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REVIEW ARTICLE

An Updated Meta-analysis of the Impact of Polymorphisms of Repair Genes XRCC1 and XRCC3 in Head and Neck Cancer

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Abstract

Head and neck cancers (HNCs) comprise malignancies occurring in upper aerodigestive tract and involve the role of both environmental and genetic factors. Familial aggregation of Squamous cell carcinoma in the Head and Neck region suggested the role of genetic predisposing factors. The DNA repair machinery plays an important role in the occurrence of HNCs. The X-ray repair cross complementing (XRCC) groups XRCC1 Arg399Gln and XRCC3 Thr241Met genes that play an important part in repairing pathways of defective DNA; are crucial for the maintenance of genomic stability and therefore could affect the HNC risk. The search was done on the polymorphisms of XRCC1 Arg399Gln and XRCC3 Thr241Met, for their influence on radiotherapy response and prognosis of patients with head and neck cancer. In this paper, with the help of inclusion and exclusion criteria, an overall of 19 efficient studies are included in pooled analyses. They consist of 7634 cases and 8955 controls on which meta-analysis has to be conducted. The proof of correlation was suspected between XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms and the chances of developing HNC in several genetic models. The detailed analyses of various subgroups that were framed in accordance to ethnicity, site of formation of tumour, year of publication, genotyping method; so far have not detected any significant association, except that oral cancer was found to be associated with Thr241Met mutation in heterozygous model. Furthermore, no significant effect of these polymorphisms along with smoking on HNC risk was detected. The current meta-analysis suggested that the XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms may be involved in generation of HNC. Also, interaction studies involving gene-gene and gene-environment factors in different populations must also be considered for conclusive interference.

Keywords: Arg399Gln, Head and neck cancer, Meta-analysis, Thr241Met, XRCC1, XRCC3

Introduction

Head and Neck cancers (HNCs) influence the upper aero-digestive tract and are one of the common malignancies worldwide. They are far reaching problem in India constituting approximately one-third of all cancer cases, in contrast to 45% in the developed world (Vokes *et al.*, 1993; Parkin *et al.*, 1999; Choudhury *et al.*, 2014). The universal factors responsible for HNCs worldwide are smoked tobacco, consumption of alcohol, etiological constituents liable for it and also smokeless tobacco, betel nut, and EpsteinBarr virus (Hiyama *et al.*, 1992; Geisler and Olshan, 2001). Due to significant alterations in environmental carcinogens, an erroneous response towards repairing of defective DNA is induced, followed by apoptosis or genetic instability and unregulated (proliferative) cell growth. The recent studies show that head and neck carcinogenesis is associated with abnormalities in DNA repair, apoptosis, carcinogen metabolism, and cell cycle control (Hoeijmakers, 2001; Pfeifer *et al.*, 2002 ; Zhong *et al.*, 2011). Treatment of Head and Neck tumours can induce additional laceration and negatively affect the quality of life of the patients. The past two decades have seen an increased risk of HNCs due to a combination of HPV infection and genetic predisposition (Marcu and Yeoh, 2009).

DNA repair pathways provide important protection against mutagenic exposures and maintain genomic integrity. Nucleotide excision repair (NER), Base excision repair (BER), and Double strand break repair (DSB), are the three essential DNA repair pathways which are significantly associated with the HNC risk. The level of mutations in several DNA repair genes OGG1, XRCC1, XRCC2 and XRCC3 have been studied in patients suffering from HNC. The present study mainly focuses on XRCC1 and XRCC3 genes which are present in BER pathway and plays extensive role in repairing of DNA injury induced by ionising radiation. The first gene which impacts the responsiveness of cells toward ionizing radiation in mammals is X-ray repair cross-complementing gene 1 (XRCC1). It encodes the proteins which interact with poly-ADP-ribose polymerase (PARP), DNA polymerase-beta (POLβ), DNA ligase III (LIG3). In

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XRCC1 gene, three prevalent SNPs (Single Nucleotide Polymorphisms) are Arg194Trp, Arg280His and Arg399Gln which undergo amino acids substitution (Fig. 1). SNPs are defined as the DNA base variants which are seen in human population. They lead to reduced DNA repair capacity which results in high mutation rate and increased cancer risk (Werbrouck *et al*., 2008). XRCC1 Arg399Gln consists of modified alleles which have transformed enzyme functions, superior ascent towards DNA adduct and a vigorous association with cancer (Yu *et al*., 2004; Choudhury *et al*., 2014).

