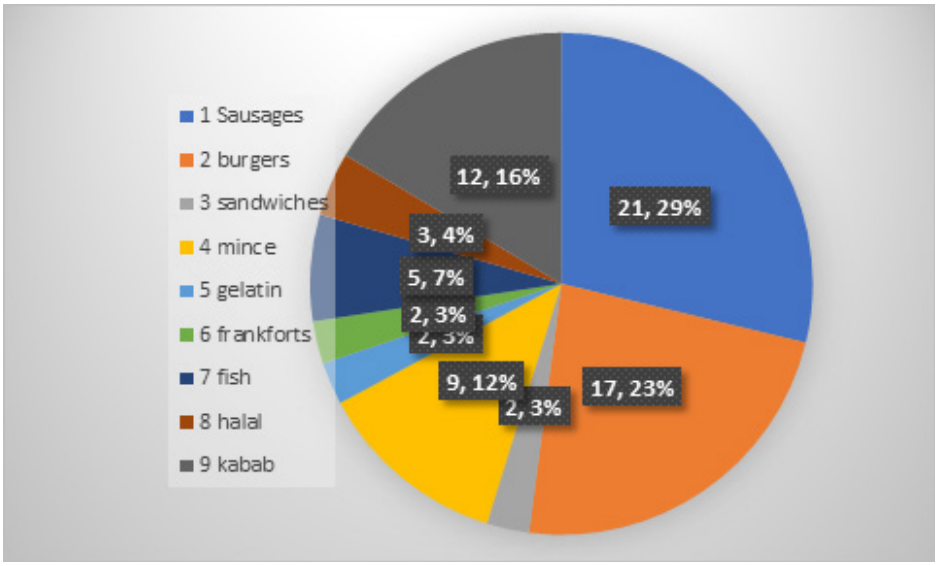

Figure 2. The number of studies conducted according to the type and amount of fraud in meat products

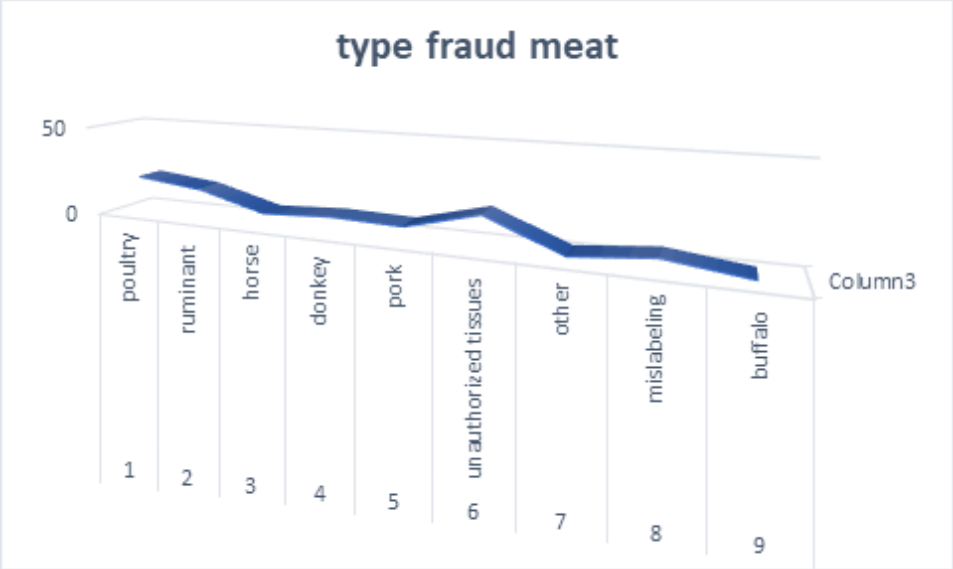

Figure 3. Number of fraud investigation articles

honest and accurate food labeling is essential to ensure consumer safety and food selection (12).

There is a requirement in meat products to specify the amount of each ingredient providing the nutrient, which is known as a Quantitative Declaration ((QUID) Quantitative Declaration). There is a requirement in meat products to specify the amount of each ingredient providing the nutrient, which is known as a Quantitative Declaration. (9). So it implies that each animal in the merchandise is depicted and determined separately. Furthermore, mechanically recycled meat ((MRM (mechanically recycled meat)and other components, such as the liver, lung, heart, or tongue, are not considered meat and must be separated (2).

A variety of 105 distinct meat sandwich items (Kufta, Havashi, and Shawarma sandwiches, 35 sandwiches of each type of product were collected in 2016 and from New Valley City of various sorts) was reviewed. A scanning electron microscope was used to detect meat theft by analyzing scanning and light. Select half of every group's samples for optical and histochemical microscopic inspection, and the remaining samples for electron microscopic investigation. Hematoxylin and eosin, PAS, trichrome, Garrett and Crossman, bromophenol blue, and ATPase were used to stain these sections (7).

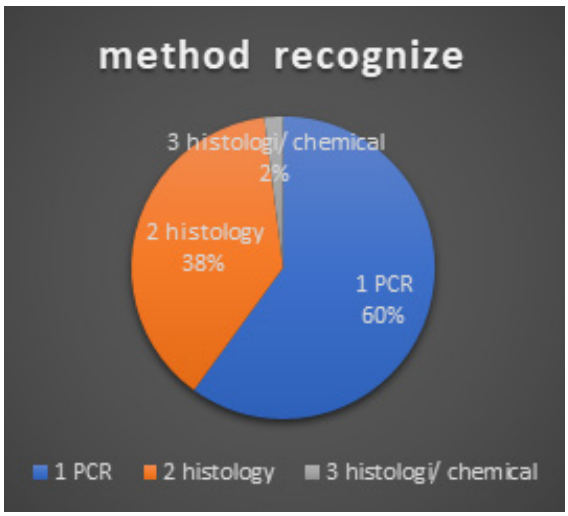

Figure 4. Method of fraud investigation articles

Histological examination indicated that skeletal muscle contains a variety of tissues, including connective tissue. lungs, ruminant stomach, enormous elastic blood vessels, cardiovascular system, adipose tissue, cartilage (hyaline and white), spongy bone, lymphoid system (spleen), plant material, mostly on sand particles Embryonic tissue in Hawawshi meat with flying muscle fiber (shrinkage) relative to light might be suspected using the enzyme ATPase Staining (rapid shrinkage). The discovery of muscle fibers in the study points to histology as a possible method of improving the quality of market meat sandwiches (7).

The identification of species in meat products is important to ensure the health of consumers. PCR amplification and species-based- Dedicated primers were used to identify horses, donkeys, pigs, and other ruminants in their raw form, and meat products. Processed: Oligo nucleotid primers were designed and patented for amplification of species-specific mitochondrial DNA sequences of each species, and samples were prepared from binary meat mixtures.

The findings revealed that the meat kinds in all chemicals were precisely determined multiplex - (PCR (polymerase chain reaction)), This product's

sensitivity was 0.001 ng, making it available to you and acceptable for usage in industrial meat products. Based on the results, the least volume of fraud was found in chicken products compared to other meat products (20). The results showed that the types of meat in all compounds were precisely determined multiplex. On 12S rRNA chicken mitochondrial genes, dedicated primers are created. On DNA isolated from 150 sausage samples, conventional PCR and SYBR Green RT-PCR were recruited. Results The presence of mislabeled chicken in sausages was discovered to be 84%, with Q-PCR technology able to reduce the load, detecting 10% to 50% chicken in products. Your method's recognition limit could be Meat authorities commonly use this method to control the quality of meat products (9). Specific analysis methodologies, sensitivity, and dependability are necessary for detecting counterfeit chemicals in food products. Some methods have defined reasons for their placement, whilst others do finger printing across the sample without a specific aim. The goal of this study is to give an overview of both targeted and non-targeted approaches developed in previous studies that focused on food quality, especially beef authentication (2).

Consumption of food from pig sources is strictly forbidden in Islam. Gelatin, taken mostly from beef and pork sources, it has many uses in food and medicine Industries to ensure the compliance of food products with solvent regulation, valid and reliable development Analytical methods are much needed. In this study, a specific polymerase chain reaction is specific Method (PCR) using mitochondrial DNA protected region (cytochrome b gene) for study- Eat the solvent origin of gelatin. After separation of DNA from gelatin powders of specified origin, Ventional PCR was performed using a specific zinc type primer on the extracted DNA. Boosted Expected PCR products of 212 and 271 DNA structure were observed for pig and bovine gelatin, respectively. Zinc sensitivity method for binary gelatin mixtures containing 0.1%, 1%, 10% and 100% (w / w) pork gelatin in cow gelatin and vice versa. If more DNA is destroyed Due to the intense processing of gelatin production, the minimum level was 0.1% by weight on the weight of both pigs and bovine gelatin was detected. In addition, eight labeled foods containing cow gelatin and Eight capsule shells were subjected to PCR. The results showed that all samples were present Bovine gelatin, and the absence of porcine gelatin were confirmed. This method is very original It is useful to check that gelatin and gelatin-containing foods are derived from solutes (43). Meat cheating is a worldwide problem Violates diet, health and religious care. Bottom Measuring the prevalence of meat scams is difficult and used Various methods have been used for this topic.

The histometric analysis demonstrated that additive bone, especially in mincemeat kebab, and skin texture did not differ significantly from the actual result in adulteration detection (6).

The detection of porcine DNA in meat extracts is critical for the halal certification of meat products. To address this issue, the creation of a true green SYBR was effective for the pig PCR method. Successful DNA isolation from meat samples had been proven to be deleterious when using particular primers for porcine mitochondrial DNA. The research indicated that green SYBR real-time PCR, could be considered a reliable method for meat solvent authenticity (4).

Conclusion

In conclusion, this study demonstrated that real-time PCR is a reliable method for recognizing fraud in meat products. However, certain improvements are required to develop these approaches. These useful tests and approaches are recommended for quality control companies.

Strict supervision of industrial meat products is necessary for quality assurance for consumers. For this quality assurance, molecular methods for detecting food fraud are highly accurate, and they have the highest detection rate.

Conflicts of interest

The authors declare no competing interests.

Authors' contributions

Drafting of the manuscript and screening the article was done by (Nourozi A). Conception and design was done by (Hashemi M). Critical revision of the manuscript for important intellectual content and double review to minimize bias was conducted by authors (Hashemi M, A. Afshari, Erfani A).

Ethics approval and consent to participate

This is a systematic review article and all ethics approval and consent of used articles was checked.

No aspect of this article was related to laboratory animals, special human illnesses, and/or the use of people's information.

Consent for publication

Our work did not include any personal data ("Not applicable").

Availability of data and materials

All data from this study are included in the published article and its supplementary files.

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Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Breast Cancer Survival Rate in Mashhad, Iran: A 10- Year Population-based Study

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ABSTRACT

Introduction: The breast cancer burden is still increasing, both in developing and developed countries. The present study was conducted to determine the survival rate of breast cancer based on tumor biological subtypes in patients referring to three referral oncology centers at Mashhad University of Medical Sciences, Mashhad, Iran.
Methods: The present population-based study was conducted at Imam Reza Hospital, Omid Hospital, and Reza Oncology Center, Mashhad University of Medical Sciences, Mashhad, Iran Demographic information, the status of biomarkers in immune-histochemical evaluation, clinical and pathological features of the tumor, type of therapy, recurrence, or death was recorded for each eligible patient.
Results: In total, 247 patients were included in the study. The mean age of patients was 48.8 ± 1.3 years. The mean time of survival was 2.64±0.13 years. In detail, the survival rate from the first year to the fifth year was reported as 100%, 96%, 91%, 90%, and 89%, respectively. The survival rate of the luminal B subtype was higher than other subtypes but the difference was not significant (P=0.7). Only in terms of metastasis, there was a significant difference between the alive and dead patients (P=0.0001).
Conclusion: Despite no significant relationship between biological markers, the breast cancer subtype, and its survival rate, the overall survival rate of the patients decreased lightly through five years. However, further studies are required to indicate more accurate data about the breast cancer survival rate in our region.

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Introduction

Breast cancer is the most common cancer in women, affecting 2.1 million women annually and causing the most cancer-related deaths among women in the US. In 2018, about 627,000 women died due to breast cancer, as this disease accounts for approximately 15% of all cancer deaths in women (1). The burden of breast cancer continues to increase in both developed and developing countries. Inadequate early screening and expensive treatment contribute to significant variations in breast cancer survival rates between countries (2). The age-standardized incidence rate of breast cancer is

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33.21 per 100,000 according to the latest national database; however, breast cancer mortality has not changed over the past 30 years in Iran; Thus, the age-standardized mortality rate was 14.2 per 100,000, with a median age of 49.84 years (3, 4).

To improve breast cancer outcomes and survival, early detection is critical (4). Traditional clinical and pathologic factors such as age, histological grade, tumor type, tumor size, and hormone receptors are often used to stratify patients into high-risk groups for treatment with adjuvant hormone therapy, radiation therapy, and/or chemotherapy. These factors accurately classify patients based on long-term follow-up studies. However, it is acknowledged that traditional prognostic factors are limited in their ability to provide reliable stratification in all patients (5). Recently, morphologic and immune-histochemical analyzes have been integrated to determine patient prognosis (6). Breast cancer has been divided into five molecular subtypes based on gene expression patterns found to have significant differences in clinical outcome including Luminal A, Luminal B, HER2, Basal- like, and normal basal-like (7). Molecular subtypes are predictive for responses to specific therapies and are prognostic for clinical outcomes (8-10).

According to the current situation, Breast cancer is a major concern in public health, and patient survival is a key indicator of overall health globally. This study aimed to determine the survival rate of breast cancer patients based on tumor biological subtypes in whom referring to Imam Reza Hospital, Omid Hospital, and Reza Oncology Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Materials and Method

Study Design

The present population-based study was conducted at Imam Reza Hospital, Omid Hospital, and Reza Oncology Center, Mashhad University of Medical Sciences, Mashhad, Iran. The study utilized the medical records of breast cancer patients who underwent mastectomy between 2008 to 2018. Patients with measured levels of biomarkers (HER2, PR, and ER) were included in the study. Patient data was extracted from archives and their survival time was calculated. All eligible patients were included in the study by census sampling method. All extracted data analyzed confidentially without mentioning the patient's name and the research has been approved by the Organizational Ethics Committee of Mashhad University of Medical Science with the code IR.MUMS.fm.REC.1396.32.

Measurements

Demographic information, the status of biomarkers (ER, PR, HER-2) in immune-histochemical evaluation, clinical and pathological features of tumors (such as type, size, degree of histology, number of lymph nodes, presence of primary metastasis), type of therapy, recurrence or death were recorded during follow-up.

Statistical Analysis

The data was analyzed by SPSS software (version 11). The t-test was applied to compare quantitative variables, while Chi-square test was applied to compare qualitative variables between two groups (dead and alive). Patient survival analysis was performed using the Kaplan-Meier method. The significance level was set at <0.05. Cox regression model was used to predict factors affecting survival.

Results

Among the 400 breast cancer patients who were referred to Imam Reza Hospital, Omid Hospital, and Reza Oncology Center, Mashhad University of Medical Sciences, Mashhad, Iran, from the beginning of 2008 to the end of 2018, 247 cases met the inclusion criteria and the information were registered. Then contacted them and their living conditions were recorded. The mean age of patients was 48.8±1.3 years and the mean weight was 67.5±1.6 kg. The participants' demographic characteristics are represented in Table 1. In terms of disease status, 202 participants (81.78%) were early stage, 24 (9.71%) recurrence of disease, and 21 (8.50%) were metastatic. Table 1 indicated the pathological status of the studied population in detail.

In immune-histochemical studies, 188 (92.6%) of patients were positive for estrogen receptor (ER) and 15 (7.4%) were negative. Progesterone receptor was negative in 23 (11.3%) of patients and positive in 181 (88.7%) of patients (Table 3). The classification of patients based on the results of the immune-histochemical test showed that most subtypes was related to luminal B (n=107; 52.5%) and then luminal A (n=66; 32.4%). In staging of disease according to table 7, the most patients were in STAGE IB (n=47; 27.3%). Metastasis was observed in 21 (10.4%) of the patients. Additionally, 172 (84.3%) of the studied population were alive (table 2).

Comparison based on demographic status showed no significant difference between living and deceased groups ($P\geq0.05$). Comparison between living and dead groups based on biological subtype, grade, hormonal receptors, familial history, Breastfeeding history, and

Table 1. Demographic and pathological characteristics of Studied Population

Variable		Mean± SD	Min-Max
Age (year)		48.78±1.34	25-84
Weight (kg)		67.55±1.61	60-168
BMI (kg/m^2)		27.77±5.11	16.38-44.37
Body surface (m^2)		1.37±0.29	1-2.42
Age at menarche (year)		12.76±1.01	10-16
Age at first pregnancy (year)		19.72±3.40	14-36
Age at last pregnancy (year)		34.48±4.17	20-43
Survival (year)		5.6±2.53	1-10
Characteristics		**Frequency**	**%**
Marital status	Single	43	17.3
	Married	204	82.7
Addiction	Drugs	1	0.4
	Cigarettes and Hookahs	4	1.6
	Alcohol	0	0
Menstrual Status	Pre-menopause	129	52.4
	Post-menopause	118	47.6
	Regular menstrual cycle	225	90.9
	Irregular menstrual cycle	22	9.1
Familial History	Positive	31	12.6
	Negative	216	87.4
Breast feeding History	Have	232	94.1
	Not have	15	5.9
Variable		**Frequency**	**%**
Disease Status	Early stage	202	81.78
	Relapsed	24	9.71
	Metastatic	21	8.50
Organ	Right breast	82	41.2
	Left breast	85	42.7
	Lymph node	2	1.0
	Unknown	30	15
Pathology	Invasive ductal carcinoma	226	91.4
	Invasive lobular carcinoma	19	7.8
	Others	2	0.8
Grade	I	31	15.5
	II	125	62.5
	III	44	22.0
Type of surgery	Modified radical mastectomy	190	77.1
	Breast conserving surgery	55	22.4
	Others	1	0.4

menstrual (pre/post menopause) status showed no considerable difference between the two groups (P≥0.05). Only in terms of metastasis, there was a significant difference between the two groups (P<0.05) (Table 3).

Additionally, the trend in overall survival of patients is shown below in Table 4. In survival assessment based on subtypes, it was found that the survival of luminal B was higher than other subtypes. But this difference was not significant (p >0.05)

Discussion

The present study was performed on 247 cases of breast cancer patients between 2008 and 2018. The mean age of patients was about 48 years. About 42% of patients had cancer in the left and 41% had it in the right breast. Invasive ductal carcinoma was reported in 91.4% of patients. In the immuno-histochemical assessment, 92.6% of participants were positive for estrogen, 88.7% for progesterone, 62.3% for HER2, and 74.9% for K167 receptor, and the most subtype was related to luminal B and then luminal A. Most treatments were neo-adjuvant-chemotherapy. 15.7% of all patients had died by the end of the study and the rest were alive.

