Role of FOXO3 Gene Polymorphism at Development of Primary Knee Osteoarthritis Disease in Iraqi Patients

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Abstract

Primary Knee osteoarthritis (PKOA) is disease occur in knee joint characterize by cartilage degradation that mediate via pro-inflammatory marker such as matrix metalloproteinase-13 (MMP-13) . Forkhead box (FoxO) proteins are a subgroup of the Forkhead family of transcription factors ,which were originally identified in Drosophila as a gene whose mutation resulted in ectopic head structures that look like a fork . Cartilage cells obtained from human joints affected by OA , the cells had a reduced activity of genes producing FoxO proteins and reduced activity of autophagy genes.

Key words :PKOA and FOXO3

Introduction

Primary Knee osteoarthritis (PKOA) is the disease occur in the knee joint .It's degenerative arthritis in cartilage matrix of knee , is a group of overlapping distinct diseases, which may have different etiologies but with similar at biologic, morphologic, and clinical outcomes (BARR & Andrew , 2019) . The most common form of arthritis is PKOA , 6%of adults are affected it and the women more affected than men at age more than 45 years .PKOA always has many soluble mediators for example prostaglandins and cytokines (KLOSE-JENSEN et al., 2019).

Forkhead box (FoxO) proteins are a subgroup of the Forkhead family of transcription factors ,which were originally identified in Drosophila as a gene whose mutation resulted in ectopic head structures that look like a fork . Cartilage cells obtained from human joints affected by OA , the cells had a reduced activity of genes producing FoxO proteins and reduced activity of autophagy genes (VARMA & Disha , 2014). A transcription factor is a special protein that binds to a specific gene in DNA and either stimulates or inhibits transcription. In this way it controls gene expression, which can be thought of as the turning on of a gene. The transcription factor makes sure that the correct gene is turned on or off at the right moment in the cell's life (LIANG et al., 2019).

Material and Methods

This study involved collected 50 PKOA grade 1 (G1) and 50 grade 4(G4) cases and 50 controls, age of the cases and controls were more than 40 years . Samples collection : Five ml of blood drawled from cases and controls collected it in EDTA tube .DNA was isolated from blood sample by DNA extraction kit (Zymo company / USA) according to special

method at kit The isolate DNA stored in -20 C until future use .The DNA sample had purity ratio 1.7 to 2 that used in PCR-RFLP method . The Gene Runner software (Version 3.05) was used to design of FOXO3 gene primer .

Primer	Sequence (5'→3') direction foxo3 gene(FORTE *et al., 2014*)	GC (%)	Tm (°C)	Size of gene bp
Forword	CAG CTT CTG AGT GAC AGA GTG	55.1	52.4	**157bp**
Reverse	TTC TTC CCT AGA GAG CAG CAG	56	52.4	

Steps	Temperature	Time	No. of Cycles
Denaturation 1	95°C	3min	1
Denaturation 2	94°C	45sec	33
Annealing	68°C	45 sec	
Extension 1	72°C	45sec	
Extension 2	72°C	7min	1

Results

This study explain increase of TT allele frequency in G1 of PKOA group when compared with control group , also increase TG allele in G4 of PKOA group when compared with control group . Table 1 and 2 .

Table 1: Genotype distribution of FOXO3 gene SNP (rs2802292) TT , TG &GG polymorphism in healthy control and patients G1

Polymorphism	**Group**	X^2	**Sig.**	**P-value**	**CI 95%**		**df**

	Control N=50				G1 N=50									
	Observed	Percentage	Expected	Percentage	Observed	Percentage	Expected	Percentage						
TT	19	38	21.25	42.5	23	46	20.79	41.58	16.17	S	0.0003	0.463 to 1.014	0.426	2
TG	31	62	23.75	47.5	16	32	23.27	46.54						
GG	0	0	5	10	11	22	5.94	11.88						
Total	50	100	50	100	50	100	50	100						
X^2	7.451				6.817									
Significant	S				S									
P-value	0.0241				0.0331									
Allele frequency														
T	0.69				0.62									
G	0.31				0.38									

Table 2 : Genotype distribution of FOXO3 gene SNP (rs2802292)TT , TG &GG polymorphism in healthy control and .patients G4

Polymorphism	Group								X^2	Sig.	P-value	CI 95%	OR	df
	Control N=50				G4 N=50									
	Observed	Percentage	Expected	Percentage	Observed	Percentage	Expected	Percentage						
TT	19	38	21.25	42.5	9	18	13.86	27.72	3.44	S	0.0434	1.006 to 2.074	2.384	1
TG	31	62	23.75	47.5	35	70	32.67	46.54						
GG	0	0	5	10	6	12	3.47	11.88						
Total	50	100	50	100	50	100	50	100						
X^2	7.451				30.87									
Significant	S				S									
P-value	0.0241				< 0.0001									

Allele frequency								
T	0.69	0.53						
G	0.31	0.47						

Discussion

Primary Knee Osteoarthritis (PKOA) is a progressive disorder occur in knee joints lead to loss of cartilage cell (degradation) gradually and development of bony spurs and cysts in Knee . This disorders is commonly with aging populations after 50 years . PKOA caused by many factors , such as trauma , gout and BMI effect , but in this study focus on PKOA that development with age without other factors therefore consider as one of age related diseases(KLOSE-JENSEN et al., 2019)

This study showed association between FoxO3gene polymorphisms with PKOA through the mutant of T>G . This association explained when compare control with G4 PKOA and G1 with G4 PKOA and obtained significant association in this comparisons. This result demonstrate that FoxO3 gene polymorphisms can develop the PKOA , This effect occur by NO and FSH that mediate the FoxO3 expression(LEE, Ki, and et al 2019)

Our study for the first time shows that FoxO3 SNP (rs2802292) is significantly associated with PKOA in Iraqi population . In addition to the highly significant association of FoxO3 and OA , based stratification also indicated that the mutant “G” allele has a much more pronounced risk rate of OA in Iraqi populations . Increase of G allele have association with reduce FoxO3 level and KOA development . Our study is first time shows association between KOA and FoxO3 SNP (rs2802292) in Iraqi population(WILLCOX, et al., 2008)

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