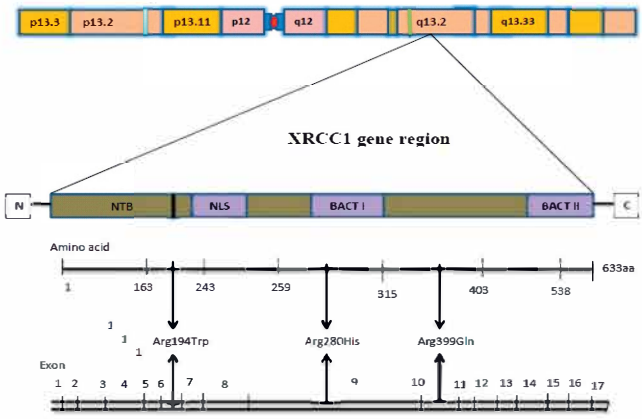

Fig. 1. Schematic diagram representing XRCC1 gene regions and showing the most commonly studied single nucleotide polymorphisms: Arg194Trp, Arg280His and Arg399Gln. (Silvia and Renata, 2010)

Another family of genes liable for reformation of DNA double strand breaks induced through normal metabolic procedures and/or exposure to ionizing radiation is X-ray repair cross-complementing group 3 (XRCC3). The gene is present in the 14q32.3 chromosome region. In homologous recombination repair system, the function of XRCC3 is not completely understandable, but it shows coherence with Rad51, which mobilizes DNA strand exchange in homologous recombination and XRCC3-deficient cell lines present altered homologous recombination repair. The polymorphism of exon 7 in XRCC3 gene favours substitution at codon 241 and therefore, change the amino acid Threonine to Methionine at position 241 (Thr241Met). This led to alteration in the enzyme function and interaction with different proteins present in DNA damage and repair. For understanding the role of XRCC1 and XRCC3 polymorphisms and investigating the role of different DNA repair pathways in relation to head and neck cancer, the risk of this cancer in connection to familiar amino acid substitution SNPs in two DNA repair genes XRCC1 (Arg399Gln) and XRCC3 (Thr241Met), in a pooled analysis of 7634 cases and 8955 controls, 19 twenty case-control studies were examined.

Materials and Methods

Strategy to Conduct Search for Research Articles

A systematic literature search of Elsevier bio base, the PubMed, EMBASE, Web of Science, and Medline databases has been conducted. These were operated to analyse studies done so far using the following search words either alone or in combination: head and neck, oral, pharynx, larynx, nasopharynx, cancer, tumour, carcinoma, X-ray repair cross complementing group 1, XRCC1, Arg399Gln, X-ray repair cross complementing group 3, XRCC3, Thr241Met, polymorphism, variant variation, allele and genotype. Research works that were published from the earliest entry points till 2019 were considered. Additional researches were considered by manual searching of references as original and review articles.

Inclusion and Exclusion Criteria

The preference was given to the most significant and reviewed articles and abstracts independently by two authors. All the chosen studies included the following measures: (a) publication in English, (b) study must consisted of data which helps to calculate odds ratio for HNC cancer (c) analysis of case-control studies which included correlation between XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) polymorphism and the risk of HNC, (d) genotype frequencies must be described and its classification in controls was in in Hardy-Weinberg equilibrium (HWE), and (e) when numerous publications were present on the same data or overlying data, the best or most recent publication was selected. The major exclusion criteria were as follows: (a) papers characterized as reviews, abstracts or case reports, (b) unpublished papers and reports, and (c) meta-analysis done on less than 100 case control studies.

Data Extraction

All worthy data was differentiated from best publications in accordance of inclusion criteria by two independent authors. If results were dissimilar, the data is repeatedly checked and discussed to come to accordance. The elicited data from preferred articles consisted of the first authors name, year of publication, ethnicity, and country of origin, genotyping methods, source of control, tumour site, number of cases and controls and traits of sample populations. Tumour sites were classified as oral, laryngeal, pharyngeal and diversified HNCs.

