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Original Article

Possible association of Vitamin D receptor, caudal-related homeobox 2 polymorphism with the risk of cancer

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ABSTRACT

Objective: This study was conducted to find out the possible association of Vitamin D receptor, caudal-related homeobox 2 (VDR-Cdx2) polymorphism with cancer in the given study group.

Methods: In this study, 151 subjects (84 cases and 67 controls) were recruited from two local tertiary care hospitals of Karachi, Pakistan, suffering from various cancers including gastric cancer (GC), rectal cancer (RC), colon cancer (CC), and multiple myeloma followed by ethical approval from institutions and informed consent from all the participants. The genotyping of VDR-Cdx2 polymorphism was performed using tetra-primer amplification refractory mutation system-polymerase chain reaction method. The genotypic assortment/distribution in the control and disease groups was according to Hardy-Weinberg's equilibrium.

Results: The genotype frequencies of VDR-Cdx2 polymorphism in cancer patients were observed as: AA 1.2%, AG 32%, and GG 66.8% while in control group as; AA 7.5%, AG 50.7%, and GG was 41.8%. The results unveil that the genotype VDR-Cdx2 was found significantly different in cancer and control group ($P < 0.01$). The AG and GG genotypes were found to be associated with the cancer ($P < 0.05$). Therefore, these genotypes may be considered as the risk factors for cancer. However, the frequencies of "A" and "G" alleles were not significantly different between two groups.

Conclusion: The observed single-nucleotide polymorphism of VDR-Cdx2 gene may be considered as a risk factor for the cancer in this study group. The AG and GG genotypes established an association with various cancers including GC, RC, CC, and multiple myeloma in Pakistani population. Further investigations examining large data are required to compare the role of VDR-Cdx2 polymorphism in cancer etiology in related population.

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Introduction

Cancer causes a significant global disease burden causing a disease burden of more than 18 million in the year 2018–2019.[1-3] This has been recently reported that some cancer types are reducing or stabilizing to the year 2020 but still the overall number of cancer cases and deaths progressively increasing.[4,5]

Vitamin D has shown assorted roles in diverse physiological functions in the body. Calcitriol, the biologically active and naturally occurring secosteroid hormone, has been involved in the regulation of immune response, bone metabolism, cell proliferation, differentiation of various cell types, and many more functions.[6]

The role of Vitamin D receptor (VDR) polymorphism is one of the attention-seeking points, despite proper sunlight exposure. The association of VDR gene polymorphisms has been established with several forms of cancer and other chronic diseases. Most studied polymorphism of VDR is caudal-related homeobox 2 (Cdx-2) (rs11568820), FokI (rs10735810), ApaI (rs7975232), TaqI (rs731236), and BsmI (rs1544410).[7]

A VDR polymorphism found in promoter region is the Cdx-2 polymorphism (rs 11568820).[8] A Cdx-2, intestine-specific transcription factor, is found at the promoter region of exon 1 in VDR gene. This transcriptional factor is responsible for cellular proliferation and differentiation.[9] The gene expression of Cdx-2 has been regulated by the interaction of functional enhancer elements of VDR gene in small intestine.[10] It has been

implicated in homeostatic function in cellular development, and its impairment may result in tumor progression.

Single-nucleotide polymorphism (SNP) (rs 11568820) is a G>A sequence diversification at promoter region in the gene, at Cdx2 binding site, an intestinal specific transcription factor.[11] This A to G base replacement affects the Cdx2 binding site and decreases the transcriptional activity of VDR.[4] The available literature on the incidence of cancer and VDR Cdx-2 polymorphism is rudimentary. Most of researchers came with conflicting results regarding this SNP in various cancers. Number of studies found no association with cancer risk such as breast cancer, prostate cancer, basal cell carcinoma and squamous cell carcinoma, and colorectal cancer in some ethnicities.[12-15] Some studies are, however, evident about the correlation of cancer with respective SNP such as prostate cancer and ovarian cancer in related populations.[16,17,18]

This study was aimed to unravel the possible association of Cdx-2 polymorphism with cancer types related to digestive tract including oral cancer, gastric cancer (GC), colon cancer (CC), and liver cancer, which is more prevalent in our society.

Materials and Methods

This study included various types of cancer patients (including GC, rectal cancer (RC), CC, and multiple myeloma [MM]) as cases (n = 84) and healthy individual as controls (n = 67) in accordance with the WHO. Cancer patients were recruited from two tertiary care hospitals, Jinnah Postgraduate Medical Center and Darul Sehat Oncology Hospital located in Karachi, Pakistan, followed by ethical approval from institutions and informed consent from all the participants. Normal adult healthy volunteers were selected randomly as control subjects.

