ORIGINAL ARTICLE

Growth Differentiation Factor 5 (GDF5) Polymorphism and Risk of Knee Osteoarthritis: A Case-Control Study

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Abstract

Aim: The present case-control study was designed to determine the association of GDF5 gene Polymorphism with risk of occurrence of Osteoarthritis especially knee osteoarthritis and to find any significant association of the polymorphism with the severity of the disease. **Material and Methods:** 200 samples were collected out of which 100 were confirmed cases of Knee osteoarthritis and 100 were healthy controls which conducted at VMMC and Safdarjung Hospital New Delhi. **Results:** On evaluation it was found that there was a significant difference in genotypic distribution between the case and control groups ($\chi 2$= 5.016, p=0.04). The wild type C allele was higher in control group than cases which is statistically significant (OR= 1.679, CI (95%)= 1.114-2.530, p= 0.006). Other genetic comparison also revealed significant results, there by resulting in a conclusion that GDF gene polymorphism increases the risk of occurrence of Knee osteoarthritis. But on comparison between various sub groups of cases based on KL grade, none of the association was statistically significant showing that the grading score has no association (p>0.05) with GDF5 polymorphism (rs143833).**Conclusion:** Our results revealed a positive association between the GDF5 polymorphism (rs143383) and Knee OA where TT genotype has an increased risk of occurrence of OA in North Indian population, whereas no significant difference could be concluded between genotype and severity of the disease.

Keywords

Polymorphism, Osteoarthritis, Growth Differentiation Factor 5

Introduction

Osteoarthritis (OA) is a degenerative disease of the joint which is most common form of arthritis and one of the leading causes of morbidity in adult population.[1] According to World Health Organization (WHO), "9.6% male and 18%female of age group e"60 years have developed symptomatic OA worldwide". As per National Health Portal (India), "OA is the second most common rheumatologic disease with a prevalence of 22% to 39% in India.[2]

Prevalence of OA depends on factors like age, gender, and ethnicity. According to age, 43% of population with OA are e"65 years whereas 88% are e"45years, making an annual incidence of knee OA most prevalent in age group 55-65years. In terms of gender 62% of OA patients are female. Again, population with age d"45years have more incidence of OA in male and population with age >45 years develop OA in female. Basing on ethnicity, OA has a greater impact on "People with Colour" than whites.[3]

OA is a multifactorial disease, where environmental as well as genetic factor have important role play. Repeated wear and tear lead to low grade inflammation and joint

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destruction especially articular cartilage, which is a result of imbalance between cartilage damage and its repair.[4] Articular cartilage repair is performed by growth factor like Tumour Growth Factor (TGF-â1), Insulin like Growth Factor (IGF-1), Bone Morphogenetic Protein (BMP), etc. Among these growth factor, GDF-5, is a member of TGF-â family and is also one of the earlier markers of joint development.[5] Genome-wide association studies revealed that GDF-5 can be a susceptible gene for OA, there by playing a role in maintaining homeostasis of articular cartilage.[6]

Single Nucleotide Polymorphism (SNPs) are DNA variations which is common within a population.[7] Several studies have revealed that GDF-5 polymorphism especially SNP rs143383 present in 5'UTR region of GDF-5 gene is a major allele that is susceptible for OA in Asian and European population. [6,8,9]

Current treatment modalities for OA are focused on pain relief and to promote patient's day to day function rather than inhibiting OA progression or preventing its occurrence.Therefore, there is a need to other possible measures that contribute to pathophysiology of OA, inorder to develop more effective way for prognosis and treatment of the disease.

Material and Methods

The present study is a hospital-basedCase Control study which was conducted in the Biochemistry department ofVMMC and Safdarjung Hospital, New Delhi in collaboration with Department of Orthopaedics and Department of Radiology. 100 adults of both sexes with agee"45 years and diagnosed withKnee Osteoarthritis from Orthopaedics OPD were considered as Cases and 100 age and sex matched apparently healthy adults, without any history of joint pain were taken as Controls.

Inclusion Criteria

Cases of Knee Osteoarthritis which were diagnosed as per the American College of Rheumatology criteria withage e"45 years were taken.Kellgren and Lawrence scores (KL scores) were calculated on radiographs to confirm the diagnosis of OA.A cut off KL scores 2 or more was used for classification of OA.

Eclusion Criteria

Gout,Rheumatoid arthritis, H/O severe trauma to the affected joint, Congenital lower limb deformity.

Initial Screening and Evaluation

Patients fulfilling the inclusion criteria were enrolled in the study. A predesigned proforma was used to obtain information about the adult. A detailed history was taken, and examination was done to look for signs of Osteoarthritis.