Statistical Analysis

All suitable data is extracted from studies. Firstly, the Hardy Weinberg equilibrium (HWE) is applied to the controls by using the goodness-of fit test (Chi-square test or Fisher exact test). The Crude odds ratios (ORs) and

95% confidence intervals (95% CIs) were practiced for finding the stability in relationship between HNC risk and XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) polymorphisms. In current scenario various statistical models were used, such as the recessive genetic model, the allelic genetic model, and the co dominant genetic model (homozygote comparison). The statistical heterogeneity is examined by applying Cochrans Q statistic and the I2 statistic, where P<0.1 was observed as significant heterogeneity, and I2>50% indicates large heterogeneity. A random-effects model (the Der Simonian and Laird method) was utilized, if heterogeneity is founded, otherwise, a fixed-effects model (the Mantel-Haenszel method) was selected as appropriate. Results stability was determined by performing sensitivity analysis. A funnel plot, Beggs rank correlation method and the Eggers weighted regression method was approved for statistically assess publication bias ($P < 0.05$ was considered statistically significant). All analyses were implemented by using IBM SPSS statistics (version 20.0) by IBM Corp US and R programming.

Results and Discussion

Study Characteristics

On the basis of our search strategy, 42 studies or research articles were found important for further analysis. Out of which 7 research papers were excluded as they dont contain important information related to our study. Alternatively, 10 more studies were removed on the basis of genotypic data. Only the excellent data were chosen for the study so, on the basis of irrelevant information, copied data and no relation between gene polymorphisms and HNC, 5 more studies were excluded. The research papers or studies left with usable and important information were 19 on which the present study was conducted (Fig. 2).

