FREQUENCY OF HYPOMAGNESEMIA IN RHEUMATOID ARTHRITIS PATIENTS WITH TRPM6 GENE POLYMORPHISM IN PESHAWAR

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ABSTRACT

Objectives: The goal of the current study was to find the frequency of hypomagnesemia in rheumatoid arthritis (RA) patients and possible association between TRPM6 gene polymorphism and onset of rheumatoid arthritis.

Material and Methods: A cross-sectional analysis was performed from June 2019 till May 2020. 150 patients and 150 control samples were included in the study from assigned health care facilities of Peshawar and blood samples and other information was collected. The experimental work was performed in Department of Physiology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. Non-probability sampling technique was used, and written informed consent was obtained from the subjects. Serum magnesium levels were measured. A modified salting out protocol was used to extract DNA. The TRPM6 gene polymorphism was genotyped using the ARMS-PCR (amplification refractory mutation system polymerase chain reaction) method. The Chi-square test was used to determine the relationship between TRPM6 genotypes and rheumatoid arthritis disease. The odds ratio at the 95 percent confidence level was used to calculate the risk of rheumatoid arthritis.

Results: According to our results, serum magnesium level was found normal in 110 (36.67%) patients with RA. Hypomagnesemia was found in 35 (11.67%) while magnesium levels of 05 (1.67%) patients were found to be at the borderline. A significant association was found between TRPM6 gene polymorphism (rs2274924) and hypomagnesemia in patients with RA (*p*-value <0.05) but was not a risk factor for RA in predisposed people (odds Ratio = 0.23).

Conclusion: Hypomagnesemia and TRPM6 gene polymorphism were found to be common in rheumatoid arthritis patients in Peshawar. However, large-scale study is required to further investigate the risk of RA in patients with TRPM6 gene polymorphism.

Key Words: Autoimmune diseases, Rheumatoid arthritis, TRPM6, prevalence, hypomagnesemia, Peshawar.

Introduction

Rheumatoid arthritis (RA) is a widespread inflammatory disease that causes chronic organ dysfunction, early death, and a socioeconomic burden on patient [(1)]. Because of the deregulation of immune system functions that occurs as the disease advances to severe stages, individuals with RA are at a high risk of death [(2)].

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The global prevalence of the disease is estimated to be around 1% [(3)] and Although the specific aetiology of the disease is unknown, recent breakthroughs have revealed that the condition is related with a complex set of genetic and environmental risk factors [(4)]. Discrepancies in immunomodulatory pathways also contribute significantly to the onset of illness [(5)]. During initial stages of the disease, synoviocytes interact with immune cells such macrophages, natural killer cells, T and B lymphocytes to cause chronic synovial inflammation. Complex interplay between complement and pro-inflammatory proteins promotes illness progression in sensitive individuals. [(6)].

The link between nutritional components of food and the development of normal immunity is a well-established natural phenomena. However, the relationship between nutritional

deficits in the body and autoimmune immunomodulation is a relatively new notion [7]. Fernandes et al. demonstrated the relationship between nutrients and immune system performance for the first time in 1976 [8]. According to Hayashi et al., patients with RA had a decreased intake of fish oil and other unsaturated fatty acids, but an appropriate diet of omega-3 and other monounsaturated fatty acids can assist to minimise joint inflammation and other disease activities [9]. Zinc also play a critical role in the development of the immune system. Paola et al. revealed that zinc deficiency increases cytokine and other pro-inflammatory protein synthesis, resulting in persistent joint inflammation [10]. Selenium is a naturally occurring trace element that has been proven to have antioxidant activity against reactive oxygen species. It also participate in the regulation of appropriate immunological responses. Angelica et al. reported that a selenium-rich diet aided in the treatment of many inflammatory disorders in animal models via modifying immune system functions [11].