The normal healthy individuals with no known history and symptoms of cancer were selected as controls. Cancer patients were selected with known history based on previous medical records and investigations. All selected patients were those who have received standard chemotherapy and synchronous cancer was excluded.

This study was approved by the Ethical Review Board of Departmental Research Committee (DRC-00826/KU/2015), Department of Biochemistry, University of Karachi. All samples were collected from the subjects followed by receiving written informed consent.

A 3 ml of venous blood sample was collected in ethylenediaminetetraacetic acid (EDTA) (as anticoagulant) containing Vacutainer (BD, USA.) from each subject. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood using salting out protocol followed by proteinase K digestion.[15] Finally, the extracted genomic DNA was resuspended in 10 mM Tris-EDTA buffer (pH 8.0) and stored at –80°C for perusal of genetic evaluation.

Genotyping of Cdx2 (rs11568820) in VDR gene was carried out by tetra-amplification refectory system-polymerase chain reaction (T-ARMS-PCR).[19] The "forward (F) primer (5'-AGGATAGAGAAAATAATAGAAAACATT-3')" and "Reverse (R) primer (5'-AACCCATAATAAGAAATAAGTTTTTAC-3')" specifically amplify the G allele, with a product size of 110 bps. The "F primer (5'-TCCTGAGTAAACTAGGTCACAA-3')" and "R primer (5'-ACGTTAAGTTCAGAAAGATTAATTC-3')" pairs specifically amplify the A allele, with a product size of 235 bps. An internal control of 297 bps was amplified using the G-F and A-R primers.[20]

The reaction was performed by GoTaq Green Master mix, in a total volume of 25 µl. Briefly, "12.5 µl of 1x Master Mix, 0.5 µl of each pair of primer (10 µmol), 2 µl of genomic DNA, and 8.5 µl" of water were amplified using an automated thermal cycler (BIO-GENER, China) under the subsequent conditions described below are: Initial denaturation was done at "95°C (2 min), 29 cycles of 95°C (25 s), 58°C (30 s), and 72°C (60 s), was followed by an extension at 72°C for 5 min." The resultant amplicons were electrophoresed with the ladder of 100 bp (Fermentas, USA) on 2% agarose gel at 80 V for 40 min. Gel images were obtained using the Gel Doc (Bio-Rad, USA) Quantity One software (version 4.5.1).

Statistical analyses

Statistical analyses were carried out using SPSS (version 19). $P < 0.05$ was considered as statistical significance. Hardy-Weinberg equilibrium (HWE) was analyzed using Chi-square test. Pearson Chi-square and odd ratios with 95% confidence interval (CI) were computed to determine the corroborating relation among cancer and VDR-Cdx2 polymorphisms.

Results

The genotypic and allelic distribution of Cdx2 polymorphism in controls and respective cancer types is shown in the results below.

Table 1 depicts that all the genotypes including AA, AG, and GG of control and case samples are significantly in compliance with HWE.

Table 2 shows the genotypic and allelic distribution. The genotype frequencies of Cdx2 polymorphism in cancer patients were AA 1.2%, AG 32%, and GG 66.8% while in control group were observed as; AA 7.5%, AG 50.7%, and GG was 41.8%, respectively.

The above results depict a significant association between Cdx2 polymorphism and cancer, while an odds ratio through binary logistic regression analysis of 3.0 2.17 (1.12–4.21; 95% CI) was observed for the AG while 0.35 (018–0.69; 95% CI) for GG genotype, respectively. However, AA genotype

did not show any significant association. These observed results also indicate the significant odds ratio for A allele, 2.44 (1.13–5.26; 95% CI) and G allele 0.17 (0.08–0.36; 95% CI), respectively.

Table 3 has shown the distribution among the research group of cancer patients which including GC, RC, CC, and MM.

Figure 1 shows the genotyping product of VDR-Cdx2 by T-ARMS PCR on 2% agarose gel. Lane 1 is showing 100 bp ladder. A 297 bps band is of internal control while A allele is having a band of 235 bps and G allele is having a band of 110 bps. Lanes 2 and 4 are showing AG genotypes, lanes 3–5 and 7–8 are showing GG genotypes.

Figure 2 shows the genotyping product of VDR-Cdx2 by T-ARMS PCR on 2% agarose gel. In upper lane 8, a 100 bp ladder is shown. The 297 bps band is of internal control while A allele is having a band of 235 bps and G allele is having a band of 110 bps.