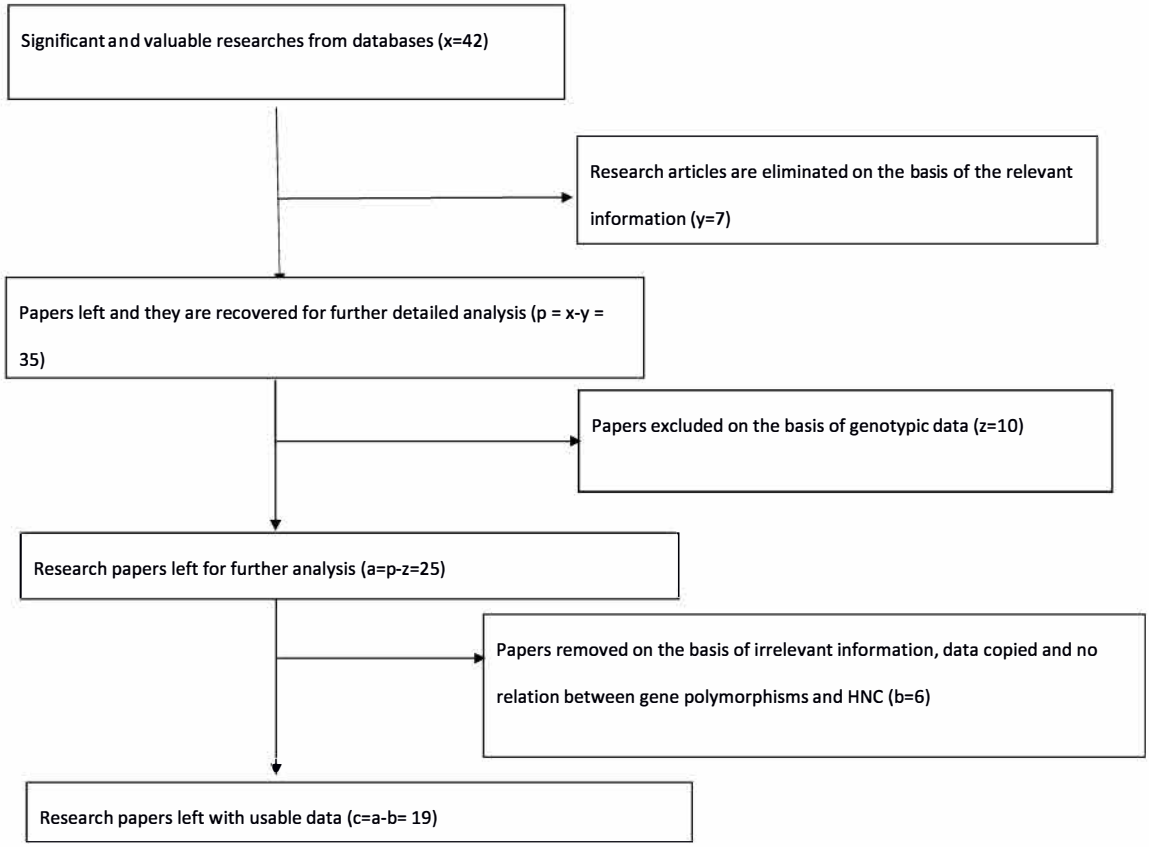

Fig. 2. Flowchart of search pattern used for conducting following study

Meta-analysis was performed on 19 studies consisting of 7634 cases and 8955 controls on the basis of inclusion criteria. The features are arranged in Table 1. Out of 19 studies on which the meta-analysis was performed consist of 9 Caucasians, 8 Asians and 2 mixed populations. 13 studies were centred towards the correlation between XRCC1 Arg399Gln polymorphism and HNC risk and rest 6 targeted towards the relationship between XRCC3 and Thr241Met. In these studies, four types of genotyping methods were used such as PCR-RFLP, iPLEX, ARMS-PCR and TaqMan method. The controls of 13 studies conducted were taken from hospitals and 6 controls were taken from public.

Table 1. Main characteristics of studies included in the Meta-analysis

Author	Year	Country	Ethnicity	Tumor site	Gene polymorphism	Source of control	Sample size (case/ control)	Genotyping methods
Laantri *et al.*	2011	Morocco	African	Nasopharynx	XRCC1	Not	512/477	Taqman
Huang *et al.*	2011	China	Asian	Nasopharynx	XRCC1	Health	4245/4310	PCR-RFLP
Avci *et al.*	2017	Turkey	Turkish	Oral	XRCC1 XRCC3	Hospital	108/102	PCR-RFLP
Kostrzewska-Poczekaj	2013	Poland	Caucasian	HNC	XRCC1 XRCC3	Hospital	293/160	PCR-RFLP
Dos Reis *et al.*	2013	Brazil	Caucasian	OSCC	XRCC3	public based	144/144	PCR-RFLP
Kayani *et al.*	2014	Islamabad	Asian	HNC	XRCC3	Hospital	200/150	PCR-RFLP
Halkova *et al.*	2016	Czech	Caucasian	thyroid	XRCC1	Hospital	209/374	PCR-RFLP
Wang *et al.*	2015b	China	Asian	thyroid	XRCC1	Hospital	276/552	PCR-RFLP
Khlifi *et al.*	2014	Tunisia	Caucasian	HNC	XRCC1	public based	169/261	PCR-RFLP
Khaled et al.	2011	Saudi Arabia	Caucasian	HNC	XRCC1 XRCC3	public based	156/251	PCR-RFLP
Fard-Esfahani *et al.*	2011	Iran	Asian	thyroid	XRCC1	Hospital	482/570	PCR-RFLP
Kumar *et al.*	2012	India	Asian	HNC	XRCC1	public based	278/278	PCR-RFLP
Csejtei *et al.*	2009	Hungary	Caucasian	HNC	XRCC1	Hospital	108/102	PCR-RFLP
Gracia-Quispes *et al.*	2011	Spain	Caucasian	thyroid	XRCC1	Hospital	402/479	iPLEX
Bashir *et al.*	2017	Pakistan	Asian	thyroid	XRCC1	Hospital	456/400	ARMS-PCR
Zhou *et.al.*	2009	mixed	Asian	oral	XRCC1	Hospital	1326/3130	PCR-RFLP
Sliwinski *et al.*	2010	polish	Caucasian	HNC	XRCC3	Hospital	288/353	PCR-RFLP
Mahjabeen *et.al.*	2013	Pakistan	Asian	HNC	XRCC1	Hospital	300/150	PCR-RFLP

Table 2. Distribution of XRCC1 Arg399Gln and XRCC3 Thr241Met among head and neck cancer patients with appropriate controls