The results showed two groups (alive and dead) were not significantly different in terms of demographic variables, family history, history

Table 2. Hormone receptor test findings and breast cancer characteristics in the studied population

Receptor type Frequency		Negative		Positive	
		%	Frequency	%	
Estrogen (ER)		15	7.4	188	92.6
Progesterone (PR)		23	11.3	181	88.7
HER2		77	37.7	127	62.3
K167		50	25.1	149	74.9
Variable			**Frequency**		**%**
Immuno-histochemical classification					
luminal A			66		32.4
luminal B			107		52.5
Triple negative			13		6.4
Unknown			18		8.8
Clinical stage					
STAGE IA			23		13.4
STAGE IB			47		27.3
STAGE IIA			48		27.9
STAGE IIB			44		25.6
STAGE IIIA			5		2.9
STAGE IIIB			2		1.2
STAGE IIIC			3		1.7
Metastasis & Local Relapse					
Have			21		10.4
Not have			181		89.6
Medication					
Neo adjuvant	Chemotherapy		124		54.4
	Hormone therapy		8		3.3
Adjuvant	Chemotherapy		76		30.9
	Hormone therapy		5		2
	Radio therapy		20		8.1
Target therapy			4		1.6
Follow up			1		0.4

of breastfeeding, and menstruation. There was no significant difference between the two groups in terms of tumor receptors status. Only in terms of metastasis, there was a significant difference between the two groups. The mean time of survival was 2.6 years. The survival rate of the luminal B subtype was higher than other subtypes but the difference was not significant.

A study of 309 women between the ages of 18 and 40, in the USA, found that the frequency of luminal B tumors (35%) was higher than luminal A (33%) and triple negative (21%) tumors, also the triple-negative subtype had a worse prognosis than other subtypes and the frequency of grade 2 was higher than other cases (11). In a study by Bucky et al. On 909 cancer patients (12), and in a study by Alvarado Cabrero et al. In Mexico on 1,320 women with cancer, similar to our findings, the frequency of the luminal B subtype was higher than other subtypes (13).

A study by Tiffanie Jones et al. Conducted in 2013 on 453 patients with grade 1 and 2 breast cancer without lymph node involvement, showed that the luminal A subtype was the most common subtype in patients, and the mean survival time in triple-negative patients was significantly lower than luminal A (6). In the study of Jenkins et al., Which was performed on 4621 patients between 1980 and 2010, the survival time of triple-negative patients was significantly less than the other two groups (14). A study published in 2017 in India reported 45.7% of patients were in the advanced stage (15). In a study in Pakistan, 60.7% of cases were in the second stage of the cancer (16). A study conducted in northeastern Iran with 797 patients found that 48.4% of them had early-stage breast cancer and 51.6% had advanced breast cancer. This result is similar to the CRC result with 54.5% early breast cancer and 45.5% late-stage cancer (17, 18). Also, several studies in Iran evaluated the prognostic value of breast cancer biomarkers (4, 19, 20). These studies showed no significant relation between tumor prognosis and biological markers. However, a recently published study showed that negative biomarkers (ER-, PR-, HER2-) were associated with worse prognosis in

Table 3. Comparison of patients based on demographic and clinical characteristics

Variable	Living Status	Frequency	Mean±SD	P-value*
Age	Alive	172	48.2093±10.62615	0.179
	Dead	32	51.875±14.42165	
BMI	Alive	170	27.547±4.73772	0.246
	Dead	31	29.0469±6.79061	
Age at onset of disease	Alive	18	46.5±15.65905	0.662
	Dead	7	43.5714±12.14986	
Age at menarche	Alive	170	12.7529±1.0252	0.788
	Dead	31	12.8065±0.98045	
Age at first pregnancy	Alive	153	19.8497±3.5444	0.233
	Dead	27	19±2.41788	
Age at last pregnancy	Alive	153	34.5098±3.9969	0.873
	Dead	27	34.3704±5.14519	

Variable		Living status		
Index	Sub index Frequency (%)	Alive Frequency (%)	Dead	P-value**
Biologic sub type	Luminal A	54(35%)	12(40%)	0.7
Luminal B	90(58%)	17(57%)		
Triple negative	12(8%)	1(3%)		
Grade	I	25(14.9%)	6(18.8%)	0.85
II	106(63.1%)	19(59.4%)		
III	37(22%)	7(21.9%)		
ER	Negative	15(8.8%)	0(0%)	0.08
Positive	156(91.2%)	32(100%)		
PR	Negative	19(11%)	4(12.5%)	0.81
Positive	153(89%)	28(87.5%)		
HER2	Negative	65(37.8%)	12(37.5%)	0.97
Positive	107(62.2%)	20(62.5%)		
K167	Negative	39(22.9%)	11(37.9%)	0.08
Positive	131(77.1%)	18(62.1%)		
Metastasis	No	161(94.7%)	20(62.5%)	0.0001
Yes	9(5.3%)	12(37.5%)		
Familial history	No	151(87.8%)	25(78.1%)	0.14
Yes	21(12.2%)	7(21.9%)		
Breastfeeding history	No	9(5.6%)	2(7.1%)	0.75
Yes	151(94.4%)	26(92.9%)		
Menstrual (pre/post menopause) status	Pre	79(55.2%)	10(40%)	0.15
Post	64(44.8%)	15(60%)		

* Independent T-test / **Chi-square test

Table 4. Overall survival based on life table results

Survival type	First year	Second year	Third year	forth year	Fifth year	Mean years of survival	SD
Overall survival	100%	96%	91%	90%	89%	2.64	0.13

all 4 subgroups. ER-HER2+ has a shorter survival time than ER+HER2- cases (21). Akbari et al. reported that in the absence of ER and Lympho-Vascular Invasion (LVI), the probability of tumor recurrence increased (22-25).

Conclusion

According to the frequency of disease progression in different Iranian studies (30-36%), it appears that the health system reported to strengthen early detection programs needs improvement. Given the key role of biomarkers such as steroid receptor, and C-erb (HER2) in adequately managing this disease, the importance of further research and standardized evaluation of biomarkers should focus on different regions of Iran (4).

This study evaluated the recurrence and survival pattern based on different subtypes of breast cancer in patients referred to the clinic of Imam Reza Hospital, Omid Hospital, and Reza Oncology Center, Mashhad University of Medical Sciences, Mashhad, Iran, from 2008 to 2018. The strength of this study was its access to relatively accurate patient records.

There was no significant difference between biological subtypes in terms of prognosis in our study which may be due to the small population we were studying which is the limitation of our study. Additionally, a large number of missed

data was another limitation of our study. The use of more samples in different regions can show this pattern in the whole country and develop a suitable guideline for the patients' diagnosis and treatment. Designing a study without time constraints, with an adequate and equal sample of each subtype can make a more accurate comparison between different subtypes in terms of recurrence and survival.

Ethics approval and consent to participate

The protocol for conducting the present study was approved by the ethics committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.fm.REC.1396.32). Written informed consent was obtained from all of the participants.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Competing interests

The authors have no competing interests to declare.

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Author contributions

M.M, H.E.G, and A.T: Conducted the main idea of the study. M.M: Supervision. A.S: Data gathering. H.E.G: Data analysis. A.M & A.S: Drafting of the manuscript. All authors reviewed and accepted the manuscript.

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Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

A Case Report of Renal Tubular Acidosis Type 1 without Glomerular Disease in an Adolescent with Pediatric-onset Systemic Lupus Erythematosus

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ABSTRACT

Introduction: Between 50-75% of children and adolescents with systemic lupus erythematosus (SLE) experience kidney involvement within the first year of diagnosis. The gold standard for diagnosing renal involvement in SLE is a renal biopsy. It is uncommon for SLE to cause isolated tubular involvement without any glomerular disease.

Case Presentation: We report an adolescent girl with a known history of systemic lupus erythematosus who presented to the emergency department with progressively worsening weakness. The diagnosis revealed that she had distal renal tubular acidosis (RTA) without any glomerular disease. Her history of nephrocalcinosis and kidney stones on renal ultrasound is most consistent with distal renal tubular acidosis diagnosis.

Conclusion: This case highlights the importance of considering renal tubular acidosis in lupus patients who experience recurrent hypokalemic episodes. When a patient presents with a normal anion gap metabolic acidosis and hyperchloremia, without evidence of gastrointestinal HCO3 loss or absorption of exogenous acid, renal tubular acidosis (RTA) should be considered.

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Introduction

The prevalence of kidney involvement in SLE is 50%-75% in children [1]. The kidneys are typically affected either at the time of diagnosis or within the first year [2]. It is rare for SLE to only affect the renal tubulointerstitium. It typically involves the glomerulus (known as "lupus nephritis") [1]. Although the prevalence of renal tubular acidosis (RTA) during SLE is not well-researched, Based on the available literature, the probability of RTA is very low [3].

When a patient with no signs of gastrointestinal HCO3 losses or absorption of exogenous acid presents with a normal anion gap metabolic acidosis plus hyperchloremia, RTA should generally be considered [4]. RTA type 1 or distal RTA is characterized by alkaline urine, low potassium levels, nephrolithiasis, and nephrocalcinosis [5].

Here we report an adolescent girl with a definite diagnosis of Systemic Lupus Erythematosus (SLE), based on the American College of Rheumatology (ACR) classification criteria, who developed acute quadriplegic weakness due to severe hypokalemia caused by distal Renal Tubular Acidosis (RTA).

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Case Presentation

The patient, an 18-year-old female with a history of SLE, presented to the emergency department in October 2023 with 10 days of progressive weakness.

She initially experienced general malaise, nausea, and lethargy. This was followed by muscle cramps and weakness in her right upper extremity, which progressively worsened. Over the next three days, she experienced weakness on her right side, including her lower extremity and pelvic muscles. She described poor appetite and persistent nausea but did not report any fever, diarrhea, or symptoms of a viral respiratory illness.

Three years ago, she was diagnosed with SLE after presenting with symptoms such as malar rash, photosensitivity, polyarthritis, fatigue, alopecia, oral ulcers, and positive anti-dsDNA and ANA antibodies. A year before her SLE diagnosis, the patient reported experiencing headaches, hair loss, and oral ulcers during the review of systems. Over the past three years, she has been hospitalized three times due to her systemic lupus erythematosus (SLE). She experienced hypokalemia during two admissions.

In October 2021, she was admitted for generalized muscle weakness and was noted to be hypokalemic with a potassium level of 2.4 mEq/L. She has been diagnosed with a flare-up of systemic lupus erythematosus (SLE) and post-viral myositis.

In February 2023, she was hospitalized again for muscle weakness. Her potassium on admission was critically low at 2.13 mEq/L but improved to 4.37 mEq/L by discharge one week later. The patient had a renal ultrasound during hospitalization which revealed medullary nephrocalcinosis. Additionally, a kidney stone measuring 5.3mm was incidentally observed.

After being discharged, the patient underwent two follow-up ultrasounds at two-month intervals. The first ultrasound, performed in May 2023, revealed the presence of microlithiasis and a 5.3 mm lower calyx stone in the right kidney. A follow-up ultrasound in October 2023 showed worsening nephrocalcinosis and new renal calculi. The radiologist suggested they were consistent with RTA or medullary spongy kidney.

During the presentation, the patient remained alert and oriented. Vital signs were stable with a blood pressure of 118/72 mm Hg, heart rate of 70 beats/min, respiratory rate of 17 breaths/min, and oxygen saturation of 98% on room air. The patient's temperature was 37.2°C. A physical examination revealed reduced muscle strength in the right upper and lower extremities. Cranial nerves, sensation, and reflexes were intact. She had scarring alopecia but no active rash or oral ulcerations.

The laboratory results indicate a significant drop in potassium levels to 2.0 mEq/L. additionally, the patient's serum bicarbonate level is 13 mEq/L, serum sodium is 142 mEq/L, chloride is 121 mEq/L, pH is 7.31, and anion gap is 10 mEq/L. Urine pH was 8 with no proteinuria. Ultrasound showed medullary nephrocalcinosis.

The patient was admitted to the ICU for cardiac monitoring due to severe hypokalemia. A nephrologist was consulted for suspected renal tubular acidosis. The patient was prescribed oral potassium citrate and spironolactone to take. After her serum potassium level was returned to 4.1 mEq/L and her muscle weakness improved, she was transferred to the rheumatology service in stable condition after 4 days.

The patient's recurrent hypokalemia and acidosis, history of kidney stones, and nephrocalcinosis on renal ultrasounds were most consistent with distal renal tubular acidosis secondary to SLE. She is currently taking oral potassium citrate, spironolactone, oral prednisolone, Mycophenolate mofetil, and hydroxychloroquine to maintain her SLE and distal RTA.

Discussion

Renal tubular acidosis (RTA) is a type of normal anion gap metabolic acidosis that can be classified into three subtypes based on the underlying cause. Type 1 or distal RTA is associated with limited urinary hydrogen secretion, type 2 or proximal RTA is characterized by decreased bicarbonate reabsorption, and type 4 RTA is caused by hypoaldosteronism. These subtypes can occur as a primary disorder or as a secondary condition due to other underlying medical conditions. [6–7]. In contrast to type 4, types 1 and 2 are characterized by hypokalemia due to excess renal loss of potassium. [8]. Type 1 RTA is commonly caused by autoimmune diseases such as SLE, Sjogren syndrome, rheumatoid arthritis, systemic sclerosis, thyroiditis, hepatitis, and primary biliary cirrhosis. This type of RTA can lead to nephrolithiasis, nephrocalcinosis, and bone disease [4, 9].

A renal biopsy is the gold standard for diagnosing kidney involvement in SLE [10]. A biopsy was not performed due to the absence of glomerular involvement symptoms, such as proteinuria and hematuria. Although glomerular involvement cannot be ruled out in the absence of symptoms, a renal biopsy was included in the patient's follow-up plan. Established the diagnosis of distal RTA with normal anion gap hyperchloremic metabolic acidosis, inappropriately alkaline urine (pH >5.5), and other symptoms [11].

Prompt potassium repletion rapidly improved weakness and nausea. However, severe hypokalemia can cause life-threatening

arrhythmias and arrest with potassium below 2.5 mmol/L. [12]. During hospitalization, potassium levels were closely monitored daily. follow-up testing was conducted after discharge to prevent recurrent hypokalemia. [13].

Hypokalemia accompanying distal RTA may be reversible by addressing acidosis alone. Patients may experience recurrent hypokalemia and require potassium supplements. Potassium citrate is the most effective method for alkalinizing urine in distal RTA. This provides both potassium and bicarbonate to resolve issues of acidosis and hypokalemia. [14–15]. Spironolactone has been shown to effectively correct hypokalemia [16]. Combining spironolactone and potassium citrate reduces doses and combats urinary potassium losses and acidosis in distal RTA. Upon discharge, our patient received spironolactone and potassium citrate in addition to previous lupus treatments.

Conclusion

This case emphasizes the importance of recognizing distal RTA as a complication of SLE, even when presenting solely as hypokalemia and paralysis. Maintaining a high index of suspicion for renal tubular acidosis (RTA) in lupus patients with recurrent hypokalemic episodes can enable early diagnosis and prevent life-threatening complications.

Authors' contributions

All authors have contributed to the manuscript. Conception and design: AM (Abdolreza Malek) & MV (Mahdieh Vahedi). Data collection: AB (Asma Batouri) & AK (Amir Muhammad khuban). Manuscript writing and review: MV & SS (Sepideh Seyedkaboli) & AK. All authors read and approved the final manuscript.

Ethics approval and consent to participate

In compliance with the Helsinki Declaration, informed assent or consent was obtained from the patient's parents.

Consent for publication

Consent was obtained from the parents of the patient. Our study doesn't include personal data.

Availability of data and materials

You can request the study's data from the corresponding author.

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The authors declare no competing interests.

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Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Effects of Abatacept in Patients with Rheumatoid Arthritis and Cancer Risk: A Systematic Review

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ABSTRACT

Introduction: As a chronic autoimmune disease, Rheumatoid arthritis (RA) affects the joints. Studies have shown a complex and challenging link between cancer and RA. However, articles claim a significant relationship between cancer and treatment with DMARDs and biological DMARDs (e.g., Abatacept); however, the results are contradictory. Accordingly, this systematic review investigates the prevalence of cancer in RA patients taking Abatacept.

Methods: We searched for articles published in four databases, namely Web of Science, Medline, PubMed, and Scopus up to September 29, 2023. The methodology followed recommendations from the Cochrane Handbook. During the search process, we selected articles using keywords such as "rheumatoid arthritis", "malignancy", and "cancer" with the Boolean operators "AND" and "OR"."

Results: A total of 12 studies were considered, the majority highlighted the effectiveness of Abatacept as an anti-RA medicine in the risk of cancer prevalence. Most of the patients investigated in the trials were female. Lung cancer was the greatest malignancy in those suffering from RA diseases. However, these investigations found no significant link between Abatacept use and cancer risk.

Conclusion: There is speculation regarding the potential use of rheumatoid arthritis drugs in treating RA and its potential association with cancer incidence. According to the findings presented in this review article, there was no statistically significant association between the utilization of Abatacept and the prevalence of cancer in patients who were administered Abatacept either as a standalone treatment or in combination with other anti-rheumatoid medications. However, it is advised that further clinical trials be conducted to thoroughly investigate this association.

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Introduction

As an inflammatory autoimmune condition, Rheumatoid arthritis (RA) initially targets hands and feet joints and in later stages affects larger joints (1). RA prevalence and incidence rates are increasing worldwide, with almost 20 million people affected globally (2). Patients with RA manifest a vast spectrum of symptoms, such as extra-articular complications, chronic joint deformities, joint stiffness, pain, and swelling. A fundamental part of RA management is medications. They can be categorized into three groups: 1) symptomatic drugs, such as

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acetaminophen and opioid analgesics, which alleviate symptoms and pain; 2) disease-modifying antirheumatic drugs (DMARDs) including biologic and nonbiologic types that treat inflammation and damage to joints; and 3) glucocorticoids, which promote symptom relief and reduce the progression of the disease (3).

There appears to be some association between RA drug treatments and an increased risk of cancer; however, such hypotheses need to be proven (4). Additionally, studies showed an association between an increased risk of malignancy and RA, especially non-melanoma skin cancer (NMSC), malignant lymphomas, and lung cancer (5-9). RA is now treated with a variety of medications, including tumor necrosis factor (TNF) antagonists, androgen deprivation therapy (ADT), Tofacitinib, Abatacept, and other biological DMARDs (bDMARDs) (10-12).

Since the introduction of bDMARDs to treat chronic rheumatoid disease, one of the critical focuses has been on a greater prevalence of cancers (13). Recently, there have been some concerns regarding the potential risk of cancer associated with the administration of bDMARDs, in particular, Abatacept, a fusion protein of CTLA-4 (T-lymphocyte-associated protein 4) (14). However, there is a dearth of data on Abatacept safety, especially from first-line studies (12). Some research indicated the probable association of Abatacept with a higher NMSC risk compared to bDMARDs (15, 16). Evidence, however, showed that physicians were prescribing Abatacept more often to older patients with more comorbid conditions and long-term diseases (3).