Information regarding age and weight of the individual, previous history of trauma to the affected joint, history of any genetic or metabolic diseases were recorded. The study was performed only after the approval of Institutional Ethics Committee. Detail information regarding the study and risk associated was conveyed to the subjects and informed consent was taken.

Sample Collection and Processing

After collection of detailed history and necessary information, 1 ml of venous blood was collected in EDTA vacutainers, under strict aseptic conditions. The blood was used to extract DNA from the leucocytes.DNA isolation was done by "QIAGEN DNA extraction Kit". The DNA was stored at -70C which was used further for amplifying the DNA by Polymerase Chain Reaction (PCR) and then subjecting it to digestion by Restriction Endonuclease**.**DNA sequences containing GDF5 gene SNP (rs143383) was amplified by PCR. The GDF5 gene (686 bp) fragment was amplified using the following primer: 5'-TAAGCCTAGAGTCCCGCTGC-3'and 5'-AGCCGCTGAATGACACCAAA-3' under the PCR conditions:Initial denaturation at 95C for 5 min, 30 cycles of the Denaturation at 95 C for 30 sec, Annealing at 55C for 30 sec, Extension at 72C for 30 sec, Final extension at 72 C for 5 min and 4C for temporary storage.The loci for GDF5 gene SNP were identified by restriction endonuclease (BsiEI): for C/T at 37C for 16 hours, which was thenelectrophoresed and visualised to produce bands at 420 and 266 bp position if homozygous alleles present for Wild type (CC) bands at420,266,686 bp position if heterozygous alleles present for Wild and Polymorphic (CT)and 686 bp position if homozygous alleles present for Polymorphic type (TT) as displayed in Fig 1.

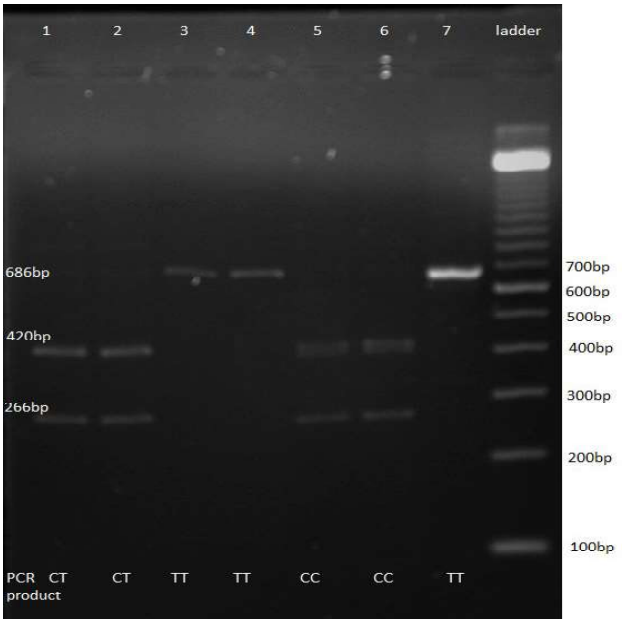

Figure 1 Showing PCR Findings

Statistical Analysis

The findings were analysed using online statistical tools as well as Microsoft Excel sheet. The Quantitative values were expressed in the form of mean ± SD and independent t-test in applied to compare the values between cases and control. Qualitative values were expressed as number (percentage) and then comparison done by Chi-square test. The Hardy–Weinberg equilibrium (HWE) is evaluated to check whether the whole population is represented by the control group. Relationship between GDF5 gene and OA is established by ORs and 95% Confidence Interval (CI). A value of $p<0.05$ is said to be statistically significant.

Results

The baseline characteristics of cases and control is represented in Table-1. According to the finding case group contains 63% female and 37% male, whereas in control group 56% female & 44% of population were males respectively. On statistical analysis, no significant difference was found among the two groups (p=0.31311). The average age of individuals in both the groups were 49.5 years and 50.4 years respectively. The frequency of smoking and drinking among cases were 17% and 39% respectively and among controls were 39% and 61% respectively. On analysis none of these parameters showed any significant difference between the 2 groups(p=0.618, p=0.471, p=0.181 respectively).But on analysing the average body weight, the case population had a mean of 78.8 kg whereascontrols had a mean of 72.7 kg, which revealed a statistically significant difference between the two groups suggesting that increase in body weight can increase the risk of occurrence of knee OA.