Table 1: Genotype distribution according to HWE

Genotype	AA	AG	GG	*P*-value
Controls *n*-value (expected value)	05 (7.22)	34 (29.55)	28 (30.22)	0.22
Cases *n*-value (expected value)	01 (2.5)	27 (23.9)	56 (57.5)	0.25

HWE: Hardy-Weinberg equilibrium (HWE $P>0.05$)

Table 2: Genotypic and allelic distribution among cancer patients and controls

Study groups	Controls (*n*=67)	Cases (*n*=84)	$\chi 2$	*P*-value	OR (95% CI)	RR (95% CI)
AA	05	01	NA	0.06	0.14 (0.01–1.31)	0.15 (0.01–1.33)
AG	34	27	5.36	0.02	2.17 (1.12–4.21)	1.57 (1.06–2.33)
GG	28	56	9.34	0.001	0.359 (0.18–0.69)	0.62 (0.45–0.86)
A	22	14.5	5.37	0.02	2.44 (1.13–5.26)	1.97 (1.09–3.54)
G	31	69.5	23.12	<0.0001	0.17 (0.08–0.36)	0.55 (0.42–0.73)

P-value (Chi-square test) $P<0.05$, OR: Odds ratio; RR: Risk ratio; CI: Confidence interval

Table 3: Distribution of cancer type and their number of cases with respective genotypes

Cancer type	Cases (*n*)	Genotypes		
		AA	AG	GG
GC	20	–	04	16
RC	18	01	17	–
CC	30	–	04	26
MM	16	–	02	14
Total	84	01	27	56

GC: Gastric cancer; RC: Rectal cancer; CC: Colon cancer; MM: Multiple myeloma

In upper lane, lanes 2, 4, 11, 12, and 14 are showing the GG genotype, while lanes 3, 5-7, 9, 10, 13, and 15 are showing AG genotype.

In lower lane, lanes 2, 4, 11, and 13 are showing AA genotypes, lanes 1, 5-8, 14-17, and 19 are showing GG genotype, while lanes 3, 4, 9, 10, and 18 are showing AG genotype and 7-8 are showing GG genotype.

Discussion

Worldwide, the Cdx-2 SNP of VDR gene has been studied with the relevance of many cancer types. Various studies have reported the polymorphism in transcriptional factor Cdx2, at promoter region of VDR binding site that alerts the resultant transcriptional activity of the VDR gene.[3-8] The higher VDR gene transcription is related with A allele. Consequently, the higher intestinal VDR expression may be a result of A allele, calcium absorption and thus an increased bone mineral density.[21,22]

A meta-analysis conducted by Huang *et al*., 2014, including case–control studies (12,906 cases and 13,700 controls), revealed that AA genotype of Cdx-2 polymorphism is associated with 16% risk of the incidence of all type of cancers especially, in African-Americans and Caucasian populations while subgroup analysis showed significant association of AA genotype with breast cancer risk.[19] It is also reported by Huang *et al*., 2014, that the genetic diversity, the correlation of Cdx-2 polymorphism on the basis ethnicity and cancer risk is different in Caucasians and African-Americans.[23]

Due to aforementioned facts, this study investigated the association of Cdx-2 polymorphism of VDR gene with cancers of digestive tract. Previously, the relation of Cdx-2 polymorphism other than breast cancer has not been reported in this population.

In this study, various cancer types (n = 84) were investigated [Table 3]. Result of this study showed that AG and GG genotypes established significant association with various type of cancers ($P < 0.05$). Whereas, AA genotype was found less frequent in this study group [Table 2]. Similar findings were reported by Torkko *et al*. in year 2008 in Hispanic White men that GG genotypes increase the risk of prostate cancer.[16]

In another study by Iqbal *et al*., 2015, conducted at South Pakistan, Cdx2 polymorphism has been studied with the relevance of breast cancer but could not found any significant association of AA genotype with it. However, the more breast cancer risk was related with GG allele (OR = 1.832, 95% CI = 0.695–4.828).[20]

Despite small sample size, the outcome of current pilot study unveils a significant correlation between cancer and VDR-Cdx2 gene polymorphism in Pakistani population, however, the reported