In general, prescribers perceive Abatacept as a first-line treatment that has a better safety profile, which may encourage their use in cancer-affected populations (12). Some of these agents are suspected to carry a cancer risk that has led researchers to require warnings (17). Patients and providers are concerned about these warnings because RA is a chronic condition requiring long-term treatment (16). The present study reviews the effect of Abatacept, an anti-rheumatoid medication, on the risk of cancer prevalence in this study.

Materials and Method

Abatacept, a class of bDMARD medications, was used to treat RA, and its effect on cancer risk was investigated in this systematic review. The research process followed the seven stages of the Cochrane Handbook for Systematic Reviews. These stages included considering the inclusion and exclusion criteria, conducting a thorough search for collecting data from the database, excluding unrelated studies, assessing the quality evaluation, retrieving data, and investigating the extracted data (18).

Inclusion and Exclusion Criteria

The eligibility criteria were determined for this study through Participants, Intervention, Comparison, and Outcome Study research. The primary inclusion criteria were RA and treatment programs, cancer incidence, sample size greater than 10, human clinical trials, and publication in English. All studies that did not use Abatacept as medicine were excluded, followed by case reports or case series, nonclinical studies, editorial letters, review articles, short communications, qualitative investigations, and meta-analyses. On the other hand, all case controls, as well as retrospective and prospective investigations on human samples evaluating RA and cancer risk, were included in the current review due to the observational purpose of the topic.

Literature search

All articles published in Web of Sciences, Medline, PubMed, and Scopus from 5 February 2022 to 29 September 2023, were searched using such keywords as “cancer,” “malignancy,” and “rheumatoid arthritis.”

Data extraction and study design

Based on a systematic review, researchers examined the effect of Abatacept therapy on cancer risk among cases with RA. In the initial phase, four selected databases (Web of Sciences, Medline, PubMed, and Scopus) were researched up until September 29th, 2023. Afterward, the number of relevant articles was determined. Following that, the relevance of titles and abstracts was assessed. The screening process considered eligibility criteria. According to the study’s purpose, full-text articles were obtained for final screening. As part of the process, articles that were duplicates, irrelevant, non-English, or lacking enough data were excluded from the research process. Two researchers carried out all of the research in the same way and independently reviewed the study titles and abstracts. Information was constantly exchanged among researchers. After agreeing on the goals, they collected the necessary information. Finally, the collected information was entered into a checklist. The PRISMA flow chart depicts all of the steps involved in selecting articles (Figure 1).

Bias risk and assessment of quality

Based on the Cochrane risk of bias tool, the bias risk in the submitted studies was determined

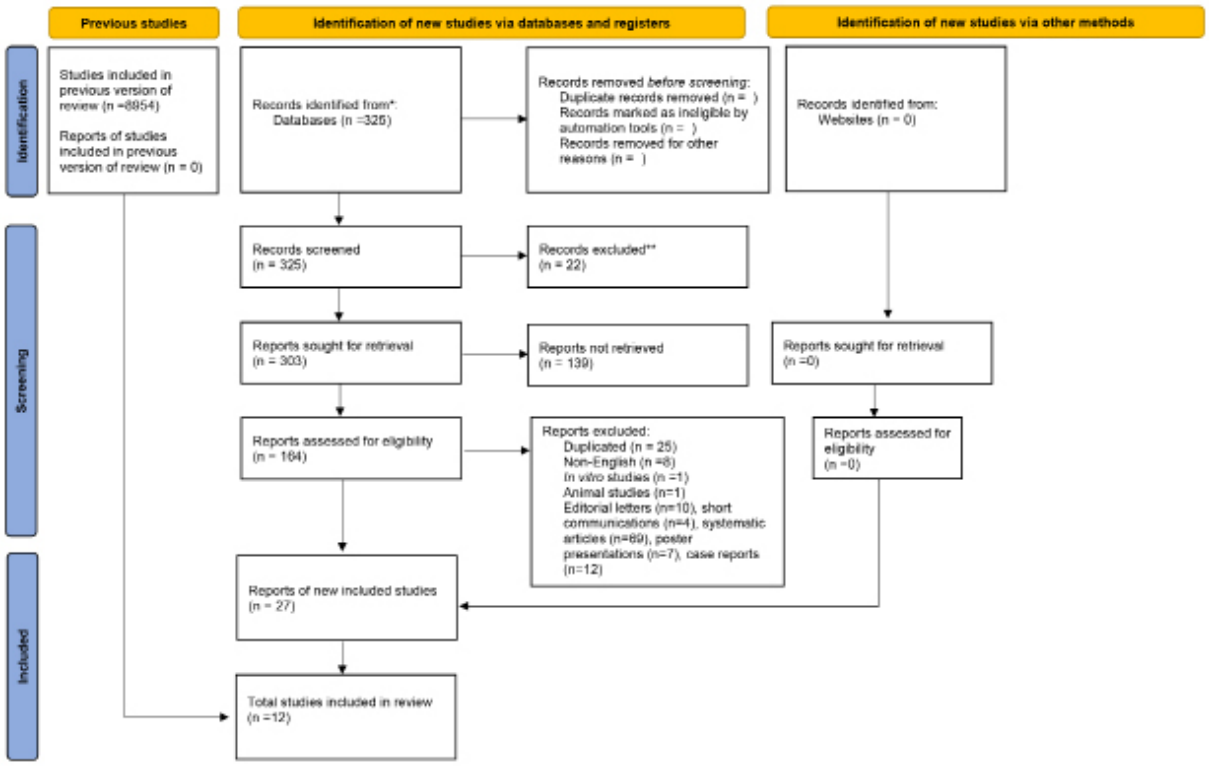

Figure 1. PRISMA flowchart showing the selection process in the study

through consideration of eight factors (18) .

A summary of results and outcomes

In total, 325 articles were found in the databases. After removing irrelevant titles and abstracts (n=139) and 25 duplicated articles, we excluded the articles written in languages other than English (n=8), followed by articles describing experimental or *in vitro* studies (n=1), animal research (n=1), editorial letters (n=10), short communications (n=4), reviews of narrative and systematic articles (n=69), poster presentations (n=7), articles with unavailable full texts (n=7), and case reports or series (n=29). Furthermore, irrelevant papers (n=28) and articles not focusing on Abatacept as an RA medication (n=134) were removed from the research process. In the end, 12 articles were included in this study, comprising retrospective observational studies [n=6; 50%] and prospective observational studies [n=6; 50%]. These studies were conducted in various regions, with the majority taking place in European countries such as France, the United Kingdom, Sweden, Germany, the Czech Republic, Denmark, Italy, and Britain. It is worth mentioning that 3 studies were conducted in the US and 1 study was conducted in Canada. Additionally, one of the submitted articles was conducted in Japan; however, no research was found to be conducted in Africa.

These studies were conducted on a total of 86,894 patients with RA who took Abatacept. All participants were over the age of 18 and the majority were female. and. The studies were conducted over a period of 9.45 years on average. The included studies investigated various types of cancer, including breast cancer (n=7; 58.33%), lymphoma (n=8; 66.66%), lung cancer (n=10; 83.33%), melanoma (n=4; 33.33%), NMSC (n=3; 25%), cervical cancer (n=2; 16.66%), central nervous system (n=2; 16.66%), ovarian (n=2; 16.66%), colon cancer (n=4; 33.33%). Additionally, one of the submitted articles investigated the prevalence of various types of cancer, including renal, hematopoietic, urinary, uterus, digestive, pancreas, liver, prostate, duodenal, thyroid, and testicular cancers. The prevalence of various cancers was investigated in two studies.

Table 1 summarizes the information obtained from each study, including the study's initiation and termination dates, number of participants, age, gender ratio, study type, cancer type, and medicine type. Table 2 shows the concomitant disease and drug prescribed for the risk of cancer in monotherapy or combination therapy. Monotherapy with Abatacept was used in all studies except for Hashimoto's study, in which patients received Abatacept in combination with tocilizumab.

Table 1. Data extracted from the included studies

Author/year	Type of study	Sample size	Age	Ratio f/m	Type of cancer	Medicine	Concomitant disease	Follow-up	Outcome	Period of patient collection
Kyung Min Ko (2023) (33)	prospective, observational cohort study,	5,023 (on Abatacept: 5)	Mean 46(37-55)	3,544 (85.5) (f)/ 599 (14.5)	Stomach, colon, lung, brain and central nervous system		Cardiovascular disease: 2226, Interstitial lung disease: 68, Pulmonary tuberculosis: 424, COPD: 168, Diabetes mellitus: 652, Liver disease: 164, Gastric ulcer: 450, Thyroid disease:588	--	Patients with RA have a lower overall risk of cancer than people in the general population. Patients who had thyroid disease and had had their RA for a longer time were more likely to get cancer than those who took hydroxychloroquine.	8 years
Teresa A Simon (2023) (34)	prospective observational	abatacept (*n* = 5182), csDMARDs (*n* = 73,755), and other b/tsDMARDs (*n* = 37,195)	≥18	—	Lung, Breast, Lymphoma	Abatacept, csDMARDs, b/tsDMARDs	Hypertension, Diabetes, Heart failure, Myocardial infarction, COPD, CKD	3.1 years for abatacept	A few incidences of lymphoma were found in a few of the registries; ARTIS (Sweden) found that abatacept had a rate ratio (RRs) of 2.8 (95% CI 1.1-6.8) compared to csDMARDs in these cases. When compared to csDMARDs and b/tsDMARDs, the abatacept pooled RRs (95% CIs) for total cancer were 1.1 (0.8-1.5) and 1.0 (0.8-1.3), respectively.	10 years
Yosuke Kunishita (2023) (35)	multicenter, retrospective study	312	70.85±11.65	263 (f)/ 49 (m)	Duodenal, Thyroid, Colon, Lung, Malignant, lymphoma, Ovarian	abatacept, anti-cyclic citrullinated peptide antibody, b/tsDMARDs, methotrexate, prednisolone, alazosulfapyridine, tacrolimus	Interstitial lung disease (n=70)	3.55 years	Patients with a history of cancer received Abatacept just as effectively and safely as those without.	12 years
Sibylle de Germay(2020) (20)	Original article	15846	≥18	3830 (24.2) (f)	Breast, lymphoma, melanoma, lung, and NMSC	Abatacept and those exposed to other bDMARDs	--	--	There was a significant association between Abatacept and increased risk of reporting melanoma in patients with RA, compared to those with other bDMARDs	10 years
Franc¸ois Montastruc (2019) (12)	Original article	64 188 patients (4328 on Abatacept vs 59 860 on other bDMARDs)	≥18	49428 (f)/ 14760 (m)	Breast, lung, lymphoma, melanoma, and NMSC	Abatacept and bDMARDs	Hypertension: 1405 (32.5%), Type 2 diabetes: 587 (13.6%), Asthma: 279 (6.4%), Chronic obstructive pulmonary disease: 294 (6.8%), Chronic kidney disease: 97 (2.2%), Leukopaenia: 30 (0.7%), Neutropaenia: 18 (0.4%), Peripheral arterial disease: 54 (1.2%), Hyperlipidaemia: 891 (20.6%), Cardiovascular disease: 1076 (24.9%), Autoimmune disease (excluding RA): 771 (17.8%)	6 m	When compared to other bDMARDs, the utilization of the Abatacept as the first bDMARDs regarding the RA treatment was correlated with a slight increase in the overall risk of cancer, specifically, non-melanoma skin cancer.	7 years

Teresa A. Simon (2019) (22)	Retrospective	n = 17,517+12,120+3354= 32991//OTHERS: 59026	≥18		Lung, lymphoma, breast, and NMSC	Abatacept versus other b/tsDMARDs	Cardiovascular disease: 64, Autoimmune diseases: 54		The specific cancers and infection risks showed no significant difference between patients in the Abatacept and other b/tsDMARDs groups in this real-world multi-database study.	8 years
T A Simon (2009) (30)	Extended report	4134	<20 - ≥75	3323 (80)	All malignancies (excluding NMSC)	Abatacept	--	2.1 year	The Abatacept CDP's IR for total malignancy (excluding NMSC), colorectal, breast, lymphoma, and lung cancers were comparable to those in a comparable RA population. The results indicate that there are no new safety signals for malignancies, which will be closely monitored.	5 years
Hjalmar Wadström (2017) (19)	Original investigation	2021	mean 61 (51-68)	20 m	Breast, colorectal, lung, and lymphoma	Abatacept	COPD: 6 %, Diabetes: 10%, IHD: 11%	--	With one exception that requires replication treatment with TNFi (as a first or second bDMARDs), it does not appear that Tocilizumab, Abatacept, or Rituximab increase the overall occurrence of malignant neoplasms; moreover, there were no signals of increased risks for specific cancer types.	3 years
Seoyoung C. Kim (2016) (21)	A cohort Study	14729	≥ 18	f	Cervical	DMARDs	Diabetes: 21%, Chronic kidney disease: 3 %, Liver disease: 8%,	--	When compared to nonbiologic DMARDs, initiation of the biologic DMARD therapy showed a correlation with a numerically significant but not statistically significant increase in the risk of high-grade cervical dysplasia or cervical cancer among women with RA.	12 years
Atsushi Hashimoto (2015) (29)	Prospective cohort	66953	mean 62.7	81.6% female and 18.4% male	Lung, gastric, breast, lymphoma, and colon, as well as overall malignancies	Non-anti-TNF biologics (including Tocilizumab and Abatacept)	--	--	Although patients with RA revealed no higher overall rate of cancer, lymphoma was significantly more common in them, compared to the general population.	9 years
Louise K Mercer (2016) (31)	Clinical and epidemiological research	1563	mean 57.4 (56-58.2)	78.4 (77-79.2) f/	Melanoma	Abatacept	--	4399 (pyr)	No evidence of an increased risk of melanoma was indicated in this large European collaborative study as a result of TNFi exposure.	9 years

Viking Huss (2021) (32)	Original article	3306	(50-68) 60	male 19%	Prostate, testicular, breast, hematopoietic, renal, lung, colorectal, ovarian, cervical, urinary, CNS, uterus, digestive, pancreas, and liver	Abatacept	Diabetes mellitus: 10%, Hypertension: 25%, IHD: 10%, CHD: 5%, COPD: 6%, Renal insufficiency: 2%, Joint replacement: 19%	years 3.8	Findings are generally reassuring for other b/tsDMARDs and site-specific risks, but they contain signals that require further investigation.	years 17

COPD: chronic obstructive pulmonary disease, IHD: ischemic heart disease

Table 2. Shows the concomitant disease, and drug prescribe the risk of cancer in the in monotherapy or combination therapy

Auther/year	Concomitant disease	Drug prescribe	Monotherapy (abatacept) OR combination (abatacept + X)	Risk of cancer in Monotherapy or combination
Kyung Min Ko (2023) (33)	Cardiovascular disease: 2226, Interstitial lung disease: 68, Pulmonary tuberculosis: 424, COPD: 168, Diabetes mellitus: 652, Liver disease: 164, Gastric ulcer: 450, Thyroid disease:588	Hydroxychloroquine (n= 1,409), Sulfasalazine (n=681), Leflunomide (n=1288), NSAIDs (n=3336), Corticosteroids (n=3063), Bisphosphonate (n=1009), Abatacept (n=2), Adalimumab (n=35), Etanercept (n=61), IL-17 inhibitors (n=1), Infliximab (n=53), Rituximab (n=13),	Monotherapy	Incidence of malignancies in people on Abatacept =0
Teresa A Simon (2023) (34)	Hypertension, Diabetes, Heart failure, Myocardial infarction, COPD, CKD	infliximab, etanercept, adalimumab, certolizumab pegol, golimumab, tocilizumab, rituximab, tofacitinib, anakinra, methotrexate, hydroxychloroquine, sulfasalazine, azathioprine, tacrolimus, gold sodium thiomalate, leflunomide, aurothioglucose, auranofin, cyclosporine, penicillamine, and cyclophosphamide	Monotherapy	Crude incidence rates (IRs) per 1000 patient-years of overall malignancy ranged from 7.6–11.4 (abatacept)
Yosuke Kunishita (2023) (35)	Interstitial lung disease (n=70)	b/tsDMARDs (n=223), methotrexate (n=134), prednisolone (n=96), salazosulfapyridine (n=35), tacrolimus (n=17)	Monotherapy	Following the use of Abatacept, the incidence of cancer was 1080.3 per 100,000 person-years.
Sibylle de Germay(2020) (20)	—	Carcinogenic drugs (n=208 patient): ethinylestradiol, estradiol, estriol, estrone, estrogen, tibolone, diethylstilbestrol, tamoxifen, busulfan, chlorambucil, mephalan, semustine, thiotepa, treosulfan, chlormethine, vincristine, procarbazine, etoposide, chlornaphazine, methoxsalen and phenacetin	Monotherapy	Mono: [ROR 0.98 (95% CI 0.91, 1.05)].
Franc¸ois Montastruc (2019) (12)	Hypertension: 1405 (32.5%), Type 2 diabetes: 587 (13.6%), Asthma: 279 (6.4%), Chronic obstructive pulmonary disease: 294 (6.8%), Chronic kidney disease: 97 (2.2%), Leukopaenia: 30 (0.7%), Neutropaenia: 18 (0.4%), Peripheral arterial disease: 54 (1.2%), Hyperlipidaemia: 891 (20.6%), Cardiovascular disease: 1076 (24.9%), Autoimmune disease (excluding RA): 771 (17.8%)	MTX: 2116 (48.9) Other csDMARDs: 1709 (39.5), Parenteral antibiotics : 208 (4.8), Oral corticosteroids: 2506 (57.9), Other corticosteroids : 1167 (27.0) NSAIDs : 1767 (40.8) , Cholesterol-lowering medication: 954 (22.0)	monotherapy	Hazard ratioadjusted (HR) 1.17; 95% CI 1.06, 1.30)
Teresa A. Simon (2019) (22)	Cardiovascular disease: 64, Autoimmune diseases: 54	csDMARDs:184, b/tsDMARDs: 150, Glucocorticoids: 186	monotherapy	(HR [95% CI] 1.09 [1.02–1.16])
T A Simon (2009) (30)	—	Oral corticosteroids: 2657 (64%), NSAID: 3113 (75%)	monotherapy	Incidence of malignancies per 100 person-years (95% CI) in the abatacept clinical trial experience: SIR 0.61, 95% CI 0.45 to 0.80)