After sodium, potassium, and calcium, magnesium is the fourth most common micronutrient in our bodies [12]. The recommended daily allowance of magnesium for an adult person is 360-400mg per day, which is entirely obtained from food [13]. Normal serum magnesium values are maintained via three mechanisms: intestine absorption, bone storage, and renal excretion. When there is a magnesium deficiency, Mg from bones is released to maintain steady serum levels [14]. Magnesium plays an important role in a variety of metabolic and cellular activities. Magnesium is a cofactor for roughly 300 enzymes involved in the metabolism of key macromolecules such carbohydrates, proteins, and lipids [15]. Similarly, magnesium is an essential mineral that plays an important role in the regulation of normal immune responses in inflammatory conditions [16]. About 80-90% of dietary Mg is absorbed in small intestine through paracellular passive mechanism while small amount is transported in blood through Transient Receptor Potential Channel Melastatin member 6 and 7 (TRPM6 and TRPM7) [17]. The main cause of hypomagnesemia related to gastrointestinal absorption is due to acute or chronic diarrhea rather than vomiting since the concentration of magnesium in lower GI tract secretion is higher than secretion of upper GI tract secretions [18]. Similarly, the use of some drugs, such as omeprazole for more than one year, may also reduce the absorptions of magnesium from GI tract by inhibition of TRPM6 and TRPM7 magnesium channels [19]. Besides this, genetic mutations in TRPM6 gene results in malfunctioning of TRPM6 channels, leads to hypomagnesemia with secondary hypocalcemia (HSH) while downregulation of TRPM7 intestinal channel results in increased magnesium influx in colon cells [20]. Research studies have shown that hypomagnesaemia in turn increases the risk of onset of pro-inflammatory mediators such as IL-1, IL-6, TNF-alpha and histamine [21, 22]. The objective of current study was to determine the frequency of hypomagnesemia in patients with RA and to find association between the TRPM6 gene polymorphism leading to onset of rheumatoid arthritis.

MATERIAL AND METHODS

This cross-sectional analysis for determination of frequency of hypomagnesemia and TRPM6 gene polymorphism in patients with RA was performed at Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, while samples were collected from OPDs of Department of Medicine, Lady Reading Hospital (LRH), Hayatabad Medical Complex and Khyber Teaching Hospital, Peshawar from June 2019 to May 2020. Gender and ages of cases and control samples were statistically compared. Informed consent for participation in the study either directly from patient or indirectly from his or her guardian was obtained. The study was approved by the ethical committee of KMU (Reference number: DIR/KMU-EB/PG/000822). A total of 300 subjects i.e., 150 diagnosed patients with RA (54 females and 96 males) and 150 healthy control subjects (80 females and 70 males) were included in the current study. Non-probability sampling technique was used while selecting the patients. The sample size was calculated according to the World Health Organization formula for sample size calculation taking 06% margin of error and with confidence interval of 95% [23]. Patients were enrolled in the study based on pre-set inclusion and exclusion criteria i.e., diagnosed patients with RA of either gender were included in the study while the patients with other bone diseases were excluded from the study. Blood samples and other information were collected from already diagnosed patients with RA. 3cc blood was withdrawn by venipuncture and transferred to EDTA coated vacutainers and kept at -4^{o}C. The collected blood samples were transported immediately to the assigned laboratory, applying standard protocols for blood sample transportation. Serum magnesium levels were measured using Diestro® Electrolyte Analyzer Machine according to the guidelines of the manufacturer and magnesium level of 1.8-2.5 mg/dL was

taken as normal while serum magnesium level in between 1.65-1.75mg/dL was taken as borderline and the values below this range were considered as hypomagnesemia. After that, DNA was extracted from whole blood by using modified salting out protocol [24] and was kept at -20ºC. For analysis of specific TRPM6 SNP (rs2274924), specific primers were designed. The PCR conditions were set as follows: 35 cycles of 60 seconds' denaturation at 95ºC, 30 seconds annealing at 59ºC and 60 seconds' extension at 72ºC. The amplification products were analyzed by loading 10µl of PCR product on 1.5% agarose gel along with 1µl of 6X loading dye and 1kb DNA ladder was used for size discrimination. The results were then visualized under ultraviolet (UV) trans-illuminator. The data was primarily recorded on a Microsoft Excel Spreadsheet. Age and gender-wise stratification were also performed. SPSS V.20 was used for statistical analysis and chi-square test was performed for finding association between alleles and genotypes. Odds ratio (ORs) was calculated with 95% confidence interval (CI).

RESULTS

The mean age of the study population was 50.60 ± 13.01 years. The study included 212 (70.66%) female while 88 (29.34%) male subjects. In control samples, the serum magnesium level was found normal in 110 (36.67%), hypomagnesemia was found in 35 (11.67%) while magnesium levels of 05 (1.67%) subjects were found to be at borderline. Similarly, in cases, the serum magnesium level was found normal in 20 (6.67%), hypomagnesemia was found in 96 (32%) while 34 (11.34%) patients had serum magnesium levels were found at borderline.

The study samples were then studied for determination of TRPM6 SNP (rs2274924) through Polmerase Chain Reaction (PCR) technique (**Fig 1**). The The chi-square (x2) test revealed a significant association between the SNP and RA (*p*-value = 0.042). We calculated the odds ratio (OR) between cases and controls to see if this significant association is a risk factor for disease onset. Our study SNP was associated with RA, but not a risk factor for onset. **Table 1** summarises the calculated odds ratio (OR) of 0.23. Our findings suggest that the SNP rs3750424 is not a risk factor for early onset RA symptoms in the Peshawar, KPK, and surrounding areas.