Comparison	Number of studies	Sample size (case/control)	Cases			Controls		
XRCC1-Arg399 Gln			**arg/arg**	**arg/gln**	**gln/gln**	**arg/arg**	**arg/gln**	**gln/gln**
Total	13	6060/7679	2012	2461	1587	2255	3158	2266
Caucasian	6	1205/1353	295	561	349	367	565	421
Asian	6	4343/5849	1443	1707	1193	1609	2430	1810
HNC	13	6060/7679	2012	2461	1587	2255	3158	2266
Smoking	13	6060/7679	2012	2461	1587	2255	3158	2266
Publication year	7	3264/3391	1491	1311	462	1592	1305	492
PCR-RFLP	10	4706/6328	1445	1916	1345	1658	2645	2025
Taqman	1	512/477	274	193	45	279	163	35
iPLEX	1	386/474	153	186	47	196	212	66
ARMS-PCR	1	456/400	140	166	150	122	138	140
XRCC3 - Thr241Met			**thr/thr**	**thr/met**	**met/met**	**thr/thr**	**thr/met**	**met/met**
Total	6	1574/1276	618	560	396	433	497	346
Caucasian	3	711/702	121	306	284	149	281	272
Asian	2	752/426	463	229	60	256	135	35
HNC	6	1574/1276	618	560	396	433	497	346
Smoking	6	1574/1276	618	560	396	433	497	346
Publication year	6	1574/1276	618	560	396	433	497	346
PCR-RFLP	5	1096/878	586	381	129	416	352	110
Taqman	0	0	0	0	0	0	0	0
iPLEX	1	478/398	32	179	267	17	145	236
ARMS-PCR	0	0	0	0	0	0	0	0

Table 3. Meta-analysis for XRCC1 polymorphism and probable risk for HNC

Comparison	Number of studies	Sample size (case/control)	Test of association			
XRCC1 Arg399Gln			**OR**	**95% CI**	**p value**	**Model**
Arg399 allele vs. Gln399						
Total	13	8955/11216	1.1954	1.0961 to 1.3038	0.001	F
Caucasian	6	1272/1397	0.99	0.8754 to 1.1236	0.89	R
Asian	6	7063/9240	1.0086	0.9504 to 1.0705	0.7773	R
HNC	14	8955/11216	1.1954	1.0961 to 1.3038	0.001	F
Smoking	14	8955/11216	1.1954	1.0961 to 1.3038	0.001	F
Publication year	14	8955/11216	1.1954	1.0961 to 1.3038	0.001	F
PCR-RFLP	11	7585/9860	0.9794	0.9572 to 1.0021	0.0745	R
Taqman	1	512/477	1.0472	0.9355 to 1.1723	0.423	R
iPLEX	1	402/479	1.0242	0.9018 to 1.1632	0.712	F
ARMS-PCR	1	456/400	1.025	0.9090 to 1.1559	0.6866	R
Arg/Arg vs.Gln/Gln						
Total	14	8955/11216	1.02	1.08 to 1.28	0.001	F
Caucasian	6	1272/1397	0.88	0.7227 to 1.0754	0.21	R
Asian	6	7063/9240	1.36	1.2288 to 1.5067	0.0001	F
HNC	14	8955/11216	1.02	1.0961 to 1.3038	0.001	F
Smoking	14	8955/11216	1.02	1.0961 to 1.3038	0.001	F
Publication year	14	8955/11216	1.02	1.0961 to 1.3038	0.001	F