Hjalmar Wadström (2017) (19)	COPD: 6 %, Diabetes: 10%, IHD: 11%	csDMARD: 56%, Prednisolone: 58%, NSAID: 43%	monotherapy	crude incidence per 100 000 person-years: 61 (1026)
Seoyoung C. Kim (2016) (21)	Diabetes: 21%, Chronic kidney disease: 3 %, Liver disease: 8%,			Risk of high-grade cervical dysplasia or cervical cancer : Hazard ratio (95% CI) : 1.25 (0.78–2.01), Incidence rate per 1,000 person-years (95% CI): 1.59 (1.20–2.11)// Rate ratio of any gynecologic procedures: Incidence rate per 1,000 person-years (95% CI): 135.8 (129.1–142.8), Rate ratio (95% CI): 0.99 (0.90–1.09)
Atsushi Hashimoto (2015) (29)	_	_	Combination: tocilizumab and abatacept	Overall incidence of malignancies: (standardized incidence rates) 0.89, 95% CI 0.82–0.97)
Louise K Mercer (2016) (31)	_	_	monotherapy	SIR : 1.6 (95% CI)
Viking Huss (2021) (32)	Diabetes mellitus: 10%, Hypertension: 25%, IHD: 10%, CHD: 5%, COPD: 6%, Renal insufficiency: 2%, Joint replacement: 19%	_	monotherapy	1.2, 95% CI: 1.0, 1.3

csDMARDs: conventional synthetic disease-modifying antirheumatic drugs, b/tsDMARDs : targeted synthetic disease-modifying antirheumatic drugs, NSAID: non-steroidal anti-inflammatory drugs, COPD: chronic obstructive pulmonary disease, IHD: ischemic heart disease

The risk of bias identification

A total of 12 articles were investigated in this study. The quality of these articles was evaluated using the Cochrane guidelines, which consist of seven domains. 'Yes' and 'No' options were employed to mark the low and high risks of bias, respectively, in the evaluation of bias risk. The term 'unclear' was used to describe a risk of bias that was unclear or unknown. Table 3 summarizes these factors related to confounders, participants' selection, the intervention measurement, missing data, selective reporting, measurement outcome, a departure from the intended intervention, and other factors. Disease activity and duration of

Table 3. Risk of bias in the included studies (risk of bias tool by Cochrane)

Auther/year	Random sequence generation	Allocation Concealment	Blinding of participant, personal	Blinding of outcome assessment	Attrition bias	Incomplete outcome data	Selective reporting	Free of other biases	Risk of bias
Kyung Min Ko (2023) (33)	No	Unclear	No	Yes	No	No	No	No	Intermediate
Teresa A Simon (2023) (34)	No	Unclear	No	Yes	No	No	No	No	Intermediate
Yosuke Kunishita (2023) (35)	No	Unclear	No	Yes	No	No	No	No	Intermediate
Sibylle de Germay(2020) (20)	Yes	Yes	No	Yes	No	No	No	No	Low
Franc¸ois Montastruc (2019) (12)	Yes	Yes	No	Yes	No	No	No	Yes	Intermediate
Teresa A. Simon (2019) (22)	Yes	Yes	No	Yes	No	No	No	Yes	Intermediate
T A Simon (2009) (30)	No	No	No	No	No	Yes	No	Yes	Intermediate
Hjalmar Wadström (2017) (19)	Yes	Yes	No	unclear	No	No	No	Yes	Intermediate
Seoyoung C. Kim (2016) (21)	Yes	Yes	No	Yes	No	No	No	Yes	Intermediate
Atsushi Hashimoto (2015) (29)	Yes	Yes	No	Yes	No	No	No	Yes	Intermediate
Louise K Mercer (2016) (31)	No	Yes	No	Yes	No	Yes	No	Yes	Intermediate
Viking Huss (2021) (32)	Yes	Yes	No	Yes	No	No	No	Yes	Intermediate

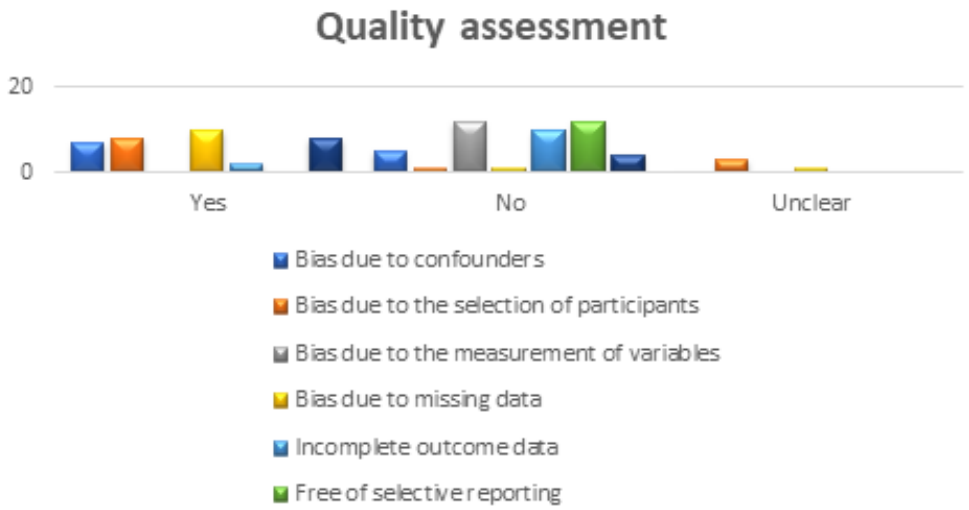

Figure 2. Quality assessment of the articles in the review process

disease were not recorded in the studies. The assessment of the selected articles' quality is presented in Figure 2.

Discussion

Several previous observational cohort studies have investigated the effect of Abatacept and other bDMARDs as first- or second-line treatments for RA on specific cancer patients. One study found no link between Abatacept use and the development of the first invasive solid or hematologic malignant neoplasm (not including NMSC). However, patients who received Abatacept revealed a higher risk of developing the first invasive squamous cell skin cancer compared to those who received TNF-α inhibitors (19). According to a study by Germay, the utilization of Abatacept is linked to higher melanoma risk in RA cases than other bDMARDs. They suggested that it was reasonable to closely monitor patients who had been exposed to Abatacept for melanoma; however, adverse reactions to this drug may occur rarely (20). Montastruc et al. demonstrated a statistically significant increase in overall cancer risk (17%) in comparison with patients who received other bDMARDs as treatment and those initiating treatment with Abatacept. Except for NMSC, the cancers under concern (lymphoma, breast, lung, and melanoma) showed no statistically significant differences. In sensitivity analyses, the results obtained from Abatacept and cancer risk stayed unchanged (12).

Molecular Mechanism of Abatacept

Inhibitory molecules, such as CTLA-4, have a key role in preventing T cells from becoming activated. According to a cohort study in the US, the overall prevalence of high cervical dysplasia or cervical cancers in the entire research cohort was estimated at 1.30 per 1,000 people/year. Furthermore, the high-grade cervical dysplasia and cervical cancer risk was 1.3 times more significant with an expansive 95% confidence level interleaved the negative value in female RA patients who started treatment with a biologic DMARD with or without a nonbiologic DMARD in comparison with patients who began their treatment with a nonbiologic DMARD only (21).

A slight improvement in total cancer risk can be attributed to Abatacept vs. other b/tsDMARD treatment that was discovered in a report by Simon et al. They utilized three large healthcare databases in the US to extract data and show safety outcomes in RA patients. However, the differences did not reach statistical significance (22).

Moreover, it is noteworthy that Abatacept has been shown to have a similar malignancy risk in some interventional trials and real-world analyses when compared to placebo or other comparators (23-25). There are several possible explanations for the modest but significant increase in average malignancy danger observed following Abatacept treatment, including Abatacept's distinctive upstream molecular mechanism and variations in patient characteristics. Abatacept is a CTLA-4 analogue. Inhibitory molecules, such as CTLA-4, can effectively prevent T cells from becoming activated. Therefore, this explains why Abatacept is being used to treat autoimmune and inflammatory diseases. Regarding the biology of cancer, the CTLA-4's role is more complicated involving tumor progression and a weakened anti-tumor response ;(26) however, there are controversies concerning this issue, and the clinical significance remains unknown (27). Furthermore, Abatacept prevents the

CD80/CD86:CD28 costimulatory signal, which is considered necessary for the activation of full T cell. As a result, this could affect immune responses to tumors while also reducing pathogenic autoimmunity (28).

Rheumatoid arthritis, cancer, and the role of race, age, and gender

The total incidence of malignancies differs in different studies, as does the incidence of each malignancy type based on racial or regional characteristics. In comparison with an age- and gender-matched Japanese population, the study of Japanese RA patients showed a slightly, however, significantly lower total incidence of all types of malignancies other than an excess of lymphoma. They also reported reduced rates of some cancer types, such as liver and colorectal, resulting in fewer malignancies overall. Hashimoto et al. suggest that it is critical to distinguish between RA characteristics (particularly long-term disease activity) and the effect of environmental or therapeutic factors, as well as comorbidities when studying the malignancy risk among RA patients. Additionally, these factors need to be looked into further. More research is needed to figure out what causes an increase in lymphoma but a decrease in the incidence of some other cancers in RA patients (29). Simon's research revealed that breast, colorectal, lung, and lymphoma incidence malignancy rates (except for NMSC) in the Abatacept clinical development program were the same as those in a comparable RA population (30).

Rheumatoid arthritis and cancer incidence: the effect of the treatment period

The findings of the study conducted by Wadström et al. were also in agreement with the preceding results. They discovered that RA patients who started therapy with TNFi or non-TNFi had the same overall cancer risk as patients with bDMARD-naive RA (19). Nonetheless, the last signal of **an** incremented **risk** of melanoma after TNFi highlighted by previous studies was not replicated across the registries in this study despite the European collaborative project of 11 registers from nine countries (31). Moreover, they found no evidence of a notable rise in both age and gender standardized incidence ratios. In terms of melanoma incidence, there was no notable difference between biologic-naive patients and those who had received TNFi, RTX, or Abatacept, or TOC.

The longest average cancer risk follow-up in RA cases treated with a b/tsDMARD (17 years) was performed by Huss et al. (32). The study found no significant increase in cancer incidence with TNF inhibitors and observed no trends with time since treatment start, time on active treatment, or age attained, in comparison with b/tsDMARD-naive cases. Even when estimates of some relative risk were (statistically significantly) higher than 1, they found no steady signal of incremented total cancer risks with other bDMARDs. Regarding the relative risk for 16 cancer sites, they found some statistically notable relationships with the TNFis Rituximab and Abatacept for urinary tract cancer.

Limitations and suggestions

According to the studies, we found no statistically notable link between the risk of Abatacept use and the prevalence of malignancy. However, studies suggest that this medicine and other effective RA prescriptions be used with greater caution. Furthermore, one of the significant flaws in the studies reported in this review article is no mention of medicine doses among patients, even when they are of different ages and genders. This could be one of the essential factors confounding the outcomes. Moreover, the use of biologics in combination with other immunosuppressive drugs can raise malignancy risk in these patients. This issue may have distorted the findings of the reviewed studies. Finally, it is recommended that clinical trials focus on the dose and duration of Abatacept and compare them to the control groups in the future.

Conclusion

In comparison to other biologic antirheumatic drugs (bDMARDs), the administration of Abatacept as the initial bDMARD in rheumatoid arthritis has shown a rise in the overall cancer risk, specifically in relation to non-melanoma skin cancer (NMSC). Nonetheless, the rate of malignancies in RA patients was not significantly greater in comparison with the general population. The obtained results indicate that there is no additional indication of safety concerns related to the development of malignancies. However, it is important to note that ongoing surveillance will be conducted to closely monitor this matter. Further studies are required to substantiate the use of Abatacept in those suffering from RA.

Conflict of interest

The authors declare no competing interests.

Authors' contributions

Drafting of the manuscript and screening the article was done by (Homapoor S, Sahebari M). Conception and design was done by (Khodashahi M, Homapoor S). Critical revision of the manuscript

for important intellectual content and double review to minimize bias was conducted by authors (Khodashahi M, Sahebari M, Homapoor S).

Ethics approval and consent to participate

This is a systematic review article and all ethics approval and consent of used articles was checked. No aspect of this article was related to laboratory animals, special human illnesses, and/or the use of people's information.

Consent for publication

"Not applicable."

Availability of data and materials

All data from this study are included in the published article and its supplementary files.

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Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Enhancing Growth in Epidermolysis Bullosa: Nutritional Supplements and Dietary Interventions for Children and Adolescents

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ABSTRACT

Introduction: Epidermolysis bullosa (EB) represents a diverse set of disorders that affect the skin and mucous membranes. Ensuring proper nutrition for children and adolescents with Epidermolysis Bullosa is a vital aspect of their treatment plan. The objective of this study is to demonstrate how nutritional intervention in a specialized nutrition clinic can enhance their well-being.

Methods: This longitudinal study was conducted over a 3-year period at Akbar Children Hospital, a tertiary facility affiliated with Mashhad University of Medical Sciences in Iran. The study included all patients diagnosed with EB based on clinical symptoms and genetic studies.

Results: In the present study, the median (25-75 IQR) age of the participants was 81.0 (36.0-156.0) months, and 19% of the participants were girls. The median (25-75 IQR) weight was 17.5 (10.8-24.5) kg, and the mean ± SD of height was 109.9 ± 31.1 cm. Among all types of malnutrition, there was only a significant association between gastrointestinal complications and BMI-for-age z-score (OR: 0.08, P-value=0.039) in the crude model. After adjustment, there was no significant association between gastrointestinal complications and malnutrition. The mean values of weight at the baseline, the first, and the second appointment of the study were 21.3, 21.2, and 24.8 kg, respectively. Moreover, the mean height at the baseline, the first, and the second appointment of the study were 109.4, 121.0, and 123.4 cm, respectively.

Conclusion: Regular clinic visits and tailored nutritional interventions positively impact EB patients, emphasizing the importance of managing anemia and deficiencies for their well-being.

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Introduction

Epidermolysis bullosa (EB) represents a diverse set of disorders that affect the skin and mucous membranes. In certain cases, it can lead to complications in organs such as the gastrointestinal tract, eyes, and genitourinary system (1). The classification of EB has been recently updated and now comprises four main categories: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and over 35 distinct subtypes of EB. Additionally, there's an exceedingly rare form called Kindler EB (KEB) (2, 3).

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The prevalence of EB varies across different countries. Globally, approximately 50 out of every 1 million live births are diagnosed with EB, affecting 9 out of every 1 million individuals. Among these cases, about 92% are categorized as EBS, 5% as DEB, 1% as JEB, and 2% remain unclassified (4). In India, the estimated incidence is around 54 per million live births, according to data from the National Epidermolysis Bullosa Registry. In Iran, due to the absence of a dedicated registry system, the prevalence is unknown, but our center sees around 60 patients. (5). Only one EB registry study publishing in 2021 demonstrated that in Iran, a total of 538 individuals with Epidermolysis Bullosa (EB) were recorded, which translates to approximately 6.72 patients per 100,000 persons. The distribution of these cases was nearly equal among males and females. Among the 103 patients for whom the disease type was determined by a pathologist, 78 patients (75.7%) were diagnosed with the dystrophic type, 13 (12.6%) with the junctional type, 9 (8.7%) with the simplex type, and 3 (2.9%) with the kindler type. The most frequently reported complaints among these patients were related to dysphagia, followed by issues with tooth damage (6).

Ensuring proper nutrition for children and adolescents with Epidermolysis Bullosa is a vital aspect of their treatment plan (7, 8). This is crucial because the condition increases their metabolic demands, placing added stress on their immune system and the healing process. Furthermore, the symptoms of the disease can hinder the intake and absorption of essential nutrients, potentially leading to inadequate growth and developmental challenges (7-10). Furthermore, malnutrition in individuals with EB is often a consequence of a combination of reduced food intake and heightened nutrient requirements. This condition can manifest as failure to thrive, delayed puberty, anemia, and a series of clinical and biological complications. These factors collectively contribute to the impediment or slowing down of the wound healing process (11). Additionally, nutritional depletion and protein-energy malnutrition can lead to changes in immunocompetence, potentially increasing the susceptibility of EB patients to secondary bacterial infections, as documented in various reports (12).

As a result, nutritional status is greatly impacted by various complications like malnutrition, anemia, infections, dental issues, and ultimately, growth problems. Therefore, nutritional support plays a pivotal role in managing these patients (7). The objective of this study is to demonstrate how nutritional intervention in a specialized nutrition clinic can enhance their well-being.

Materials and Method

Participants: This longitudinal study was conducted over a 3-year period at Akbar Children Hospital, a tertiary facility affiliated with Mashhad University of Medical Sciences in Iran. The study included all patients diagnosed with EB based on clinical symptoms and genetic studies. Patients were categorized into two major subtypes based on clinical manifestations and genetic assessments conducted by dermatologists: EBS, JEB, or DEB.

Data Collection

Medical History: A comprehensive medical history of the disease was collected using a checklist specifically designed for assessing the nutritional status of EB patients. In present study we follow up EB patients every month for one year. Additionally, we monitored the changes in weight and height at four-month intervals. This allowed us to calculate the mean weight and height every four months, and in the end, we analyzed the trends in these measurements over the course of one year of the study.

Dietary Requirements: Subsequently, the energy and protein requirements were estimated for each patient based on their assigned subtype.

Nutritional Support: The method of food administration and the need for nutritional support were determined, taking into account the patient's clinical subtype.

Micronutrient Supplementation: Micronutrient supplements, including iron (Fe), zinc (Zn), calcium (Ca), and vitamin D3, were prescribed and administered as needed. In EB patients with minimal blistering and no gastrointestinal involvement, their nutritional needs are similar to those of healthy children of the same age and sex. Here, we provide a complete algorithm outlining the nutritional management protocol for EB patients (Figure 1) (13).

Infection Control: Addressing skin infections is crucial in EB patients, as inflammation is a major factor contributing to growth retardation and the risk of skin cancer. We conducted wound exudate cultures and administered appropriate antibiotics in cases of active infection, based on antibiogram results.

Anemia: Iron deficiency anemia (IDA) is a common micronutrient deficiency in EB patients (14). In our center, we followed a specific treatment algorithm for patients with IDA (Figure 2).

Nutritional Assessment and Management: We conducted a thorough dietary history

assessment using a dedicated checklist for EB patients. This included information about food consistency, gastrointestinal complications, time taken for meals, method of food delivery, and vitamin or nutritional supplement intake.

In the next step, a 24-hour dietary recall was used to determine the typical meal pattern and median total energy consumption.

Energy Calculation: Energy needs in EB patients depend on three factors: actual weight, skin involvement, and the presence of sepsis. We calculated their energy requirements using an equation to obtain the target Energy intake. We then gradually increased their calorie intake based on their average intake from the 24-hour recall to achieve the target. The calculation for the energy needed for catch up growth is as follows:

Energy requirements (kcal) = (current weight × appropriate age for height (kcal/kg)) × ([percentage of body involved in sepsis + sepsis severity + energy requirements for catch up growth] + 1)

Here's a breakdown of the components in the formula:

- *Current weight:* The current weight which was measured in every appointment by scale.
- *Appropriate age for height:* Determine the appropriate age for the patient's current height and find the corresponding kcal/kg value from the below table.
- *Percentage of body involved in sepsis:* The percentage of body involved in sepsis were divided to three categories including; 1- body surface area (BSA) 20% = 0.19, 2- BSA 40% = 0., and 3- BSA 100% = 0.95.
- *Sepsis severity:* The sepsis severity was categorized to mild, moderate, and severe which were equal to 0.2, 0.4, and 0.8; respectively.
- *Energy Needed for catch up growth:* This represents the additional energy required for growth which equal to 0.1-0.2.