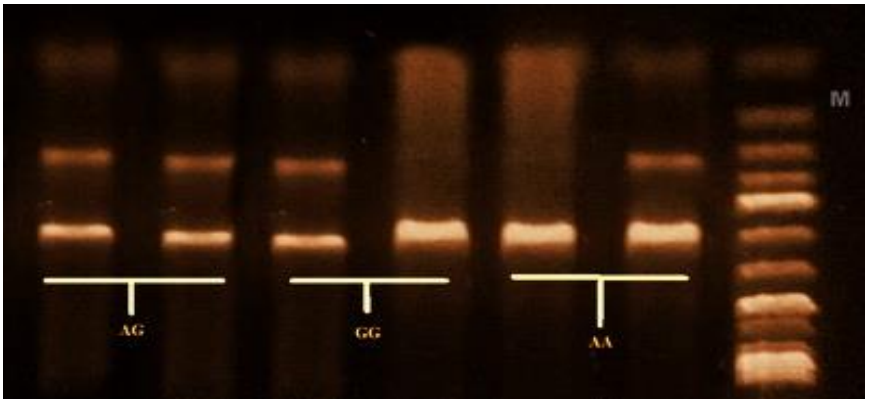

Fig.1: PCR amplification of TRPM6 gene. (Right to Left) Lane 1: DNA gene ruler ladder. Lane 2: AA, Lane 3: GG, Lane 4: AG. PCR product size of 510 bp for Allele specific,762bp for Control.

Table 1: Analysis of association of TRPM6 polymorphism (rs2274924) with RA.

SNP[1]	**Sample type**	**n[2]**	**Genotype Frequency**				
			Genotype			***p*-value[3]**	**OR (95%CI)[4]**
			AA	A/G	GG	**0.042**	0.23 (0.34-0.18)
	Cases	150	34	85	31		
	Controls	150	76	24	50		

Discussion

Even though magnesium is the second most prevalent intracellular and fourth most abundant extracellular cation after sodium, potassium, and calcium, it receives little medical attention. Because hypomagnesemia has milder symptoms than other electrolyte deficiencies, it is often misdiagnosed. Symptoms appear when serum magnesium levels are critically low [25]. Although the exact role of magnesium shortage in inflammatory reactions is uncertain, numerous pathways have been hypothesised.

Chavan et al. discovered hypomagnesemia in patients with rheumatoid arthritis when compared to healthy controls, demonstrating that a decrease in serum magnesium level is substantially connected with the onset of rheumatoid arthritis (*p*-value = < 0.01) [26]. According to Song *et al*., genetic mutations in the TRPM6 gene may result in diabetes mellitus in susceptible women whose diet lacks magnesium [27]. In current study, we also found decrease in serum magnesium levels in 32% patients suffering from RA while 11.34% patients with RA had serum magnesium level at borderline. Furthermore, 33.33% of patients with RA had normal serum magnesium levels. Our results were found to be related to a metanalysis on the prevalence of RA around the globe [28].

Genetic polymorphisms in the TRPM6 and TRPM7 genes, which regulate the magnesium transport channels in the intestine, may be involved in the onset of RA disease. When these transport channels malfunction, dietary magnesium absorption suffers. According to various studies, hypomagnesemia can cause the onset of pro-inflammatory responses that enhance the process of inflammation, particularly in rheumatoid arthritis patients who are predisposed to it [29]. In our study, we found that the most reported single nucleotide polymorphism (SNP) in TRPM6 gene i.e., rs2274924, is significantly associated with RA is susceptible patients (*p*-value = 0.042). On the other hand, at the same time, hypomagnesemia was not found to be a risk factor for the onset of rheumatoid arthritis disease in susceptible patients (OR = 0.23). As previously no such study has been conducted for determination of association between serum magnesium level and RA, our findings are first in this regard. However, our results can be indirectly correlated with the previous study performed by Weglicki et al. according to which hypomagnesemia leads to increased serum CRP levels in adult patients of metabolic syndrome [30].

Conclusion

Hypomagnesemia was seen in patients suffering from rheumatoid arthritis and a significant association was found between TRPM6 gene polymorphism and rheumatoid arthritis, but due to limited population size, it is very early to derive a conclusive statement on the involvement of hypomagnesemia in the onset of rheumatoid arthritis.

Authors Contribution

Sara Asmat (SA): Manuscript writing
Ambar Shoaib (AS): Sample collection and manuscript writing
Javaid Hassan (JH): Sample collection and laboratory work
Shahid Fareed (SF): Laboratory work and manuscript writing
Shah Hussain (SH): Results compilation and statistical analysis
Ghulam Farooq (GF): Data analysis and manuscript compilation.

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