PCR-RFLP	11	7585/9860	0.9794	0.9572 to 1.0021	0.0745	R
Taqman	1	512/477	0.9667	0.9111 to 1.0257	0.2625	R
iPLEX	1	402/479	1.05	0.8220 to 1.3516	0.6784	F
ARMS-PCR	1	456/400	1.0331	0.8816 to 1.2106	0.6875	F
Arg/Arg vs. Arg/Gln						
+ Gln/Gln						
Total	14	8955/11216	1.138	1.0594 to 1.2224	0.0004	F
Caucasian	6	1272/1397	0.7931	0.6696 to 0.9393	0.0072	F
Asian	6	7063/9240	1.3112	1.2040 to 1.4280	0.0001	F
HNC	14	8955/11216	1.138	1.0594 to 1.2224	0.0004	F
Smoking	14	8955/11216	1.138	1.0594 to 1.2224	0.0004	R
Publication year	14	8955/11216	1.138	1.0594 to 1.2224	0.0004	F
PCR-RFLP	11	7585/9860	1.1704	1.0784 to 1.2702	0.0002	F
Taqman	1	512/477	0.3301	0.2438 to 0.4470	0.0001	R
iPLEX	1	402/479	0.2216	0.1646 to 0.2983	0.0001	F
ARMS-PCR	1	456/400	1.1643	0.8672 to 1.5631	0.3115	R
XRCC3 - Thr241Met						
Total	7	1529/1731	1.1013	0.9341 to 1.2986	0.25	F
Caucasian	3	839/783	1.0781	0.7249 to 1.6034	0.7103	R
Asian	3	579/800	0.9511	0.7682 to 1.1774	0.6451	R
HNC	7	1529/1731	1.1013	0.9341 to 1.2986	0.25	F
Smoking	7	1529/1731	1.1013	0.9341 to 1.2986	0.25	R
Publication year	7	1529/1731	1.1013	0.9341 to 1.2986	0.25	R
PCR-RFLP	6	1127/1252	1.0853	0.8352 to 1.4104	0.5401	F
Taqman	0	0	0	0	0	F
iPLEX	1	402/479	1.0242	0.9018 to 1.1632	0.712	R
ARMS-PCR	0	0	0	0	0	R
Thr/Thr vs Met/Met						
Total	7	1529/1731	1.2049	1.0045 to 1.4453	0.0446	F
Caucasian	3	839/783	1.0641	0.7109 to 1.5927	0.7628	F
Asian	3	579/800	0.7778	0.5809 to 1.0414	0.0914	R
HNC	7	1529/1731	1.2049	1.0045 to 1.4453	0.0446	F
Smoking	7	1529/1731	1.2049	1.0045 to 1.4453	0.0446	R
Publication year	7	1529/1731	1.2049	1.0045 to 1.4453	0.0446	F
PCR-RFLP	6	1127/1252	1.1817	0.9006 to 1.5504	0.2283	R
Taqman	0	0	0	0	0	R
iPLEX	1	402/479	1.66	0.9006 to 3.0736	0.104	F
ARMS-PCR	0	0	0	0	0	R
Thr/Thr vs Thr/Met						
Total	7	1529/1731	1.2139	1.0290 to 1.4322	0.0215	F
Caucasian	3	839/783	0.9552	0.7428 to 1.2283	0.7209	R
Asian	3	579/800	0.7457	0.5583 to 0.9960	0.0469	R
HNC	7	1529/1731	1.2139	1.0290 to 1.4322	0.0215	F
Smoking	7	1529/1731	1.2139	1.0290 to 1.4322	0.0215	F
Publication year	7	1529/1731	1.2139	1.0290 to 1.4322	0.0215	F
PCR-RFLP	6	1127/1252	1.2477	1.0369 to 1.5013	0.0191	R
Taqman	0	0	0	0	0	R
iPLEX	1	402/479	1.5248	0.8140 to 2.8564	0.1877	F
ARMS-PCR	0	0	0	0	0	R

