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Original Research Article

TNF α 308 G/A Single Nucleotide Polymorphism in Cancer Cervix: A Risk Factor for HPV Associated Cervical Cancer

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Conflict of interest: Nil

Abstract:

Background: Cancer cervix is the second most common cancer in females in India with multi-factorial cause. Tumor necrosis factor alpha, a pro-inflammatory cytokine is implicated in the pathogenesis of cervical cancer

Aim: To analyse the presence of Single Nucleotide Polymorphism TNF α 308 G/A and to correlate its incidence in biopsy confirmed patients of cancer cervix.

Methodology: A cross sectional study which included 60 subjects with age limit of 30 to 60 years (30 study group & 30 controls). Participants were selected from OPD and inpatient ward of gynaecology department.The parameters such as FBG, Urea, Creatinine, Urine Albumin, Urine Glucose & Haemoglobin were estimated**.** Single Nucleotide Polymorphism in TNFα 308 G/A among the study groups were done using PCR.

Result: Subjects with carrier A allele had 16.7 times higher odds of having malignancy in comparison to subjects with Allele G which is statistically significant. In univariate logistic regression, the odds of having malignancy was 1.042 with every year increase in age but this association of age was not statistically significant.

Conclusion: Cancer cervix incidence is found to increase with increase in age of patients. TNF α gene polymorphism at rs1800629 is strongly associated with cancer cervix patients. The risk allele "A" and genotype " GA/AA" is more commonly associated with cancer cervix cases than healthy controls.

Keywords: Cancer cervix, Tumor necrosis factor alpha (TNF α), Polymorphism, Genotype, Urea, Creatinine, Fasting blood sugar, Hemoglobin, Urine albumin.

Introduction

Cancer cervix is the second most common cancer in females in India [1]. The peak age of incidence in India is 55 -59 years. Considerable proportion of women will usually report in the late stage of disease [2]. It is a multi-factorial causation disease. The strains such as 16 and 18 of Human Papilloma Virus (HPV) are most likely associated with cervical cancer [3]. The virus gets cleared in 70-80% of cases but in some it persists and leads to carcinogenesis.

The direct control of HPV infection by TNF-α occurs by induction of apoptosis in HPV infected cells and cervical cancer cells, stimulation of the inflammatory response through up-regulation of vascular adhesion molecules and chemokines, arresting growth of HPV infected keratinocytes, and down-regulation of HPV gene transcription. Indirect control is done by TNF-alpha mediated up-regulation of HLA class I components in non-professional antigen presenting cells [4]. Other risk factors are high parity, long-term hormonal contraceptive use, co-infection with HIV, poor personal hygiene, tobacco and smoking [5]. Tumor necrosis factor alpha is a potent immunomodulator and pro-inflammatory cytokine that is implicated in the pathogenesis of cervical cancer [6]. It plays a major role in the cell mediated immunity (CMI) of the host, thereby genetically determines the host factors in cervical cancer [7].

A promotor polymorphism (rs1800629) of TNFα results in G to A transition at nucleotide position -308 of the transcriptional start site of the gene and is positively related to regulation of TNF-α synthesis at the transcriptional level. Genotype studies of the TNF-α rs1800629 polymorphism showed that the G allele conferred two-fold lower effects on the transcription level when compared with the A allele [8]. This study is proposed to

show the association between Single Nucleotide Polymorphism in TNFα 308 G/A with cervical cancer.

Objectives: To analyse the presence of Single Nucleotide Polymorphism TNF α 308 G/A and to correlate its incidence in biopsy confirmed patients of cancer cervix.

Methodology: After getting approval from the Institutional Ethical Committee, this study was conducted at K.A.P.V. Govt. Medical College and MGMGH, Tiruchirappalli during the period of June 2016 to June 2017.

Study group: This age and sex matched cross sectional study included 60 subjects with age limit of 30 to 60 years. Out of 60, 30 belong to study group, 30 were control group. Study group participants were selected from OPD and inpatient ward of gynaecology department. The healthy individuals as control group were selected from Master Health Check-up OPD. Informed and written consent were obtained from both study and control group. People who were newly diagnosed with cervical cancer and confirmed by biopsy between the age group of 30 to 60 years were included. Self-reported cancer history, previous radiotherapy and chemotherapy for unknown diseases, family history of cancer, patients on treatment with oral contraceptive pills, smokers, people with drug abuse, alcoholics and immune suppressed individuals were excluded.