Protein Calculation: Protein intake was estimated at about 115-120% of the recommended dietary allowance (RDA) for their age and gender. In fact, 20% of the total energy needs were allocated to protein.

Figure 1. Developing a structured nutritional management algorithm for individuals with Epidermolysis Bullosa (EB)

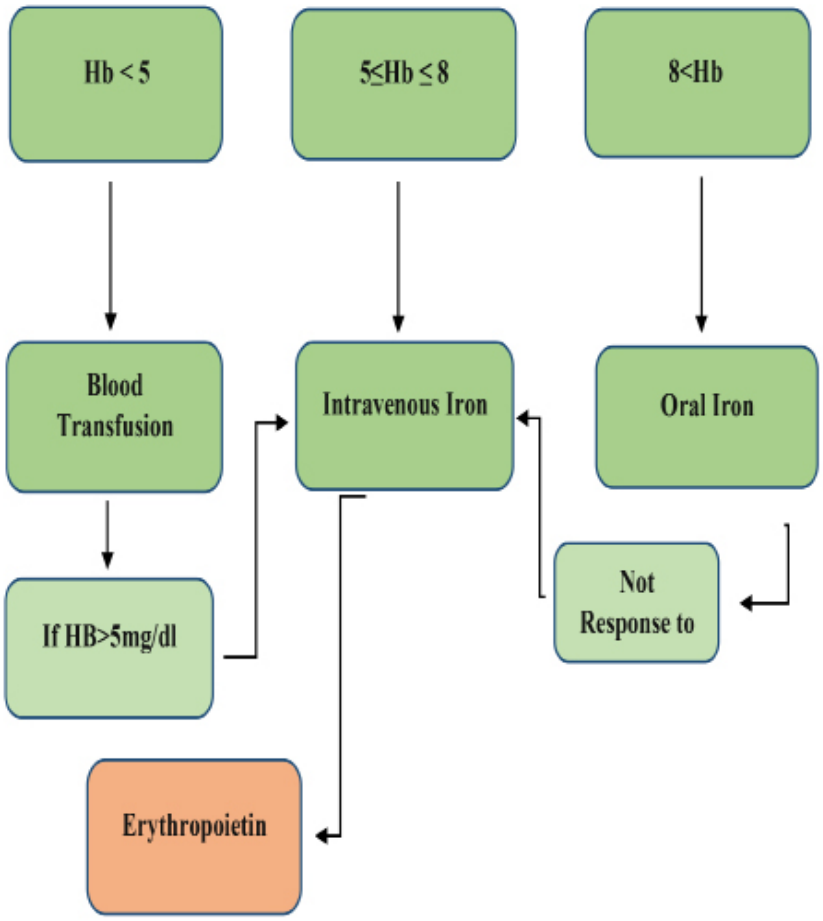

Figure 2. The treatment protocol for individuals with Epidermolysis Bullosa with Iron Deficiency Anemia (IDA)

Carbohydrates Calculation: Given that carbohydrates can increase inflammation, we restricted simple carbohydrates to less than 10% of the total energy needs. However, complex carbohydrates were increased. Specifically, complex carbohydrates with insoluble fiber were provided to patients with constipation but without dysphagia.

Fat Calculation: Around 30% of total calories were allocated from fat. To mitigate inflammation, foods enriched with omega-3 fatty acids were prescribed.

Anti-Inflammatory Diet: All these macronutrients were incorporated into anti-inflammatory diet menus tailored to each patient. The anti-inflammatory diet was designed based on the Dietary Inflammatory Index (DII).

The protocol of the present study was approved by the Institute's Ethics Committee of Mashhad University of Medical sciences (IR.MUMS.MEDICAL.REC.1401.288). The written informed was conducted the children or parents of patients (aged less than 18 years).

Results

In the present study, the median (25-75 IQR) age of the participants was 81.0 (36.0-156.0) months, and 19% of the participants were girls. Demographic, anthropometric, dietary, and clinical characteristics of the participants were presented in Table 1. The median (25-75 IQR) weight was 17.5 (10.8-24.5) kg, and the mean ± SD of height was 109.9 ± 31.1 cm.

Table 2 & 3 displays the association between the prevalence of malnutrition (by type) and sex. No significant associations were found between sex and all types of malnutrition. Furthermore, the prevalence of malnutrition based on type and severity was demonstrated in Table 4.

The comparison between energy requirement and energy intake based on the type of EB is shown in Figure 3.

Table 5 presents the association between gastrointestinal complications and malnutrition. Among all types of malnutrition, there was only a significant association between gastrointestinal complications and BMI-for-age z-score (OR: 0.08, 95% CI (0.01-0.87), P-value=0.039) in the

Table 1. Demographic, anthropometrics, dietary, and clinical characteristics of participants

variables		N (%) or Mean ± SD/ Median (25-75 IQR)
Age (month)		81.0 (36.0-156.0)
Sex	Boys	19 (40.4)
	Girls	28 (59.6)
Weight, (kg)		17.5 (10.8-24.5)
Hight, (cm)		109.9 ± 31.1
BMI		
Energy intake (kcl)		
Type of disease	Simplex	1 (2.2)
	Junctional	3 (6.5)
	Dystrophic	42 (91.3)
Mouth blister	Yes	32 (68.1)
No	15 (31.9)	
Small mouth	Yes	11 (23.4)
No	36 (76.6)	
Fixed tongue	Yes	4 (8.5)
No	43 (91.5)	
Denature tooth	Yes	28 (59.6)
No	19 (40.4)	
Reflux	Yes	15 (31.9)
No	32 (68.1)	
Dysphagia	Yes	16 (34.0)
No	31 (66.0)	
Stricture of esophagus	Yes	(14.9) 7
No	(85.1) 40	
Excess mucus excretion	Yes	(23.4) 11
No	(76.6) 36	
Regurgitation	Yes	(8.5) 4
No	(91.5) 43	
Painful defecation	Yes	(21.3) 10
No	(78.7) 37	
Bleeding with defecation	Yes	(12.8) 6
No	(87.2) 41	

crude model. After adjustment, there was no significant association between gastrointestinal complications and malnutrition (based on BMI-for-age z-score).

The mean values of weight at the baseline, the first, and the second appointment of the study were 21.3, 21.2, and 24.8 kg, respectively. Moreover, the mean height at the baseline, the first, and the second appointment of the study were 109.4, 121.0, and 123.4 cm, respectively. The trend of height changes from baseline to the second appointment of the study significantly increased based on repeated measurements of ANOVA (P-trend value were less than 0.001), while the trend of weight increased but had no significant changes (P-trend value = 0.429) (Figure 4 and Figure 5).

Among participants, the mean±SD levels of hemoglobin (Hb), RBC, MCHC, MCH, iron, and ferritin were 10.14±2.70 (g/L), 14.09±40.09 (10^{12}/L), 32.73±9.24, 23.91±11.71, 68.40±12.18

Table 2. The association between prevalence of malnutrition (by type) and sex.

Variables	Boys	Girls	P-value
WHZ, n (%)	5 (45.5)	4 (26.7)	0.476
WAZ, n (%)	3 (15.8)	5 (19.2)	0.762
HAZ, n (%)	6 (31.6)	8 (28.6)	0.636
BMIZ, n (%)	5 (31.3)	4 (20.0)	0.785

Table 3. The prevalence of malnutrition by type and severity

variables	Type of malnutrition			
	WHZ	WAZ	HAZ	BMIZ
Mild, n (%)	0	0	0	0
Moderate, n (%)	7 (14.9)	24 (51.1)	16 (34.0)	18 (38.3)
Severe, n (%)	19 (40.4)	21 (44.7)	28 (59.6)	18 (38.3)
All severity, n (%)	26 (55.3)	45 (95.7)	44 (93.6)	36 (76.6)

Table 4. The association between gastrointestinal complications and malnutrition

Variable	OR (95 % CI)	P-value
WHZ		
Model[1]	0.27 (0.03-2.02)	0.201
Model[2]	0.01 (0.01-1.48)	0.094
Model[3]	0.07 (0.003-2.02)	0.123
WAZ		
Model[1]	1.09 (0.11-10.88)	0.939
Model[2]	0.98 (0.08-11.48)	0.991
Model[3]	0.64 (0.05-8.82)	0.740
HAZ		
Model[1]	2.60 (0.27-24.65)	0.405
Model[2]	2.88 (0.26-32.11)	0.391
Model[3]	1.97 (0.15-25.44)	0.602
BMI for age z-score		
Model[1]	0.08 (0.01-0.87)	0.039
Model[2]	0.11 (0.01-2.14)	0.146
Model[3]	0.21 (0.01-6.84)	0.384

Regression logistic.
Model[1]: Crude.
Model[2]: adjusted for type.
Model[3]: Model[2] + additionally adjusted for energy intake.

Table 5. The levels of biochemical markers in EB patients.

Lab marker	Mean±SD	Minimum	Maximum
Hb (g/L)	10.14±2.70	4.30	13.60
RBC x (10^{12}/L)	14.09±40.09	3.40	208.0
MCHC	32.73±9.24	23.40	78.70
MCH	23.91±11.71	10.30	79.20
Iron (µg/dl)	68.40±12.18	38.20	96.90
Ferritin (ng/mL)	35.19±27.0	1.70	112.00

(µg/dl), and 35.19±27.0 (g/mL), respectively. The details of the levels of laboratory markers were presented in Table 5.

Discussion

This recent study was conducted to investigate the nutritional status of Epidermolysis bullosa (EB) patients referred to the nutrition clinic and examine the nutritional changes of these patients in relation to referral, intervention, and nutritional monitoring.

In the present study, gastrointestinal complications were not related to BMI-for-age z-score as an indicator related to nutritional status. Contrary to this result, in the study of Reimer et al. (15), the complications of EB patients, including esophageal stenosis, dilatation, and complications related to the side effects of gastrostomy, as well as laboratory markers indicating inflammation and anemia, were related to the developmental status of the patients.

In our study, EB patients were visited in the nutrition clinic regularly every month and were

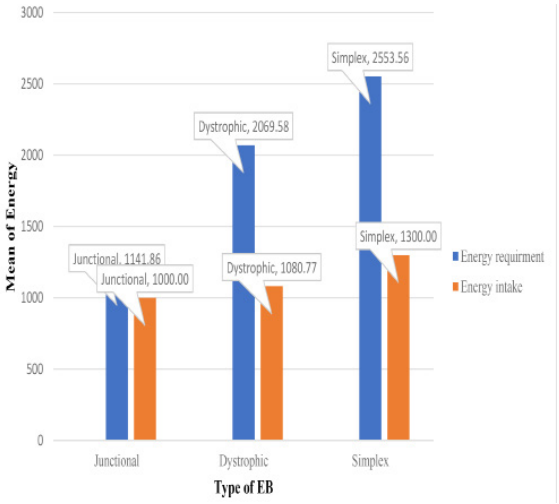

Figure 3. The comparison between energy requirement and energy intake based on type of EB

monitored and received necessary interventions. These interventions included the assessment of nutritional needs and gastrointestinal complications and taking appropriate measures to optimize the nutritional status; the trend of the height of the patients increased significantly compared to the baseline in the second appointment, while the trend of weight was not significant. These findings could be connected to a medical condition that has a greater impact on weight fluctuations (failure to thrive) than on changes in height. In this regard, in the study by Colomb et. al.(16), on severe generalized recessive dystrophic epidermolysis bullosa, who were not able to receive enough food orally, gastrostomy insertion was performed, which led to an increase in weight-for-height and height-for-age; also, people who were less than 10 years old had normal maturity experienced, but the insertion of a gastrostomy could not improve the skin condition of the patients. Similarly, in Haynes et al. study (17), the use of a gastrostomy could lead to an increase in weight standard deviation scores and height standard deviation scores, on average, by 0.9 SDS and 0.42 SDS, respectively. Our results might be related to the disease, which influenced weight gain due to more rapid changes in weight than in height.

Enhancing the growth of these individuals presents a challenge, primarily due to their substantial energy requirements that cannot be met through oral nutrition alone. In this regard, it appears that a sustainable approach, such as the implementation of gastrostomy, could offer a solution with long-lasting effectiveness. Numerous research studies have explored the impacts of PEG (percutaneous endoscopic gastrostomy) in patients with epidermolysis

bullosa (EB). Lynne Hubbard et al (18), presented body mass index (BMI), weight, and height centiles at birth, at gastrostomy placement, and at the age of 18 years. In this pilot study, two groups of EB patients were compared as follows: 12 patients with a mean of 14.5 years who had gastrostomy as group 1, and 5 patients with 18 years who had declined gastrostomy placement as group 2. As a result, compared with group 2, the mean of BMI, weight, and height centiles in the group were significantly higher; and half of group 1 had improved their centile position. In another study conducted by Hubbard et al. in 2014 (19), gastrostomy implantation led to an improvement in the patient's quality of life. The reason for the positive effect of nutritional interventions aimed at optimizing the intake of calories and protein, especially gastrostomy, on the nutritional status of patients can be due to the existence of chronic malnutrition, digestive symptoms that reduce food intake, and chronic inflammation in these patients, as the study of the cohort of Reimer et al. (2020) (15) illustrated that over 50% of children had wasting and/or stunting. At our institution, the routine implementation of this procedure is not standard practice, and as a result, none of our patients have undergone PEG implantation, which may influence the ultimate outcomes.

Epidermolysis bullosa, particularly recessive dystrophic EB (RDEB), often causes chronic anemia due to a complex interplay of factors like iron deficiency, systemic inflammation, poor nutrition, and anemia of inflammation from skin ulcers (20). Gastrointestinal problems further complicate management. Diagnosing iron deficiency is challenging due to poor oral intake, reduced iron absorption, and inconclusive ferritin markers. STfR levels remain unaffected by systemic inflammation, aiding in distinguishing iron-deficiency anemia from inflammation-related anemia using the STfR/ferritin ratio. Standard oral iron therapy may have limited effectiveness, necessitating enteral absorption tests and, in severe cases, erythropoietin. Vitamin C supplements improve iron absorption (21), and monitoring for cardiac toxicity during iron therapy is essential . Tailored approaches are essential for comprehensive anemia management in RDEB patients (15). In EB patients, anemia is a life-threatening problem that is induced by various causes, including iron deficiency, inflammation, poor nutritional status, and blood loss from the wound (15). In Pope et al. reported that hemoglobin (Hb) levels below 10g/dl make wound healing difficult in patients with venous ulcers secondary to decreased tissue oxygenation (11). In our study, among all patients, 24.6% participants were in Hb > 10 g/dl category, 8.7% were included in 8 ≤ Hb ≤10 g/dl category, and 10.1% were belong to hemoglobin 8 < g/dl category. Reimer et al.'s study recommends oral or intravenous iron in patients with iron deficiency anemia; however, iron supplementation was not suggested due to complications such as constipation and gastrointestinal symptoms (15). The strategy for iron deficiency treatment is still under discussion and appropriate management should be considered (15).

Strengths

The strengths of this study include being the first to investigate the relationship between regular visits to a nutrition clinic by EB patients and the nutritional interventions and monitoring that result from these visits. It is also the first study conducted on the Iranian population of patients with epidermolysis bullosa. This study examined the energy requirements of patients with EB and the energy they received based on their disease subgroup, including dystrophic epidermolysis bullosa (DEB), junctional epidermolysis bullosa (JEB), and epidermolysis bullosa simplex.

Limitations

This longitudinal study has several limitations, including a small sample size, no assessment of blood micronutrient status, including vitamins and minerals, a relatively brief duration of the study, existence of some confounding factors such as age, sex, growth hormone levels and nutrients and economic status, and lack of determining the severity of damage to digestive tract tissues. Furthermore, a lack of detailed specification regarding all the interventions conducted individually, the impossibility of using gastrostomy as a supplementary feeding route due to the unwillingness and economic status of the patients, and a failure to distinguish variations in the number of changes among the different types of EB diseases were other limitations of this study.

Recommendations

To address these limitations, it is suggested that future studies should involve larger sample sizes and extend the study duration. Additionally, they should take into account the differences between various types of EB diseases to gain a more comprehensive understanding of the effectiveness of nutritional interventions in improving undernutrition and alleviating disease symptoms. This would contribute to a more robust and nuanced evaluation of the impact of

nutritional management on EB patients.

Conclusion

Patients with Epidermolysis Bullosa benefit from routine clinic visits, and tailored nutritional interventions such as providing nutritional support to ensure sufficient energy and protein intake, prescribing nutrient supplements according to the needs and conditions of each patient, and other necessary measures, as well as managing anemia and deficiencies for their well-being.

Abbreviation

EB: Epidermolysis Bullosa
EBS: Epidermolysis Bullosa Simplex
JEB: Junctional Epidermolysis Bullosa
DEB: Dystrophic Epidermolysis Bullosa
KEB: Kindler Epidermolysis Bullosa
RDEB: Recessive Dystrophic Epidermolysis Bullosa
IDA: Iron Deficiency Anemia
Hb: Hemoglobin

Authors' Contributions

The authors hereby appreciate the great contributions of the study participants. Overall, S.T. and P.R., and H.K. supervised the project and approved the final version of the manuscript to be submitted. P.R., S.T., H.K., F.H. and M.S. designed the research. P.R. analyzed and interpreted the data; S.T. critically reviewed the manuscript; P.R, F.H., and M.S. drafted the initial manuscript.

Ethics approval and consent to participate

The protocol of the present study was approved by the Institute's Ethics Committee of Mashhad University of Medical sciences (IR.MUMS.MEDICAL.REC.1401.288). The written informed was conducted the children or parents of patients (aged less than 18 years).

Consent of publication

Not applicable.

Competing of interest

We do not have any conflict of interest (financial or other) other than those declared. All of authors have read the final version of the manuscript and the corresponding author responsible for what is said in it.

Availability of data and materials

All data from this study are included in the publication article.

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Reviews in Clinical Medicine

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The Association of Overweight and Obesity with Menarche Age in Girls Aged 11-15 Years in Iran; A Cross-sectional Study

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ABSTRACT

Introduction: Epidemiologic studies have shown a discrepancy between overweight and puberty processes. This cross-sectional study was aimed to clarify these associations in the Iranian girl population.

Methods: A total of 1300 girls aged 11-15 years were randomly selected from Mashhad, in the northeast of Iran, using a multi-stage cluster sampling method. The demographic data were collected and weight, height, and waist circumference (WC) were measured, then Body Mass Index (BMI) and Waist-to-Hip ratio were calculated. Overweight and obesity were defined based on WHO reference data. Central obesity was defined as ≥90th percentile of WC. Linear regression and unconditional binary logistic regression were performed to investigate the association between socio-demographic parameters and age at menarche in months, puberty categories, and menarche age groups (<12 vs. ≥12 years) respectively.