Association of rs143383 with Knee Osteoarthritis:

All the 200 blood samples were genotyped, which yield a concordance rate of 100%. The genotype frequency in case group for TT is 25%, for CT is 36%, for CC is 39%, whereas among control, the genotype frequency for TT is 14%, for CT is 34% and CC is 52%. The Hardy Weinberg Equilibrium Test shows no significant difference between cases and control group with a p-value=1. There was significant difference in genotypic distribution with ÷2= 5.016, p=0.04. The wild type C allele was higher in control group than cases which is statistically significant (OR= 1.679, CI(95%)= 1.114-2.530, p= 0.006). On verifying the other gene model, it was observed that TT/CT genotype vs CC genotype was found to have significantly high incidence among cases than control with OR=1.694, CI(95%)=0.966-2.971, p=0.032. Another gene model illustrated that TT genotype vs CT/CC was found to be highly significant in cases than control (OR= 2.047, CI(95%)=0.993- 4.223, p=0.026), all of which shows similar result of increased risk of knee OA. The above results were shown in Table:2.

Stratification Analysis:

The cases were categorized based on the K-L grade scale of diagnosis of a OA into two groups. Where Group

Table 1: The Baseline characteristics of case and control

	CASES	CONTROL	χ2/ t-test	p-value
AGE (mean± SD)	49.56± 1.15	50.46± 1.64	15.4766	0.6182
SEX				
Male	37%	44%	1.1016	0.3133
Female	63%	56%		
Avg. Body weight (Kg)	78.85±1.156	72.76±1.934	27.028	<0.001*
SMOKING				
yes	17%	21%	0.5198	0.471
No	83%	79%		
DRINKING				
yes	39%	30%	1.7922	0.181
No	61%	70%		
GENOTYPE				
TT	25%	14%	5.016	0.04*
CT	36%	34%		
CC	39%	52%		

Table 2: Association of rs143383 with knee osteoarthritis

	CASES	CONTROL	ODD'S RATIO (95%CI)	P-VALUE
TT Vs CC	**25/39**	**14/52**	2.381 (1.097-5.167)	0.0141*
TT Vs CT	**25/36**	**14/34**	1.686 (0.754-3.772)	0.1015
TT/CT Vs CC	**61/39**	**48/52**	1.694 (0.966-2.971)	0.032*
TT Vs CT/CC	**25/75**	**14/86**	2.047 (0.993-4.223)	0.026*
T Vs C	**86/114**	**62/138**	1.679 (1.114-2.530)	0.006*

Table 3: Stratification Analysis

CASES	TT	CT	CC	T allele	C allele
Grade I/II	15	18	19	28	56
Grade III/IV	10	18	20	38	58
OR (95%CI)	1.578 (0.571-4.366)	1.052 (0.425-2.605)	1	0.763 (0.414-1.406)	1
p-value	0.189	0.455		0.192	

1 contains the subjects with K-L grade I and II, group 2 constitute the subjects with K-L grade III and IV. Various genotypic analysis was evaluated among the two groups like TT vs CC (p=0.189), CT vs CC (p=0.455), T allele vs C allele (p=0.192). None of the association was statistically significant showing that the grading score has no association (p>0.05) with GDF5 polymorphism (rs143833). Details shown in *Table:3.*

Discussion

In this present study, we concluded that the polymorphic genotype TT allele increases the risk of knee OA. As well as there were association between GDF5 polymorphism and Average Body weight for increasing the risk of occurrence of knee OA.

It is a well-known fact that OA is a multifactorial disease where genes and environmental factors, both play a very crucial role in determining the risk of occurrence of the disease. Again it is very widely acknowledged that the SNPs in certain genes are associated with risk of OA. There genes are important structural and ECM related factors or signalling molecules which are implicated in maintenance of articular cartilage and joint.[1,10] For the past decade, it was known that the initial indicator of joint development is the appearance of Interzone at the tentative joint site where it serves as joint progenitor.[11] This interzone is derived from mesenchymal tissue and expresses GDF5 which act as a marker of interzone.[12]. Further a genome-wide association studies revealed that in later stage of life, GDF5 a member of TGF-â superfamily is responsible for maintenance of the homeostasis of articular cartilage suggesting it to be a susceptible gene for OA.[6]

Many genetic studies have revealed that OA is a multigene disorder, where there are many contradictory results, like study done by Pan F et al stated that there is difference in association between GDF5 variants and OA of joint like knee, hip and hand.[13] Another study performed on European population stated that non-significant association exist between hip OA and rs143383 [14] where as in Asian population significant association of GDF5 with knee OA was found only in individual aged >60 years but no significant association found in population aged<60 years.[15] On contrary, study done by Miyamoto *et al* suggested that a decline in GDF5 expression is linked to pathogenesis of OA.[6] Moreover study performed by Abdul *et al* revealed a possible association between GDF5 polymorphism with severity of Knee OA, which can be a prognostic marker for patients with high risk of disease progression.[16]

These variation in results can be attributed by several

reasons like different study population leading to variation in genetic makeup. Different lifestyle practices, eating habits and other environmental factors. Again, some studies suggest that GDF5 expression can be influenced by DNA methylation [17] and these methylation levels can be different for different joint tissue.[18] Hence type of joint specific OA can also be a factor for varied results. The present study also has several limitations, some of those are smaller sample size, other is like previous studies, the present study design is also case-control, which has a limitation in identifying cause-effect relationship, again this study is primarily focused on patients with Knee OA, so other joint specific arthritis also needs to be evaluated.