Sample collection & Study parameters:

Under aseptic precautions, totally 4 ml of blood was collected from study group and controls by vene puncture. Among them 2 ml of blood was collected in a tube containing pro-coagulant and 0.2 ml of blood samples were collected in plain tube and were centrifuged at 1000 X g for 15 minutes. Serum was separated from the cells and used for measuring serum urea and creatinine. Fasting blood samples and spot urine sample was collected to determine the presence of albumin and glucose.

DNA is extracted from the 2ml whole blood sample obtained from both study group and controls by using vacuum manifold (spin protocol). After DNA extraction, it is amplified by using Polymerase Chain Reaction and then to the amplified product the Restriction enzyme is added and run in submarine electrophoresis to visualise the band.

The parameters such as FBG (Trinders, Endpoint, Fixed time), Urea (Urease GLDH Method), Creatinine (Jaffe's Kinetic Method), Urine Albumin (Dipstick method), Urine Glucose (Dipstick method) & Haemoglobin (Cyanmethemoglobin method) were estimated by using fully and semi-Automated Analyzers.

Results and statistical analysis:

In our study 30 cancer cervix cases and 30 apparently healthy controls were analysed for genotype distribution of TNF α 308 G/A gene. Association of each genotype with its clinical and biochemical parameters was studied. Statistical analysis was done using SPSS software. The biochemical parameters between cancer cervix cases and healthy controls were analysed by using students t test. The frequency of Genotype distribution between cases and controls were compared by using Chi-square ($\chi 2$) test. In logistic regression analysis, Odds ratio with two tailed p values and 95% confidence intervals (CI) were calculated. Level of significance for p-value was set at point < 0.05.

Table 1: Distribution of the cases according to cancer cervix staging (n=30)

Staging	Cases N (%)	Percent
stage IIA	13	43.3
stage IIB	10	33.3
stage IIIA	7	23.3
Total	30	100.0

The majority of the cases were in stage 2 (76.6%) while the remaining was in stage IIIA.

Table 2: Comparison of study parameters among cases and controls (n=60)

Study parameters	Group	Mean	Std. Deviation	Mean difference	p value	95% confidence interval
Mean Hb (gms %)	Cases	9.620	0.9297	0.723	*0.004*	-1.19 to -0.247
	Controls	10.343	0.9115			
Mean urea (mg %)	Cases	43.17	6.828	0.03	0.985	-3.5 to 3.44
	Controls	43.20	6.609			
Mean creatinine (mg %)	Cases	1.090	0.3717	0.17	0.058	-0.09 to 0.330
	Controls	0.920	0.2325			
Mean glucose (mg %)	Cases	108.57	26.038	7.80	0.151	-2.91 to 18.51
	Controls	100.77	13.492			

Table 3: Genotype distribution of the study population (n=60)

Genotype	Cases N (%)	Controls N (%)	Total N (%)
Homozygous GG	18 (60)	29 (96.7)	47 (78.3)
Heterozygous GA	9 (30)	0 (0)	9 (15)
Homozygous AA	3 (10)	1 (3.3)	4 (6.7)
Total	30 (100)	30 (100)	60 (100)

Chi-square p value: *0.002.* The difference in distribution of genotype between the cases and controls was statistically significant with cases having relatively high proportion of Homozygous AA and Heterozygous GA genotype than the controls.

Table 4: Distribution of the study population according to the Single Nucleotide Polymorphism of TNF α 308 by PCR- RFLP (n=60)

Allele	Cases N (%)	Controls N (%)	Total N (%)
G	19 (63.3)	29 (96.7)	48 (80)
A	11 (36.7)	1 (3.3)	12 (20)
Total	30 (100)	30 (100)	60 (100)

Chi-square value: 10.417 p value: *0.001.* The difference in distribution of Allele G and carrier A between the cases and controls was statistically significant with cases having relatively high proportion of carrier A allele than the controls (36.7% vs 3.3%).