Results: The prevalence of overweight, obesity, and abdominal fat distribution were 11.5%, 10.3%, and 10.5% respectively. Menarche was experienced by 63% of subjects at the mean age of 12.24±0.98 years. Regression tests revealed that the odds of menarche occurrence at the age of 12 or more was significantly lower in girls with higher BMI (OR: 0.31, 95%CI: 0.22-0.43) than their leaner counterparts (P< 0.001).

Conclusion: The findings suggest that being overweight or obese is a possible predictor of experiencing menarche at a younger age.

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Introduction

Puberty, as an important part of human reproductive life, is the end point of complex series of developmental events by which children obtain secondary sexual characteristics (1, 2). Normally the onset of puberty (the age of which 95% of children attain Tanner Stage 2) among girls occurs during ages of 8-13 years with the average of 11. Menarche, as the end stage of puberty in girls, usually occurs about 2-3 years after thelarche (3). Timing of normal pubertal maturation has received more attention over the past several years, because of its association with health and psychosocial problems (4).

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Some studies have shown a significant decline in age of pubertal maturation from the late 19th century to the end of 20th (1, 5-7). Comparing data from National Health Examination Survey (NHES) cycles I and III indicated that the average age at menarche in US decreased from 12.75 to 12.54 years (8). Similar results were reported by Bogalusa heart study (9). Such reduction in the age of menarche also has been reported among Iranian women by 0.15 years per decade (10). Recent large studies suggested that this secular trend of the pubertal age is continuing (11, 12), and generally attributing to substantial improvement in socio-economic conditions, nutritional status, sanitation and general health (13-16).

Entering puberty at younger ages in girls appears to be a risk factor for psychological problems (anxiety, negative self-image) (4), breast cancer (4, 17, 18), diabetes (19, 20), and higher sexual activity as they mature physically at a time when they are immature mentally. Furthermore children with earlier puberty are often shorter because of accelerated bone maturation and early epiphyseal fusion (21).

The "Critical Weight" hypothesis suggested by Frisch and Revelle several decades ago proposed that the attaining of a certain minimum weight or body fat percentage is necessary for pubertal development and menstrual function (13, 22-25). Decreasing age of puberty onset over the time seems to be concurrent with the global increasing prevalence of overweight and obesity (26, 27).

Obesity is a major health problem that is growing to epidemic problem worldwide in both developed and developing countries (2, 28), such as Iran. Childhood obesity is associated with lots of medical complications and to subsequent increase obesity-related morbidity and mortality (29). Besides, excess adiposity may also influence pubertal development (2, 11). Girls with heavier weight are more likely to reach menarche at a younger age than normal weight girls (30-32). Previous studies have examined the relationship between obesity and menarcheal timing (33, 34). Therefore the aim of this study was to investigate the association of Body Mass Index (BMI), Waist Circumference (WC) and Waist to hip ratio (WHR) as markers of fatness, with the occurrence of menarche among a sample of Iranian adolescence girls.

Materials and Method

Sample population

Across sectional study was carried out on 1300 healthy girls, in Mashhad, a large city in North-east of Iran. Subjects were selected from all seven urban educational regions, 2-3 schools of each region based on student population (totally 17 schools). A multistage stratified clustered sampling method was used. The exclusion criteria were presence of any chronic disease which may affect growth or cause delayed puberty (7). Additionally if the subject had not remembered the time of her first menstruation she ruled out too. Informed consent was obtained from the study participants or their parents. This study was designed based on the ethical standards of the Helsinki Declaration and was approved by the ethical committee and research council of the Mashhad University of Medical Sciences.

Demographic data

Every participant underwent medical examination, if she was eligible she completed a brief demographic questionnaire containing personal information (birth date, age, school grade, parents education and occupation). The students were asked to specify whether they had experienced menarche at the time of interview, and if the answer was positive menarche age was recorded.

Anthropometric measurements

Height and weight were measured by trained staffs to the nearest 0.1 cm using a portable stadiometer (Seca 216, Germany) and the nearest 0.1 kg using a balanced portable digital weight scale (Beurer BF66, Germany) while children wearing light indoor clothing and without shoes. Waist circumference was obtained over the unclothed abdomen at the narrowest point between the rib cage and the superior border of the iliac crest (35), using a non-elastic flexible tape and measurements were recorded to the nearest 0.1 cm. All pieces of equipments were calibrated daily. In all subjects BMI was calculated using weight (kg)/height^2 (m). Overweight and obesity were then defined based on BMI percentiles of WHO (World Health Organization) references for age and sex (36), as more than or equal to 85th and 95th BMI percentiles, respectively. Abdominal obesity was also determined as WC≥90 percentile for age and sex (36-38).

Statistical analysis

Normality of data was assessed using the Kolmogorov-Smirnov test. The data were represented by frequency (percent) and mean (SD) for qualitative and quantitative variables respectively. Chi-square test and Independent-sample t-test were used to compare between qualitative and quantitative variables, respectively. To investigate the association between age at

menarche in months and socio-demographic parameters, as binary variables, uni-and multivariate linear regression were performed. Socio-demographic variables were included BMI, WC, WHR, mother's and father's education. Unconditional binary logistic regression was used to assess the relationship between puberty categories (Non-pubertal vs. pubertal groups) and socio-demographic variables. The same test analyzed the relationship between menarcheal age groups (<12 years vs. ≥12 years) and socio-demographic factors. The last two tests were also performed in uni- and multivariate analyses for estimating un-adjusted and adjusted Odds Ratios (ORs), respectively and the 95% confidence intervals (CI). Statistical analysis was performed using SPSS for windows version 16.0 (SPSS Inc., Chicago, Illinois, USA). A probability of P=0.05 was considered statistically significant.

Results

Sample population characteristics:

The mean age of the sample population was 13.23±1.02years, and the mean and SD of BMI, WC and WHR were 19.9±3.5, 67.2±7.7 cm and 0.77±0.06 cm respectively. In total 63% of participants had experienced menarche with the average age of 146.9±11.37 months (12.24±0.98 years). The total prevalence of overweight and obesity were 11.5% and 10.3% respectively based on BMI percentiles of WHO references for age and sex. As shown in Table 1 abdominal obesity, WHR, puberty occurrence and age at menarche showed significant differences in two obesity categories (P<0.001).

Relationship of socio-demographic variables with menarche age

Compared to non-pubertal group, mean BMI, WC and WHR were higher in pubertal counterparts (20.7±3.4vs. 18.5±3.2kg/m^2, P<0.001), (68.7±7.5 vs.64.9±7.6cm, P<0.001) and (0.77±0.06 vs.0.78±0.06) respectively (Figure-1).

Table-2 demonstrates associations between age at menarche in months and socio-demographic variables using linear regression before and after adjusting for possible confounders. Age at menarche decreased significantly by 5.49 months in overweight and obese girls compared to the reference group. Similarly, girls with abdominal obesity or WC of above 90th percentile had experienced menarche 2.86 months earlier. Regarding to the parental education level, there was a significant reverse association between mother's education level and menarche timing.

Using a model of stepwise multivariate regression analysis with all variables being entered the model, only BMI and WC remained in the final model.

We also categorized menarche age into two age groups, younger than 12 and 12 or more, using logistic regression analysis to assess the relationship between socio-demographic characteristics and age at menarche (Table-3).

The findings showed that all obesity indexes were significantly associated with age at menarche. According to BMI categories, overweight and obesity lowered probability of being in the group with higher age at menarche (OR: 0.32, 95%CI: 0.24-0.43, P<0.001). The odds of having higher menarche timing among girls with WC above the 90th percentile was 0.47 times

Table 1. Socio-demographic characteristics of girls aged 11-15 in Mashhad

Variable	Obesity categories		P-value
	Normal weight n (%)	Overweight & obese n (%)	
Abdominal obesity			<0.001*
Yes	23(2.3)	111(40.8)	
No	958(97.7)	161(59.2)	
WHR			<0.001*
<0.80	749(76.4)	145(53.3)	
≥0.80	232(23.6)	127(46.7)	
Puberty			<0.001*
Yes	593(60.3)	217(78.9)	
No	390(39.7)	58(21.1)	
Menarche age(months)	148.39±11.68	142.90±11.02	<0.001†
Father's education			
Non-university educated	756(76.9)	211(76.7)	0.950*
University educated	227(23.1)	64(23.3)	
Mother's education			0.270*
Non-university educated	825(83.9)	223(81.1)	
University educated	158(16.1)	52(18.9)	

† Independent-samples t-test
* Chi-Square Tests

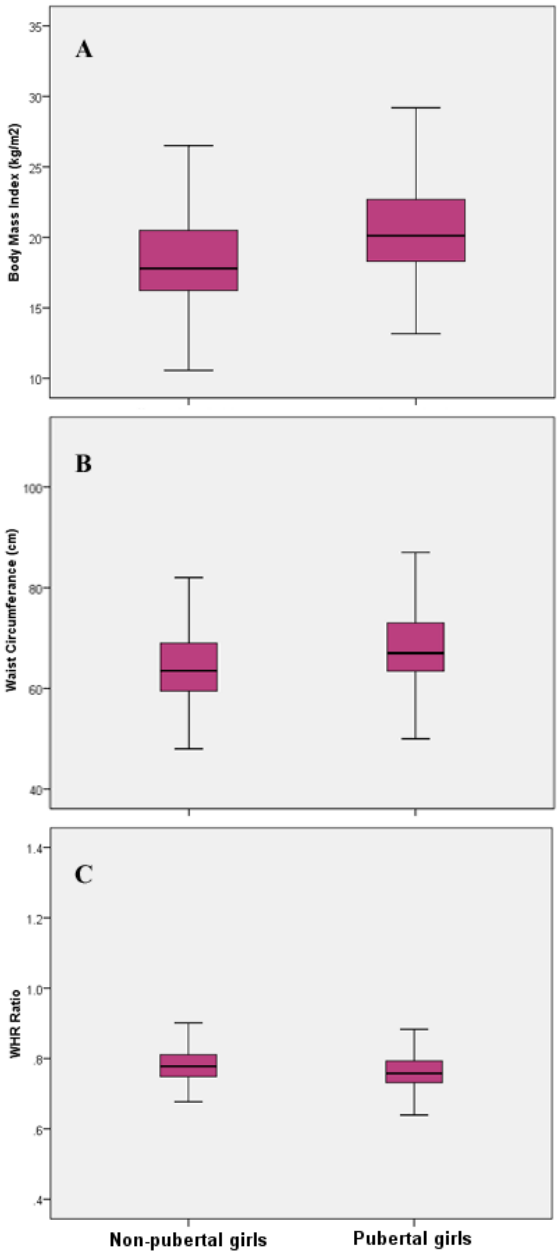

Figure 1. Mean Body Mass Index (A), Waist Circumference (B) and Waist-to-Hip Ratio (C) based on puberty status in girls aged 11-15 in Mashhad

(95%CI: 0.32-0.67, P<0.001) of the contemporary girls with WC less than 90th percentile. WHR had reverse association with menarche timing (OR: 1.42, 95%CI: 1.07-1.89, P<0.001) and higher WHR increased the possibility of being in the group with menarche occurrence at 12 or more years old. In adjusted model only BMI (OR: 0.31, 95%CI: 0.22-0.43, P<0.001) and WHR (OR: 2.08, 95%CI: 1.48-2.92, P<0.001) were significantly associated with age at menarche.

Relationship of socio-demographic variables with pubertal status

In different classification, participants were divided in to pubertal and non-pubertal groups based on menarche occurrence. In the Logistic regression model, all obesity indicators and father's education level showed significant correlation with menarche. BMI (OR:2.46 ,95%CI: 1.79-3.38 , P<0.001) and WC (OR:1.78 ,95%CI: 1.18-2.67 , P=0.006) had positive relation , while WHR (OR:0.55 ,95%CI: 0.43-0.71, P<0.001)and father's education (OR:0.68 ,95%CI: 0.53-0.89, P=0.004)were negatively correlated with puberty. Puberty was significantly associated with obesity status (OR: 2.63, 95%CI: 1.81-3.82, P<0.001) and WHR (OR: 0.41, 95%CI: 0.31-0.54, P<0.001), but not anymore with abdominal obesity after adjusting in multivariate model (Table-4).

Discussion

We conducted the current study to estimate the average age at menarche in a sample of Iranian girls and to assess the association of obesity indexes with it. Menarche was occurred at the age of 13.23±1.02 yrs in our study sample which was more than menarcheal age of Tehranian girls but less than age at menarche in all other provinces (39). Iran has different ethnicities (Persian, Azeri, kurd, Arab, and Gilaki) living in various geographic regions with different climates. All these, besides the socioeconomic differences and industrialized lifestyle in metropolitan cities such as Tehran (the capital of Iran) could cause the differences in maturation time (40). The mean age at menarche in the United States is 12.7 yrs (8), Germany 12.8 yrs (41), Turkey 12.4 yrs (7), Kuwait 12.41 yrs (42), United Kingdom 12.9 yrs (43), Nigeria 13.2 yrs, India 13.8 yrs (44), and in Ethiopia 15.8 yrs (45). It seems that lower socio-economic conditions and growth retardation might lead to slower puberty process and delay in maturation. Our results suggest that there is a significant inverse relationship between BMI and both menarche occurrence and mean age at menarche in adjusted and unadjusted analysis, which is in line with previous studies that indicate the same association between weight and puberty (7, 46, 47). A similar cross-sectional study in Iran showed that overweight and obese girls reached pubertal age earlier than normal weight ones (6). In one study that conducted on 811 French Canadian girls, there was an association between overweight and both early and late maturation in girls (48). Another study on comparison of normal weight versus overweight and obese girls indicated that body fat associate with early puberty (34). In a cross-sectional study in Kuwait, Al-Awadhi, et al. (49), concluded that there is an inverse association between high BMI and age at menarche. A retrospective study on Korean

Table 2. Association between age at menarche and socio-demographic variables, using uni- and multivariate linear regression

Variables	Unadjusted			Adjusted*		
	B(95% CI)	Beta	Pvalue	B(95% CI)	Beta	Pvalue
Step 1 **Overweight & Obesity**						
BMI †per < 85th (Reference)						
BMI per ≥ 85th	-5.49(-7.28,-3.70)	-0.21	<0.001	-6.17(-8.28,-4.06)	-0.23	<0.001
Abdominal Obesity						
WC†per < 90th (Reference)						
WC per ≥90th	-2.86(-5.03,-0.41)	-0.08	0.022	1.43(-1.40,4.26)	0.04	0.320
Step 2 **Waist-to-Hip Ratio**						
< 0.80 (Reference)						
≥ 0.80	1.70(-0.19,3.58)	0.06	0.078			
Mother's Schooling						
Non-university Educated (Reference)						
University Educated	-2.50(-4.65,-0.35)	-0.08	0.023			
Father's Schooling						
Non-university Educated (Reference)						
University Educated	-0.94(-2.90,1.01)	-0.03	0.345			

Dependent variable: Age at menarche in month , All significant and non-significant variables from univariate tests were entered in the multivariate analyses (2 steps hierarchical modeling) to control their confounding effect by adjusting.
*Adjusted R^2
† BMI: Body Mass Index, WC: Waist Circumference

middle school students, revealed that the girls with early menarche have more body weight and BMI comparing to those with late menarche (50). In contrast, a research suggested that there is no correlation at population level between BMI and age at menarche (51).

Use of BMI alone has some limitations in children, because the relation between the fat and fat free mass varies at different ages, so WC is suggested to be superior to the BMI for predicting obesity-related health diseases (52). Also we assessed the relationship of WC with menarcheal age.

Similar positive relations were found between abdominal obesity and both puberty occurrence and menarche age in univariate but not multivariate model. It seems that WC association with menarche is indirectly, via girls' weight.

Table 3. Association between different groups of age at menarche and socio-demographic variables, using uni- and multivariate logistic regression

Variables	Unadjusted		Adjusted	
	OR(95% CI)	P-value	OR(95% CI)	P-value
Step 1 **Overweight & Obesity**				
BMI per < 85th (Reference)				
BMI per ≥ 85th	0.32(0.24,0.43)	< 0.001	0.31(0.22,0.43)	< 0.001
Abdominal Obesity				
WC per < 90th (Reference)				
WC per ≥90th	0.47(0.32,0.67)	< 0.001	0.69(0.42,1.12)	0.128
Step 2 **Waist-to-Hip Ratio**				
< 0.80 (Reference)				
≥ 0.80	1.42(1.07,1.89)	0.015	2.08(1.48,2.92)	< 0.001
Mother's Schooling				
Non-university Educated (Reference)				
University Educated	0.74(0.54,1.01)	0.058	0.69(0.49,0,95)	0.250
Father's Schooling				
Non-university Educated (Reference)				
University Educated	1.01(0.76,1.34)	0.966		

Dependent variable: Age at menarche in two categories (<12 years as reference vs. ≥12 years), All significant and non-significant variables from univariate tests were entered in the multivariate analyses (2 steps hierarchical modeling) to control their confounding effect by adjusting
† BMI : Body Mass Index, WC: Waist Circumference

Table 4. Association between puberty status and socio-demographic variables, using uni- and multivariate logistic regression

Variables	Unadjusted		Adjusted	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Step 1				
Overweight & Obesity				
BMI per < 85th (Reference)				
BMI per ≥ 85th	2.46(1.79,3.38)	<0.001	2.63(1.81,3.82)	<0.001
Abdominal Obesity				
WC per < 90th (Reference)				
WC per ≥90th	1.78 (1.18,2.67)	0.006	1.53(0.92,2.55)	0.102
Step 2				
Waist-to-Hip Ratio				
< 0.80 (Reference)				
≥ 0.80	0.55(0.43,0.71)	<0.001	0.41(0.31,0.54)	<0.001
Mother's Schooling				
Non-university Educated (Reference)				
University Educated	0.83(0.62,1.11)	0.202		
Father's Schooling				
Non-university Educated (Reference)				
University Educated	0.98(0.73,1.08)	0.070		

Dependent variable: Puberty status based on menarche occurrence (non-pubertal girls as reference vs. pubertal girls), All significant and non-significant variables from univariate tests were entered in the multivariate analyses (2 steps hierarchical modeling) to control their confounding effect by adjusting.
† BMI : Body Mass Index, WC: Waist Circumference

There was a tendency for those in the younger age at menarche to have a higher WC percentile. Some studies reported that WC is positively correlates with puberty timing (53). In a study probability of getting early puberty in Girls aged 7-9 with greater WC, was higher than those with less WC (35). Surprisingly, WHR had reverse association with both probability of having menarche and age at menarche, in comparison to BMI and WC. Samples with lower WHR were more likely to have menarche in younger ages.