Conclusion:

To conclude, our results revealed a positive association between the GDF5 polymorphism (rs143383) and Knee OA where TT genotype has an increased risk of occurrence of OA in North Indian population, whereas no significant difference could be concluded between genotype and severity of the disease. But study on a larger population and multiple ethnic groups and joints is required for further exploration of the association and derive conclusion of the molecular mechanism involved in pathogenesis of OA.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

References

1. Chen D, Shen J, Zhao W, Wang T, Han L, Hamilton JL, et al. Osteoarthritis: toward a comprehensive understanding of pathological mechanism. Bone Res 2017;5(1):16044.
2. Pal CP, Singh P, Chaturvedi S, Pruthi KK, Vij A. Epidemiology of knee osteoarthritis in India and related factors. Indian J Orthop 2016 ;50(5):518-522.
3. Olen J, Schieb L, Hootman JM. Differences in the prevalence and severity of arthritis among racial/ ethnic groups in the United States, National Health Interview Survey, 2002, 2003, and 2006. Prev Chronic Dis 2010;7(3):A64.
4. Reynard LN, Bui C, Syddall CM, Loughlin J. CpG methylation regulates allelic expression of GDF5 by modulating binding of SP1 & SP3 repressor proteins to the Osteoarthritis susceptibility SNP rs143383. Human Genetics 2014;133(8):1059-1073.
5. Sun K, Guo J, Yao X, Guo Z, Guo F. Growth differentiation factor 5 in cartilage and osteoarthritis: A possible therapeutic candidate. Cell Prolif 2021;54(3):e12998.
6. Miyamoto Y, Mabuchi A, Shi D. A functional polymorphism in the 5' UTR of GDF5 is associated with susceptibility to osteoarthritis. Nat Genet 2007;39:529 -33.
7. Köberle B, Koch B, Fischer BM, Hartwig A. Single nucleotide polymorphisms in DNA repair genes and putative cancer risk. Arch Toxicol 2016; 90:2369 -2388.
8. Chapman K, Takahashi A, Meulenbelt I. A meta analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5' UTR of GDF5 with osteoarthritis susceptibility. Hum Mol Genet 2008;17:1497 -1504.
9. Southam L, Rodriguez Lopez J, Wilkins JM. An SNP in the 52 UTR of GDF5 is associated with osteoarthritis susceptibility in Europeans and within vivo differences in allelic expression in articular cartilage. Hum Mol Genet 2007; 16:2226 -32.
10. Zhang R, Yao J, Xu P et al. A comprehensive meta-analysis of association between genetic variants of GDF5 and osteoarthritis of the knee, hip, and hand. Inflamm Res. 2015; 64: 405–414.
11. Holder N. An experimental investigation into the early development of the chick elbow joint. J Embryol Exp Morphol. 1977;39:115.
12. Shwartz Y, Viukov S, Krief S, Zelzer E. Joint development involves a continuous influx of Gdf5 positive cells. Cell Rep. 2016;15:2577 2587.
13. Pan F, Tian J, Winzenberg T, Ding C, Jones G. Association between GDF5 rs143383 polymorphism and knee osteoarthritis: an updated meta analysis based on 23,995 subjects. BMC Musculoskel Dis 2014; 15:404.
14. Zhang R, Yao J, Xu P. A comprehensive meta analysis of association between genetic variants of GDF5 and osteoarthritis of the knee, hip and hand. Inflamm Res 2015; 64:405-14.
15. Zhang S, Wang J, Ji H, Jia H, Guan D. Interaction between GDF5 gene polymorphisms and environment factors increased the risk of knee osteoarthritis: a case control study. Bioscience Rep 2019; 39: R20182423.
16. Abd EM, Abdelaleem EA, Mohamed RA. Genetic influence of growth and differentiation factor 5 gene polymorphism (+104T/C) on the development of knee osteoarthritis and its association with disease severity. Eur J Rheumatol 2017; 4: 98–103.
17. Reynard LN, Bui C, Canty-Laird EG, Young D.A,Loughlin J.Expression of the osteoarthritis-associated gene GDF5 is modulated epigenetically by DNA methylation. Hum Mol Genet 2011; 20: 3450–60.
18. Syddall, C.M, Reynard, L.N, Young, D.A, Loughlin J. The identification of trans-acting factors that regulate the expression of GDF5 via the osteoarthritis susceptibility SNP rs143383. PLoS Genet 2013; 9: e1003557.