Table 5: Logistic regression of carrier A allele for predicting cancer cervix as dependent variable (n=60)

Independent variable (N)		Odds Ratio for malignancy (OR)	95% Confidence Interval for OR	p value
Allele	A	16.789	2.001 to 140.89	**0.009**
	G	1	-	
Age	In years	1.042	0.956 to 1.135	0.349

During univariate logistic regression, subjects with carrier A allele had 16.7 times higher odds of having malignancy in comparison to subjects with Allele G and this association of allele A was found to be statistically significant. During univariate logistic regression, considering age as continuous variable the odds of having malignancy was 1.042 with every year increase in age but this association of age was not statistically significant. Hence it can be stated that identifying carrier A allele can be used to predict the malignant nature of cervical lesions given the current study findings.

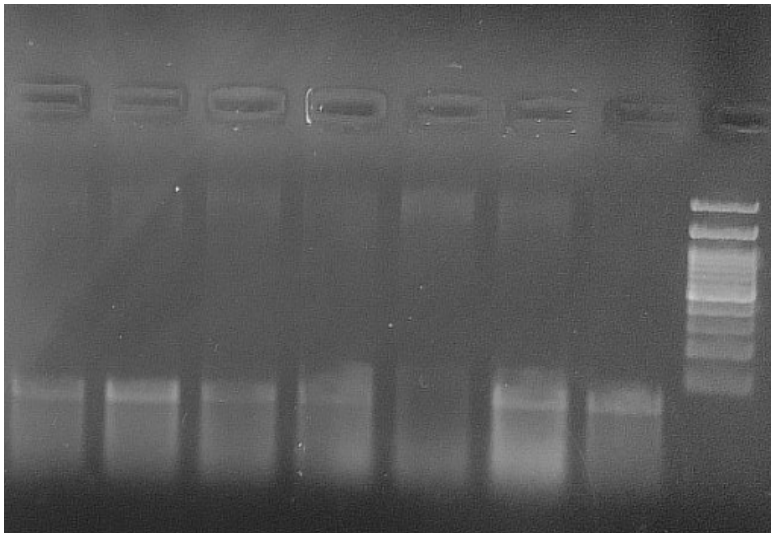

Figure 1: shows GG in lanes 1,2,3,7 & GA in lanes 4,5,6

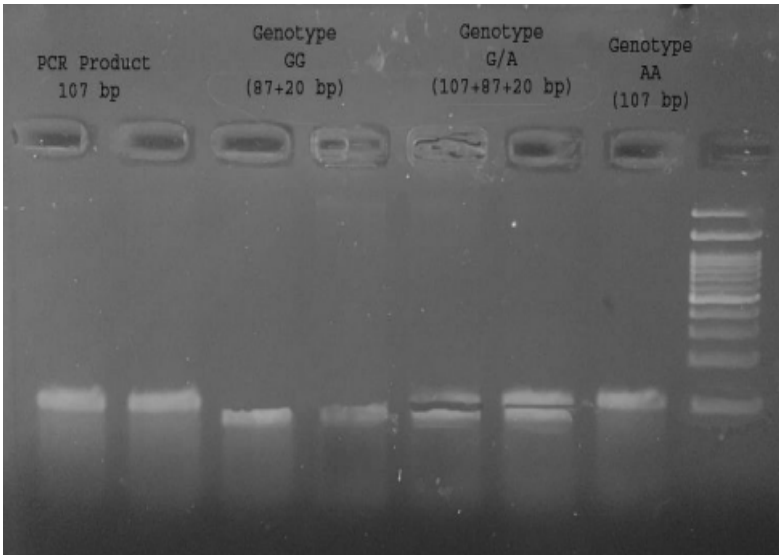

Figure 2: shows the PCR product genotypes GG, G/A & AA

Discussion

Cervical cancer is the third most common cancer and the fourth leading cause of cancer death among females, accounting for nearly 10% of the total newly-diagnosed cancer cases and 8% of the total cancer deaths. Global incidence of cervical cancer has increased from about 378,000 cases per year in 1980 to about 454,000 cases per year in 2010. In India, incidence is 13–24 lakhs per year [9] and 75% are in the advanced stage.