In our study only mothers' educational level in univariate analysis was reversely related to menarcheal age, but it did not show any association with obesity status as well as puberty occurrence. Socio-economic factors could play an important role on weight status and menarcheal age. A study in the United States attained different results with a negative relationship between children's BMI and their parents' education (54). Some researches parallel to our results, have shown that menarcheal age decreases as socio-economic status improves (15, 55, 56), in contrast, some studies did not find any significant differences in menarcheal age between social classes (7, 57). It seems that higher educational level of parents, especially mothers, affects family nutritional behaviors, but children are also affected by their friends, environment and multimedia.

The role of body weight as an accelerator factor on the developmental process which was suggested by Frisch and Revelle (58, 59), several decades ago as "Critical Weight" hypothesis, now is well-accepted by the discovery of the leptin (adipocyte-derived hormone), (2, 59, 60). Leptin is secreted from fat tissue so its blood concentration is in direct proportion to the amount of total body fat mass. Other major effect of Leptin is controlling the energy stores in the adipose tissue by appetite reduction and increased thermogenesis (2). Therefore Leptin's role as an essential mediator of the impact of body fat mass on the onset of puberty can explain our results that higher BMI and more WC tended to be associated with an earlier puberty.

Our study had several limitations. First, it was based on a cross-sectional data, so the subjects' body weight at the menarche time was not available, therefore we could not determine the causal relationship between obesity and puberty. Also we had to rely on the subject's memory on menarche time. Similar to many other studies, our study did not include boys, because it was not a cohort study and there is no such an easy indicator (age at menarche in girls) event of puberty in boys. Although some studies emphasize that BMI can affect puberty duration, besides puberty onset, as our study was not a cohort study we only considered the age of menarche, because it is easy to measure and self-reported data are more reliable (61-63). Although we were not able to report the mean age of thelarche, pubarche and duration of

puberty process in comparison to other studies, as some studies showed that the duration of the pubertal transition has increased because of the decline at the age of breast development, not age of menarche (64). We did not measure body fat mass (FM) and fat free mass (FFM), previous studies suggested that menarcheal age was more related to FM/m^2 than to BMI (65). Further studies may need to distinct how other factors such as genetic, dietary patterns, environment and geographic locations linked to the puberty onset in girls.

Conclusion

Experiencing earlier menarche parallel to the increasing of body weight emphasizes the need for early prevention and treatment programs for childhood obesity. Longitudinal investigations can determine causal effect between obesity and puberty.

Conflict of interest

All Authors including Author A, Author B, Author C, Author D and Author E declare that they have no conflict of interest.

Abbreviations

BMI: Body Mass Index, NHES: National Health Examination Survey, WC: Waist Circumference, WHO: World Health Organization, WHR: Waist to hip ratio

Compliance with Ethical Standards

Funding

This study was funded by Deputy for research at Mashhad University of Medical Sciences.

Ethical approval

All procedures performed in the current study were in accordance with the ethical standards of the Ethics Committee of Mashhad University of Medical Sciences (Mashhad, Iran) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Iron Load Evaluation of Adrenal Glands and Kidneys by using MRI T2* In Iranian Thalassemia Patients

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ABSTRACT

Introduction: Multi-organ iron load is prevalent crucial side effect in thalassemic patients due to repeated transfusions, and high intestinal iron absorption. MRI T2* has demonstrated its potency as a non-invasive technique for the imaging of hemosiderosis in thalassemia. We aim to investigate the iron load of adrenal glands and kidneys using MRI T2* in adult thalassemia patients and evaluate the serum ferritin correlation of with kidneys, heart, liver, and adrenal glands' iron load.

Methods: Thirty-five thalassemia major (TM) and thalassemia intermediate (TI) patients (age range 18-50 years) from Zafar thalassemia Clinic, were recruited in this survey from September 2019 to October 2020. Magnetic Resonance Imaging (MRI) was used to map iron overload in several organs' regions of interest (ROIs) using fast-gradient-echo multi-echo T2*sequences protocol. T-test and chi-square analysis were done.

Results: Nine (25.7%) patients had left Kidney T2* less than 36ms which could indicate abnormal renal iron load while this was 8 (22.9%) for the right kidney. In the left and right adrenal glands, these numbers were 31 (88.6%) and 29 (82.9%), respectively, below the normal threshold.

Conclusion: Adrenal gland and renal iron overloads were detected in MRI images of thalassemic patients. Correlation for serum ferritin levels and kidney and adrenal glands T2* was found weakly negative. Non-invasive monitoring of the internal organs' hemosiderosis using MRI T2* was found to be beneficial for iron-chelating optimization and preventing irreversible tissue damage.

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Introduction

Thalassemia is an inherited hemoglobinopathy with high global prevalence [1-4]. This disorder has a place to a group of hereditary blood disorders by reduced (β+) or absent (β0) synthesis of the hemoglobin beta globin chains that lead to reduced red blood cells and anemia [4]. The phenotypes of hereditary heterozygous compound beta-thalasseamia incorporate

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severe, transfusion-dependent thalassemia major, or intermediate spectrum.

Patients with TM customarily show noteworthy anemia in early age and require periodical long-life blood transfusion and iron chelation treatment to survive, whereas thalassemia intermedia patients might not [1, 4, 5]. Due to regular blood transfusion, hemosiderosis happens in organs such as the heart, liver, kidneys, and tiny endocrines, causing tissue damage and, in the long run, organ dysfunction. Although iron chelators progress, the survival of TM patients, multi-organ hemosiderosis is still notable [5-7]. While frequent estimation of serum ferritin offers a simple and accessible strategy for quantifying the iron burden, some studies have reported that there is a more prominent correlation between liver hemosiderosis and body iron index and ferritin [8-10]. Liver biopsy as the gold standard assessment method is invasive and not easy to be done for most patients' target organs like the heart, or kidney [4, 8-10].

The MRI protocol (gradient echo T2* and T2 spin echo or R2 relaxometry) was developed in the early 1980s for the non-invasive assessment of iron burden in internal organs. Iron overload in tissues produces local disturbances in the magnetic field so that higher amounts of organ iron content lead to more magnetic field disturbance. This indicates that the deposition of iron in tissues leads to a decrease in field homogeneity and a low T2* signal on MRI [3, 9-12]. Moreover, a superconducting quantum interference device, which is an important, and non-invasive method, has also been introduced for measuring iron overload, which enables researchers to study the effect of iron overload in hemosiderosis patients. However, this method is not accessible to many centers since it illustrates a few typical signatures of such artifacts in the raw data [13-15]. Quantifying liver and cardiac iron using this approach had a significant impact on the early detection and the treatment of hemosiderosis, treatment modifications, and prevention of tissue iron toxicity like cardiomyopathy due to iron overload [8, 16-20]. Global multi-center investigations have demonstrated that myocardial T2* is a significant prognostic indicator for early detection of cardiac dysfunction. Therefore, this technique has great potential for wider application in chelation regimens optimization and prevention of heart failure to increase survival rates [21, 16].

A study in 1994 indicated low signal intensity of the renal cortex on T2- weighted images in serious hemolytic anemias, due to iron deposition [22]. Information on the renal or adrenal glands iron monitoring is scarce in studies where kidneys or adrenal glands' iron overload in β-thalassaemia patients have been checked [3, 23-26]. Severe iron burden due to regular transfusions, long-lasting chelating regimen, and anemia are the main factors for renal dysfunction [26-29]. Studies conducted by Hashemieh et al. and Meloni et al. assessed iron overload in kidneys in TM and TI patients, using MRI T2* techniques. They analyzed the correlation of serum ferritin level, and the iron overload of the heart and kidney [3, 30,31].

Following these surveys, we first conducted a retrospective cross-sectional to assess the renal T2* MRI iron load of 821 thalassemic patients for a more detailed analysis of renal iron load monitoring [32]. Iron overload in the adrenal glands has been studied histologically in patients with hemosiderosis [33]. Moreover, functional alterations in the adrenal glands due to iron burden have been indicated in previous studies except in Iran [34, 35]. To our knowledge, only Drakonaki et al. and Guzelbey et al. have quantitatively studied iron deposition in the adrenal glands using MRI [33-34]. Control and case groups have been assessed and compared according to adrenal gland signal intensity values.

This seems to be the first limited study for quantification of both kidneys and adrenal glands of Iranian thalassemia patients. It might be an estimation of these organs' hemosiderosis. We assessed both kidneys and adrenals hemosiderosis by an accurate non-invasive method to check if our thalassemic patients might be at risk. The aim was to determine T2* values of both kidneys and adrenals as an index of iron overload in Iranian TM and TI patients. The study also aimed to investigate the correlation between serum ferritin and hemosiderosis in the kidney, adrenal glands, myocardium, and liver.

Materials and Method

Participants: A cross-sectional study was conducted at the referral Imaging Complex, Tehran, Iran, from September 2019 to October 2020. Iron overload assessment using T2* MRI is performed in this center annually. The study was approved by the Ethics Committee of the Iranian Blood Transfusion Organization (IR.TMI.REC.1396.023), and followed the Helsinki Declaration principles. Informed consent was obtained from all the patients. The inclusion criteria were thalassemia-diagnosed patients aged between 18 and 50 years. Also, patients with renal dysfunction, cardiomyopathy, possible liver and adrenal gland disorders, and diuretic treatment were excluded.

A total of 35 TM and TI patients who met the inclusion criteria were recruited in this study. TM patients were on regular transfusions with the frequency of 2 to 4 weeks. TI patients are considered independent of regular transfusion except in specific conditions. The patients mainly were on iron chelating therapy with Desferioxamine or its combination therapy. Demographic data of gender, age, type of thalassemia, height, weight, age of diagnosis, recent serum ferritin level, and splenectomy status were extracted from the medical records.

For conducting the MRI imaging and taking blood samples, the time elapsed after transfusion was considered at least 10 days for the study.

Magnetic Resonance Imaging: Patients were scanned with a 1.5T MR Scanner (Achieva A-series Philips, Netherlands). A standard radiofrequency body coil was used in all measurements. The Royal Brompton protocol based on a single-breath multi-echo fast gradient-echo sequence was used for T2* measurements. The liver, kidneys, and adrenal glands' T2* values were determined by imaging a single trans-axial slice (10 mm) through the center of the liver and kidneys for the measurement of myocardial T2*. Scans were synchronized to the cardiac cycle using standard ECG gating. A single 10 mm-thick, short-axis, mid-ventricular slice positioned halfway between the base and the apex of the left ventricle (LV) was acquired. Echo images for the liver, kidneys, and adrenal glands were 12, while it was 8 for the heart. T2* values were calculated for patients using CMR-based in-house software (Pardis Noor Medical Imaging Center, Tehran, Iran), validated by a standard iron phantom. The assessment and analysis of liver iron content were based on the method of Prof. Pennell, while the classification of cardiac and hepatic iron overload was applied based on Garbowski updates [36, 37]. A homogeneous full-thickness region of interest in the liver, kidney, and adrenal gland parenchyma was selected in the ventricular septum as shown in Fig 1, in which the ROIs in abdominal cross-section MRI T2* for iron overload processing and calculation have been marked as [1] adrenal gland and [2] kidney. We measured the average intensity of the area in each image and made a plot showing how it changed with the echo time(TE). T2* values were calculated in three different ROIs and were averaged to achieve a representative value for the kidney. The threshold level of kidney T2* relaxation time was determined based on the reported value in the literature, indicating that less than 36 ms is considered as a pathological value [30,31]. Also, the threshold was less than 34.81 ms for adrenal glands [34].

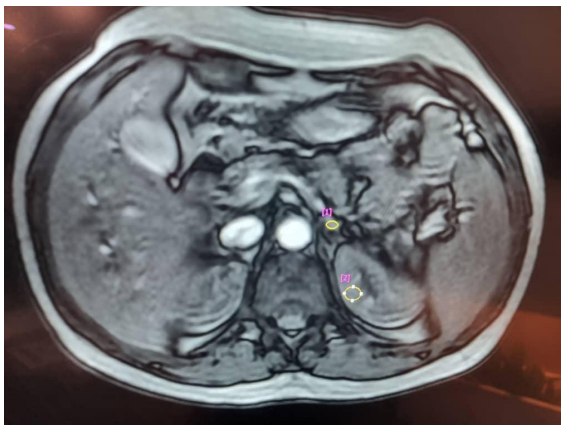

Figure 1. ROIs in abdominal cross-section MRI T2* for iron overload processing and calculation: [1] Adrenal gland and [2] Kidney

Statistical analysis: Quantitative and qualitative data analyzed and were described as mean±standard deviation and frequency and percentage respectively. The normality was checked via the Shapiro Wilks test. The independent samples t-test, paired samples t-test, and Chi-square were used to for comparisons. The correlation between the variables was evaluated by Pearson's correlation coefficient. The level of significance was equal to 0.05 and the confidence interval was 95%. For all statistics analyses, SPSS (version 26) software were applied.

Results

The demographic information of the 35 thalassemic patients is summarized in Tables 1(a, b). As it can be seen, of the details regarding age, type of thalassemia, Hb, Ferritin, diagnosis age blood transfusion interval, etc., are presented quantitatively.

The mean T2*values of the heart and liver were 26.76±8.33 and 6.94±6.02, respectively. Also, 74.28% of the studied patients had normal myocardial iron T2*, while only 58.7% of them had a hepatic iron load in the mild to severe class. The LIC of the patients was also 9.14±9.90 mg/g/dry weight. Table 2 shows the left and right kidneys and adrenal glands of thalassemia patients.

Nine patients (25.7%) had left kidney T2*<36ms which might indicate abnormal renal iron load. This was 8 (22.9%) for the right kidney T2*. The T2* values of adrenal glands were 31(88.6%) and 29 (82.9%) below the normal threshold (34.81 ± 8.74 ms), respectively, for the left and right adrenal glands. Mean T2* values of right and left kidneys and the adrenal glands in both intermedia thalassemia vs. thalassemia major have been assessed and presented in Fig. 2.

A negative weak correlation was found

Table 1 (a). Descriptive clinical demographic data information of the patients

	N	Minimum	Maximum	Mean	Std. Deviation
Age	35	18	50	34.54	9.690
Diagnosis/months	35	3	240	64.29	71.943
Start of Treatment/ month	35	6	240	66.80	71.974
transfusion days	32	20	90	28	16
Start of Iron Chelation/months	35	0	504	99.57	108.867
Desferal dose/ week	35	0	30	16.57	7.429
oral dose/day	35	0	1500	171.77	484.081
Hb g/dL	35	7.1	10.7	8.9	1.8
Ferritin	35	234	6000	1455.91	1209.496

Table 1 (b). Descriptive clinical demographic data information of the patients

		N	%
Sex	Male	8	%22.9
	Female	27	77.1
Thalassemia	Major	24	68.6
	Intermedia	11	31.4
History of Splenectomy	No	9	25.7
	Yes	26	74.3
	Yes	26	74.3
Cardiac iron overload	Normal (>20ms)	28	80.0
	Mild(15-20 ms)	4	11.4
	Moderate(10-15ms)	2	5.7
	Severe(<10ms)	1	2.9
Hepatic iron overload	Normal (>17ms)	4	11.4
	Mild (>6.2ms)	12	34.3
	Moderate(3.1-6.2ms)	11	31.4
	Severe (2.1-3.1)	3	8.6
	very severe (<2.1)	5	14.3

between serum ferritin levels and kidney T2* relaxation time values (r1=-0.343, and r2=-0.348, P-value<0.001, respectively, for the left and right kidneys) while a weak negative correlation was found between serum ferritin levels and adrenal gland T2* ms (r3= -0.214, and r4= -0.43 P-value<0.001, respectively, for the left and right ones).

The analyzed data for Pearson correlations (Significant at the 0.05 level, 2-tailed) for cardiac and hepatic T2* milliseconds with both adrenal glands and kidneys indicated a significant correlation between hepatic with Left/right adrenal glands iron overload (0.369*), while there was no significant correlation with adrenal

Table 2. Comparison of left and right adrenals T2*

	Mean±SD		p-value
	left	right	
T2* adrenal (ms)	25.31±6.84	26.92±8.27	0.230
T2* kidney (ms)	51.65±20.92	52.73±20.47	0.670

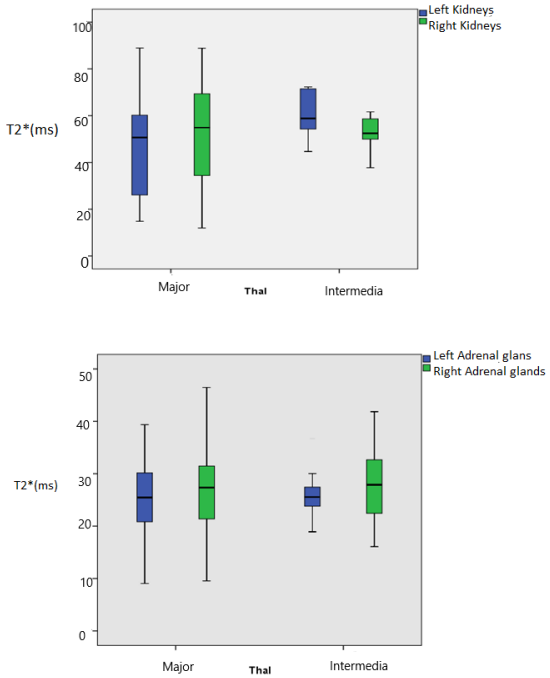

Figure 2. Box plot of kidneys and adrenal glands T2* relaxation time compared in thalasseamia groups

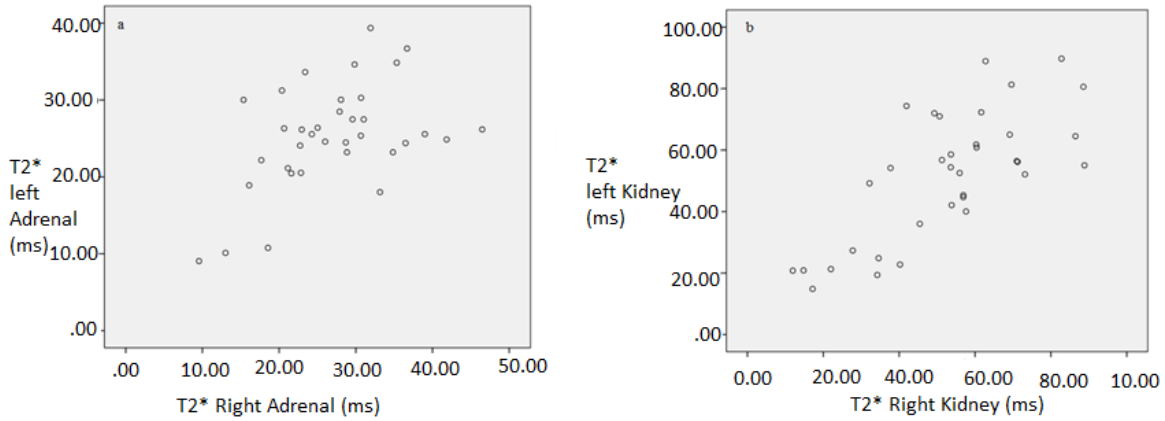

Figure 3. Scatter plot of kidney T2* relaxation time of a) both adrenal glands, b) both kidneys

glands or kidneys and heart iron load.