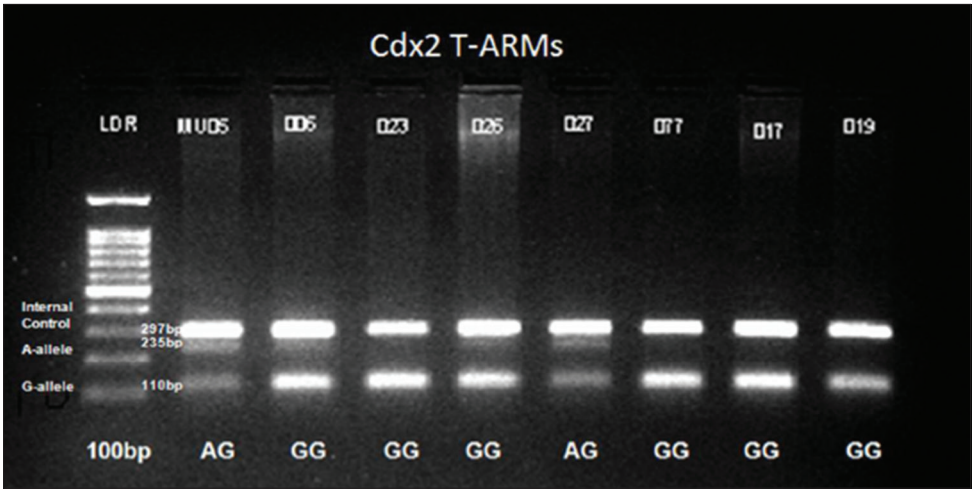

Figure 1: Electrophoresis of Vitamin D receptor-caudal-related homeobox 2 single-nucleotide polymorphism genotyping by tetra-amplification refectory system-polymerase chain reaction on 2% agarose gel

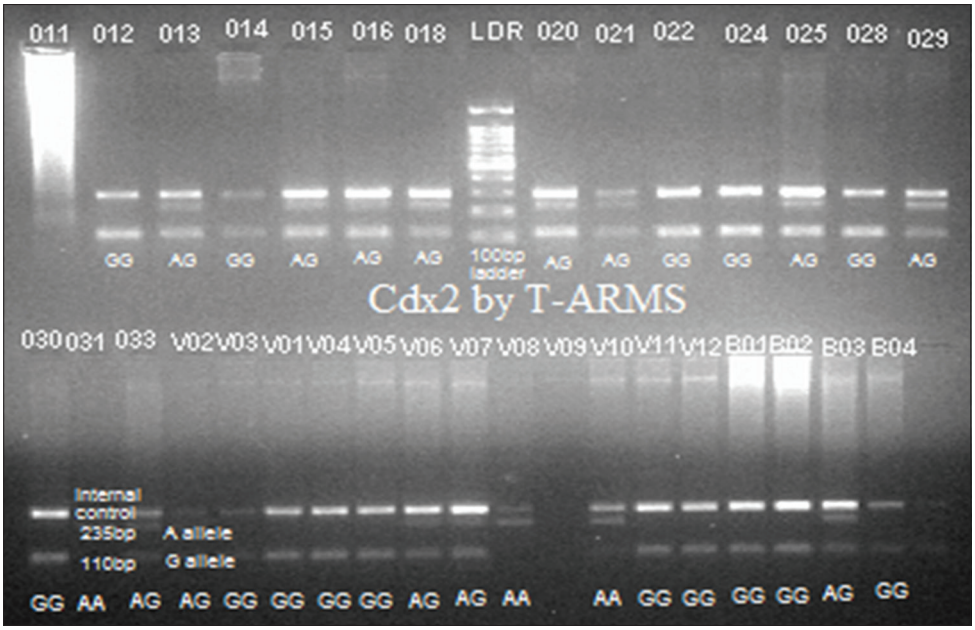

Figure 2: Electrophoresis of Vitamin D receptor-caudal-related homeobox 2 single-nucleotide polymorphism genotyping by tetra-amplification refectory system-polymerase chain reaction on 2% agarose gel

literature about the relationship between incidence of cancer and VDR Cdx2 polymorphisms in various populations are divergent.[20-24] The results of this study will add valuable knowledge and signify the need of the exploration of more data with large sample size.

Conclusion

A significant association has been established by the current pilot or preliminary study in cancer patients on observed SNP of VDR-Cdx2 gene polymorphism. The AG and GG genotypes established significant association with various cancers including oral cancer, GC, CC, and liver cancer in Pakistani population. It has provided us the notion to conduct further large-scale study with large sample size to further affirm this relation.

Authors' Declaration Statements

Ethics approval and consent to participate

This study was approved by the Ethical Review Board of Departmental Research Committee (DRC-00826/KU/2015), Department of Biochemistry, University of Karachi. All samples were collected from the subjects followed by receiving written informed consent.

Availability of data and material

The data used in this study are available and will be provided by the corresponding author on a reasonable request.

Competing interest

None to declare.

Funding statement

Not applicable.

Authors' Contributions

MR refined the research idea, collected, modified and performed the experimental and bench work, SMS helped in refining the research idea, helped in collection and execution of experimental and bench work, analysed the data and helped in finalising the conclusions, TM conceived the research idea, drafted the proposal, finalised the protocols, finalized the conclusions and approved the draft manuscript.

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