Among several risk factors for cancer cervix, Human Papilloma Virus (HPV) infection is a main cause of cervical lesions. Among them HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are considered carcinogenic [10]. Among these carcinogenic viruses, highest risk [11] viruses are found to be 16, 18, 31, 45 types [12]. In 70–90% of HPV-infected individuals the virus is naturally cleared while in small percentage of patients persistent infection with HR-HPV such as HPV type 16 and 18 lead to the development of cervical intraepithelial neoplastic lesion (CIN), a precursor of cervical cancer.

Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine has been involved in the clearance of HPV infection by inducing the inflammatory response in the host, arresting the growth of HPV infected keratinocytes and down regulating the HPV gene transcription13. Persistence of the HPV infection in cervical lesion is due to the modulation in immune response of host due to change in TNF α secretion.

TNF-α rs1800629 is the most studied polymorphism, which is a G to A transition in the promoter at position 308 and is associated with the abnormal levels of TNFα expression. Therefore, TNF-α rs1800629 polymorphism at 308 position is related to cervical cancer [13]. In present study, it is found that the majority of the cases were found to be in stage 2 (76.6%) and the remaining were in stage 3.

The cancer cervix patients were found to have the mean haemoglobin levels as 9.620 and when compared to controls (10.343) which was found statistically significant [14]. These findings were in aoncordance with studies done by Maroie barkati et al., Serkies et al., Obermeir et al., and G. Dreyer et al., [15]

In the present study, the mean urea levels for cases and controls were 43.17 and 43.20 with the p value of 0.985 and 95 % CI range from - 3.5 to 3.44, which is not statistically significant. The mean creatinine levels for patients and controls are 1.090 and 0.920 respectively , whose p value is 0.058 and CI range from - 0.09 to 0.330, which is not statistically significant and replicated by the study done by Abdus salam et al., in 2017 [16]. Also 76.2% patients were in stage 2 with no involvement of adjacent organs, hence the renal function is not impaired as in stage 3a and 3b [17].

The mean blood glucose value for patients and controls were 108.57 and 100.77, with a p value of 0.151and 95% CI is - 2.91 to 18.51, which is not statistically significant and supported by the study done by Jing li et al., in the year 2018 [18].

But there was a significant difference (Chi square p value 0.002) in TNFα–308 carrier A (GA/AA) genotype distribution between cases and controls with 40% (11/30) in cases and 11% (1/30) in controls. Among the cases, Homozygous GG percent was 60%, heterozygous GA percent was 30%, and homozygous AA percent was 10%. In controls, Homozygous GG percent was 96.7 %, heterozygous GA percent was 0% and homozygous AA percent was 4% with a p value 0.002, which is significant and was correlated with the study of Indhu kohaar et al, 2007 [19]. In concordance with

the study of Zang hl et al., 2013 [20], our study also shows that there is association of distribution of allele G and allele A among cases and controls, relatively higher proportion of A allele in cases ,whose Chi square value is 10.417 and p value is 0.001 which is statistically significant.

By logistic regression analysis of carrier allele A proportion is found to be more in cases and the odds ratio for malignancy is 16.789, 95% CI ranges between 2.001 to 140.89 and p value is 0.009, which is statistically significant.

Conclusion

From the above discussion with regard to the results of the study, the conclusions arrived at are: Cancer cervix incidence is found to increase with increase in age of patients TNF α gene polymorphism at rs1800629 is strongly associated with cancer cervix patients. The risk allele "A" and genotype "GA/AA" is more commonly associated with Cancer cervix cases than healthy controls.

Limitations: TNF α level if estimated it would give the more precised results for this Single Nucleotide Polymorphism of TNF α. HPV DNA can also be extracted for this study so that the risk factor is completely evaluated.

Scope for further study: Along with VIA -VILI, colposcopy guided biopsy this can also be used for screening purposes Its assessment could be beneficial in early detection of this pathological state and prevention of its unfavourable consequences.

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