Scatter plots of T2* relaxation time of both kidneys and adrenal glands T2* relaxation time are presented in Fig. 3. Based on this scatter plot, 85.75% of the involved patients had abnormal iron load or hemosiderosis of both adrenal glands. This was found to be 24.3% for hemosiderosis of both kidneys while 20% and 88.6% of the patients had abnormal cardiac and hepatic iron overload.

Discussion

Beta thalassemia is a global prevalent hereditary hemoglobinopathy [2]. In spite of long-life treatment care of blood transfusion and iron chelation, and longer life expectancy in beta-thalassemia patients compared to the past, therapeutic-related complications like cell damage of organs and endocrines remain [2, 6]. Cell damage from heavy iron burden and hemosiderosis in thalassemic patients seems to lead to injuries in vital organs or endocrine glands like kidneys and adrenal glands [25, 36]. Chronic anemia and hypoxia in thalassemic patients in addition to iron overload might cause oxidative stress, lipid peroxidation, and irreversible cell damage [36-38]. After the introduction of the MRI T2* method to assess hemosiderosis in the tissues, several extensive studies have been published on the heart and liver while fewer details are available concerning other organs and endocrine glands [9, 16-21, 39]. Hence, MRI has been introduced as an essential tool in the management of patients with thalassemia, given the limitations of current metrics to assess iron storage in different organs. Due to its non-ionizing property, evaluations using MRI T2* are simple, fast, and with no radiation. The milligram of dry iron in liver (liver iron concentration, LIC) can be accurately evaluated by T2* and T2 techniques, with high reproducibility and correlation [36-40]. Now, we are accustomed to MRI T2* measurements of the heart and liver as we know the cutoffs for heart failure. Adrenal gland is one of the most important endocrine glands that might suffer from inefficiencies in thalassemia. Some of the symptoms of adrenal gland insufficiency are arthralgia, muscle pain, chronic fatigue and gastrointestinal complaints [33]. Iron overload in adrenal glands might be detectable using MRI T2* as hypo-intensity images. A few surveys have showed a correlation between the liver and adrenal gland hemosiderosis. Moreover, no significant correlation has been reported between adrenal gland signal intensity and patient age or serum ferritin level [33, 34]. Regarding the kidneys, although there are some studies, apparently none of them have studied the renal cutoff correlated with renal function [39, 40]. While there are numerous studies on hepatic and cardiac iron overload assessment in Iran and worldwide, there are limited hemosiderosis quantifying data for kidneys and adrenal glands in Iranian thalassemia patients. This study was conducted to assess both kidneys and adrenals hemosiderosis by an accurate non-invasive MRIT2* for potential risk. The aim was to investigate the pattern of iron load in both kidneys and adrenal glands in Iranian TM, TI patients. We studied the correlation between renal, adrenal glands hemosiderosis with serum ferritin, and T2* values of the liver and heart iron overload. The results of this study showed a weak negative correlation between kidney T2* relaxation time and serum ferritin, with a weak correlation observed between kidney T2* relaxation time and liver and heart T2* relaxation time. The results indicated that the hepatic hemosiderosis seems to be more prevalent in thalassemic patients. It appears that the mechanisms and dynamics of the absorption, storage and elimination of iron in these tissues are different,

especially in the transfusion-dependent patients [3, 9]. Therefore, applying the MRI imaging T2* technique highlights the need for the clinicians to quantitatively estimate organs at risk by iron hemosiderosis monitoring. In addition, 24.3% of the patients in our study had iron hemosiderosis in both kidneys with T2* values less than renal threshold (22.85%, 25.71% for the right and left kidneys, respectively). This seems to be in accordance with the results reported by Meloni et al. who found that 33.6% of their thalassemia population had a pathological value (T2*<36 ms) of kidney iron deposition [30,31, 39]. In addition, the percentage of the abnormal iron load in both right and left adrenal glands was calculated at 82.85% which might show that a considerable number of the patients suffered from iron overload in adrenal glands. The data reported by Meloni et al. study have also shown a significant correlation between iron load in the adrenal gland and the liver, which is consistent with our findings [33, 34].

Although various studies have been conducted on monitoring cardiac and hepatic iron load of the thalassemia patients by non-invasive MRI methods to become the standard of care [41], fewer studies are available for renal, especially adrenal glands' hemosiderosis in thalassemia using T2* technique of MRI. In Iran, we have just found a study for determining the prevalence of adrenal insufficiency in children with β-thalassemia major. The results of this study showed that in patients with a normal baseline cortisol level, the low-dose test could efficiently detect hidden secondary adrenal insufficiency [42].

In this imaging study, we observed that the prevalence of iron deposition was approximately 24.3% in both kidneys and 82.85% in adrenal glands in thalassemia patients. Non-invasive MRI T2* method provides promising results for the evaluation of iron burdens in internal tissues and organs. It is promising for non-invasive detection of the adrenal insufficiency in thalassemia patients, based on monitoring the iron overload in them at various times.

Our study suffers from a number of limitations. We conducted this limited population study due to the expenses involved in T2* imaging which are not covered by insurance and limited research grants. Hence, more extensive and multi-center studies are recommended for more accurate understanding. Finally, only the MRI T2* iron load calculations and analysis were used for this study. For deeper findings, the application of R2 relaxation protocol using MRI is recommended for investigating renal or adrenal glands' hemosiderosis in thalassemic patients compared to the T2* measurements. In addition, it might be a beneficial suggestion to have MRI iron overload assessment and correlations with more reliable clinical tests in normal thalassemia patients and those who suffer from dysfunctions of kidneys or adrenal glands.

Conclusion

This MRI technique can assist the clinicians in early detection of renal or some endocrine complications in beta-thalassemia patients based on iron overload assessment of adrenal glands and kidney. The early diagnosis of renal and adrenal iron overload complications might shed a light for specialists on iron chelating optimization influence treatment strategies and patient outcomes while preventing tissue damage in organs and glands due to toxic iron. Similar to the toxicity and function failure of the vital tissues, it might be also developed in renal and adrenal glands and in this way some earlier care might help prevent kidney failure in thalassemic patients.

Ethics approval and consent to participate

The study was approved by the Iranian Blood Transfusion Organization Ethics Committee (IR.TMI.REC.1396.023).

Consent for publication

"Not applicable."

Availability of data and materials

All data from this study are included in the published article.

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Abbreviations

Confidence Interval: CI
Liver Iron Content: LIC
Magnetic Resonance Imaging: MRI
Millisecond: ms
Red Blood Cells: RBC
Region of Interest: ROI
Superconducting quantum interference device: SQUID
Thalassemia Intermediate: TI
Thalassemia Major: TM

Authors' contribution

Shirkavand A, Ph.D.: Medical Physics researcher, data collection, drafting and revising the

manuscript.

Razaghi Z, Ph.D.: Statistics methodologist, analyzing and interpreting the data, supervising the analysis in drafting and revision.

Akhlaghpoor S MD: Radiology concepts and design, essential reagents or tools, draft plan and revising the manuscript.

Azarkeivan A MD: Hematology clinical specialist of thalassemia patients, assisted in essential interpretation of the data and drafting and revision of the manuscript.

Karimi M MD: Consultant in the field of hematology and thalassemia, English edition and revision of the manuscript.

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Mashhad University of Medical Sciences (MUMS)

Reviews in Clinical Medicine

Clinical Research Development Center Ghaem Hospital

Effect of Zinc Supplementation on Anthropometric Parameters of Male School Children

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ABSTRACT

Introduction: Zinc has a key role in reproductive physiology, immune modulation, growth, and development.
To determine the effect of zinc supplements on the anthropometry of healthy 6-year-old children.
Methods: In this double-blind placebo-controlled trial was carried out on 40 children 6-7 years old. The intervention group (n=20) received 20mg of oral zinc sulfate syrup and the control group (n=20) received a placebo daily in the same buttle and same test for 6 months duration of study. Serum zinc levels and anthropometric measurements (weight, height, head circumference, and arm circumference) were measured before and after intervention. Zinc deficiency was defined as serum zinc level < 9.9 µmol/l.
Results: Serum zinc level did not differ between the two groups (P=0.86). Zinc supplementation resulted in a significant increase in height (P= 0.008).
Conclusion: This study showed that zinc supplements have a significant increase in the length of male 6-year-old children.

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Introduction

It is essential that Vitamins as Minerals are important for growth and metabolism. There is deficiency of vitamins and minerals, particularly vitamin A, iodine, iron, and zinc (1) in more than 2 billion people.

As zinc is present in more than two hundred specific enzymes and also a factor for structural ion in transcription there for will be an essential trace elements for humans (2, 3).

The first health concern and major attention for zinc deficiency was recognized in 1961 (4, 5). It is estimated that one-third of the world population lives in countries with a high prevalence of zinc deficiency, which was found to be responsible for 0.4 million child deaths in 2008 (6, 7). It is estimated that 17.3% of the world population, ranging from 7.5% in developed countries to 30% in South Asia, are at risk for inadequate zinc intake (8). The vulnerable populations include infants, young children, and pregnant and lactating women due to high zinc requirements at these critical stages of life (9, 10).

Zinc deficiency is reported to be associated with impairment of growth, testicular functions, appetite, and sense of taste as well as delay in wound healing, immune resistance, and impaired memory (11). Zinc deficiency

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also interferes with the metabolism of thyroid hormones, androgens, and growth hormone (GH) (11). It was shown that the insulin-derived growth Fctor-1 (EGF-1) is decreased in zinc deficiency regardless of the energy intake (12, 13). IGF-1 receptor activation is responsible for changes in cell cycle and proliferation through tyrosine phosphorylation as a result of increased tyrosine kinase activity (12, 13). On the other hand, IGF-1 increases protein and collagen synthesis through increasing cellular thymidine uptake (12, 13). Regardless of the described mechanisms of action of zinc on growth, the findings of human studies are controversial. Some studies reported positive effects of zinc supplementation on growth in various groups of zinc-deficient children (14-16), this effect was not observed in other studies (17, 18). This study aimed to determine the effect of zinc supplementation on the physical growth of six-year-old male children. This difference might, in part, be due to the cut-offs used for detecting zinc deficiency ranging from 9.9 to 10.7 µmol/l for children under the age of 10 years (19, 20). It is hypothesized that zinc supplementation may not have substantial clinical effects in children with normal serum zinc levels. This study aimed to assess the children by testing the effect of providing 6 months of zinc supplementation on their anthropometric validity. The novelty of our study is the same sex and same age in samples.

Materials and Method

Subjects and methods

This study was a double-blind clinical trial conducted on male 6 to 7-year-old (first-grade) primary school students. Subjects were selected based on cluster random sampling, after obtaining approval from the Education organ of Khorasan Razavi province, from primary schools of the region (Region Five). Written informed consent was obtained from the parents or legal guardians of the subjects.

Procedure

All studied children were subjected to medical history and clinical assessment. Healthy male children between 6 and 7 years old were included in this study. Children with a history of preterm birth, chronic systemic disease, bone dysplasia, dysmorphic syndromes, chronic malabsorption, other nutrition deficiencies, and a history of previous use of zinc supplements were excluded. Parents and laboratory staff were blinded to treatment assignment.

A total of 40 subjects were recruited for this study. Subjects were randomly assigned into intervention and control groups each consisting of 20 subjects. A single daily dose of 20 mg zinc sulfate syrup was administered orally to the intervention group for 6 months. The control group received a placebo similar to the zinc sulfate syrup in shape and tested as a case group for 6 months. It was made by a pharmacies as our coworker.

Measurements

Anthropometric characteristics, including weight, height, head, and arm circumference were measured at baseline and after intervention. Height was measured to the nearest 1.0 mm with a Harpenden wall mounted stadiometer and weight to the nearest 0.1 kg on electronic bathroom scales. Head and arm circumferences were manually measured by tape.

All blood samples were taken in the morning in a non-fasting state. Serum zinc levels were measured at baseline and 12 hours after the last dose of zinc sulfate by manual colorimetric method technique (13). Zinc deficiency was defined as a serum zinc level of less than 9.9 µmol/l (21, 22).

This study was approved by the ethical committee of the Mashhad University of Medical Science, Iran. IRCT 138711021162N9

Statistical analysis

Continuous data were presented as mean and standard deviation (SD) while frequency and percentage were used to describe categorical variables. The mean difference between the baseline and the final assessment was assessed using an independent student t-test. The results were analyzed using the statistical package for the social science (SPSS) software version 10.00 (Echosoft Corp; USA, 2005). A p-value of less than 0.05 was considered significant.

Results

The range of weight in children in the case group was 17.1 kg minimum and 24.6 kg maximum and in the control group was 17.2 kg minimum and 27 kg maximum. Two groups were the same with normal distribution in variable weight (p= 0.56), length (p= 0.16), head circumference (p= 0.48), arm circumference (p=0.31), and zinc serum level (p= 0.78). Although there was no significant difference between the two groups in weight (p=0.97), head circumference (p=0.21), and arm circumference(p=0.06) after the intervention, a significant difference was found in liner growth (P= 0.008) (Table 1).

Serum zinc levels were within normal limits and did not differ between the two groups at baseline and after intervention (P=0.86) (Table 2).

Table 1: Anthropometric characters of two groups

Variable		Case group	Control group	p
Weight (kg)	Before	20.37± 2.21	20.92± 1.98	P= 0.97
	After	21.65 ± 3.02	21.90± 1.96	
Height (cm)	Before	116.67± 5.70	117.50± 2.80	P= 0.01*
	After	122.93 ± 5.52	122.97± 3.80	
Head circumference (cm)	Before	49.79± 1.57	50.58± 1.41	P= 0.21
	After	51.26± 1.46	51.68± 1.26	
Arm circumference (cm)	Before	15.91± 1.04	16.09± 0.93	P= 0.06
	After	16.79± 1.27	16.57± 1.16	

* Significant difference

Table 2: Serum zinc levels (µg/dl) before and after zinc supplementation

Time	Case group	Control group	p
Before intervention	90.87±29.20	76.09±36.55	P= 0.86
After intervention	170.04±83.01	117.60±33.48	

Discussion

This study showed no plasma zinc level in cut-off zinc deficiency. The prevalence of zinc deficiency in Iran was reported between 5.9% and 43% (23-26). The first and foremost clinical manifestation of zinc deficiency is the reduction in the velocity of physical growth in neonates and children (27,28). Hamza et al in Egypt (2012), studied short stature and growth retardation in zinc-deficient children (29). They explained this finding by the low intakes of animal products and animal protein due to the low socio-economic status of the population. In addition, zinc intake was not only low, but its bioavailability was poor because of the high phytate, fiber, and tea content of the diet among the Egyptian population (29). The exact mechanism of zinc deficiency and zinc supplementation on growth hormone secretion, serum IGF-1 levels, and growth is not well delineated (12, 14, 30). Das et al (2013) explained that the effect of zinc on growth is due to the synthesis of collagen, osteocalcin, and differentiation of chondrocytes, osteoblasts, and fibroblasts (30). Cesur et al, (2009) found that serum IGF-1 and insulin-like growth factor binding protein-3 (IGFBP-3) were below normal reference ranges in 96.6% and 100% of their short-statured zinc-deficient children (31).

This study showed that zinc supplementation had no influence on weight, head, and arm circumference, but significant differences were found in liner growth in normal 6-year-old children. Although many studies in different countries have shown a positive association between zinc supplementation and physical growth in children the observed effect of zinc supplementation on various indicators of physical growth was inconsistent (26, 27, 32, 33). It has been shown in different studies that zinc supplementation increases height and weight (26, 34, 35). For instance, Brown et al (2002) in a meta-analysis showed a highly significant aggressive effect size of 0.350 (95% CI: 0.189, 0.511) for height, 0.309 (95% CI: 0.178, 0.439) for weight and≈ 0 for weight- for height increments (28). Masoodpoor found that zinc supplementation improved weight and height in underweight stunted children (36). In contrast in a recent systematic review by Pimpin et al. (2016) zinc supplementation was associated only with a 0.69 cm increase in height (95%CI: 0.14, 1.25) and height for age Z score by 0.09 units (95%CI: 0.07, 0.12) (33). But despite our study, Kikafulnda et al in Uganda, no effect of zinc on liner growth was found (37).

In this study administration of zinc supplement for 6 months resulted in a significant increase in linear growth compared to the control group, which was in line with the findings of a previous study by Hakimi et al. (2006) that found a 2.7 ± 2.5 cm increment in linear growth in Iranian children who were supplemented with zinc for a period of 1 to 0 months (26). In contrast to the study by Hakimi et al. (2006), where 10 out of 42 subjects were zinc deficient, in the current study, all subjects had normal zinc levels. Although Hakimi et al. (2006) reported an improvement in weight and height in zinc zinc-deficient group compared to zinc adequate group, they failed to find a significant association between plasma zinc levels at baseline and growth increment (26). Similar to the findings of the aforementioned study, the current study also found an increase in linear growth with zinc supplementation regardless of the plasma zinc level. This observed increase in linear growth regardless of detectable zinc deficiency might be due to the different cut-off values for plasma zinc levels. While the World Health Organization has defined a zinc deficiency cut-off of 9.9 µmol/l for children under 10 years

of age, a cut-off of 10.7 µmol/l has been described and used for detecting zinc deficiency in some studies and references (19, 20). On the grounds of these findings it might be hypothesized that although all subjects had plasma zinc levels above the deficiency cut-off, a mild state of zinc deficiency might have been present in the subjects. In other words, the current cut-off for serum zinc levels may only indicate severe zinc deficiency and might not be applicable in identifying children at risk for zinc deficiency. The limitation of the study is that there is an obstacle for the education organization to enter schools for research with more samples and different age groups. On the other hand Lack of complete control of diet despite health and nutritional advice to parents. Uncertainty about the exact intake of zinc supplement or placebo during the 6 months despite repeated reassurances in multiple meetings or phone calls with parents.

Conclusion

This study showed that zinc supplements have a significant increase in the length of male 6-year-old children.

Authors' contributions

AM and EKH participated in the design of the study. Statistical analyses were conducted by MS , AJE and ASF. All authors contributed to the interpretation of the results. NP, AM and AV. contributed to the drafting of the manuscript. All authors also contributed to the critical revision of the manuscript for important intellectual content, approved the final version, and are accountable for the integrity of its content.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Mashhad University of Medical Science .The researchers obtained informed consent from the participating patients. The study was registered in the Iranian Clinical Trial Registration Center (IRCT138711021162N9).

Consent for publication

"Not applicable."

Conflict of interest

The authors declare that no financial or other conflict of interest exists about the content of the paper.

Availability of data and materials

You can request the study's data from the corresponding author.

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