Abbreviations: CI= confidence interval; F= fixed-effects model; OR= odds ratio; R= random-effects model.

Results and Discussion

The Risk of XRCC1 Arg399Gln Substitution for Development of HNC in Total Population

13 studies were conducted consisting of 6060 cases and 7679 controls that help us to find the relationship between HNC risk factor and XRCC1 Arg399Gln (Table 1) The relation between Arg399Gln and HNC was observed in co dominant model and not in dominant and recessive models. In the co-dominant model (Gln/Gln versus Arg/Arg, OR =1.02, 95% CI: 1.08 to 1.28, P = 0.001, Table 3), the homozygous genotype Gln/Gln showed a significant increased risk of HNC. The great difference was present between the frequency of the XRCC1 Arg399Gln polymorphism between Caucasians and Asians (41.7% vs. 19.1%, P= 0.12). In this study, the correlation between alternative genotypes of XRCC1, Arg399Gln polymorphism and HNC under co dominant model were obtained. But, heterogeneity in all the genetic models was found. The Forest plot of Homozygous, Heterozygous and Dominant model are given in Fig. (3, 4 and 5 respectively).

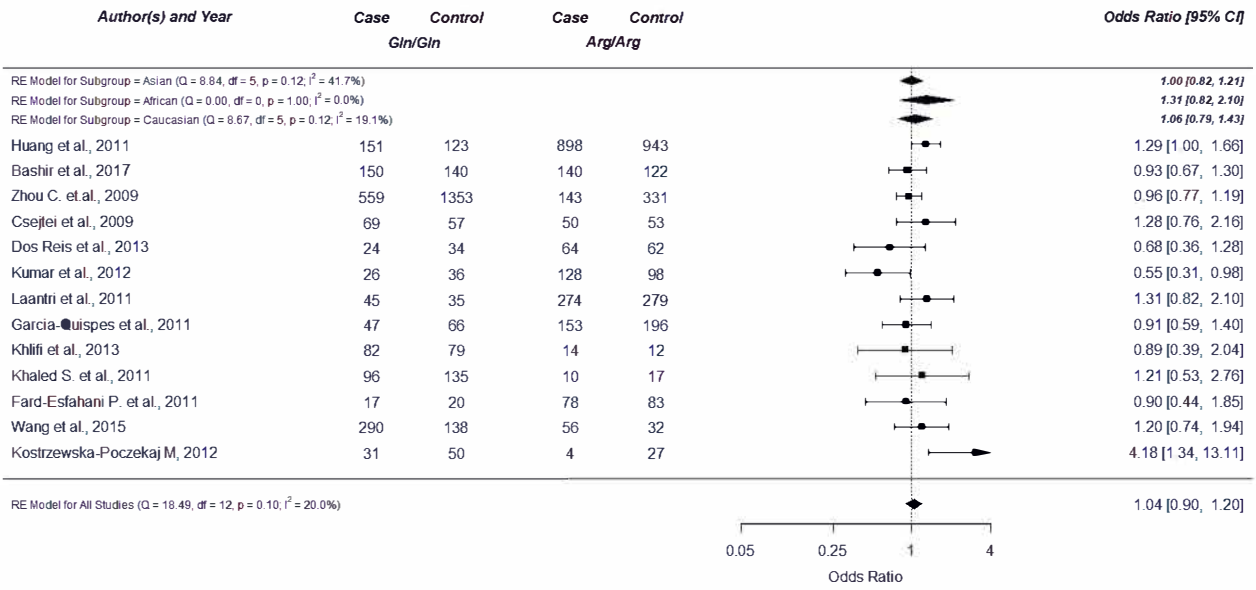

Fig. 3. Forest Plot for Homozygous (Arg/Arg vs Gln/Gln)

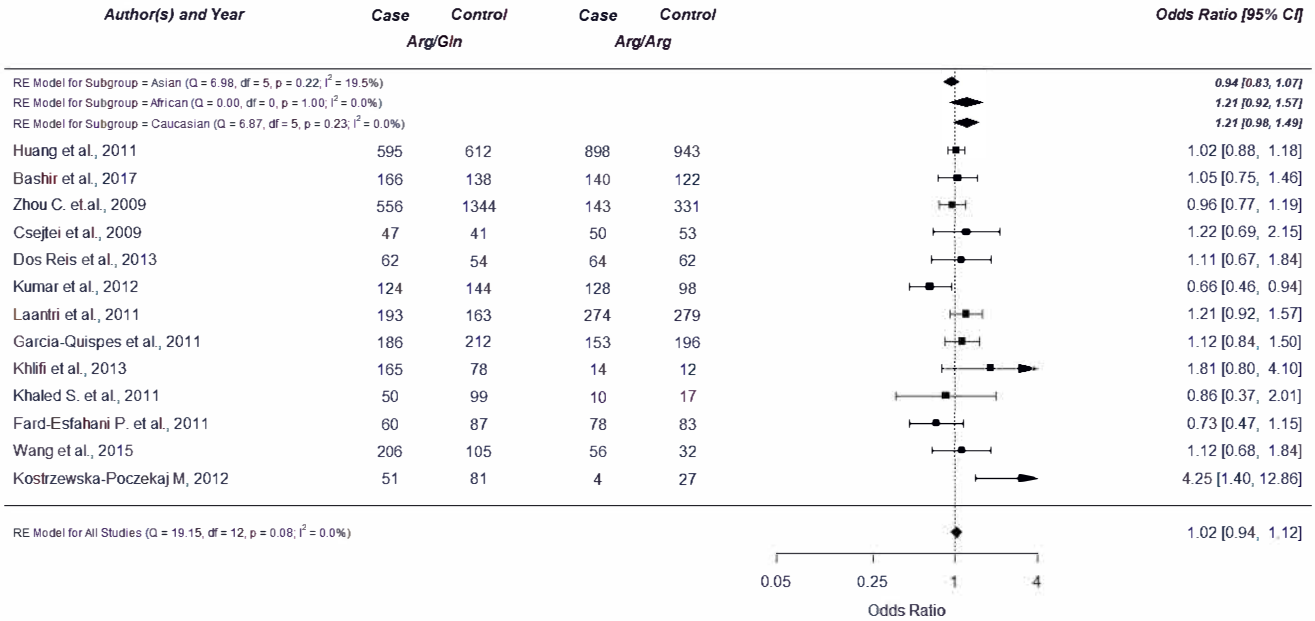

Fig. 4. Forest Plot for Heterozygous (Arg/Arg vs Arg/Gln)

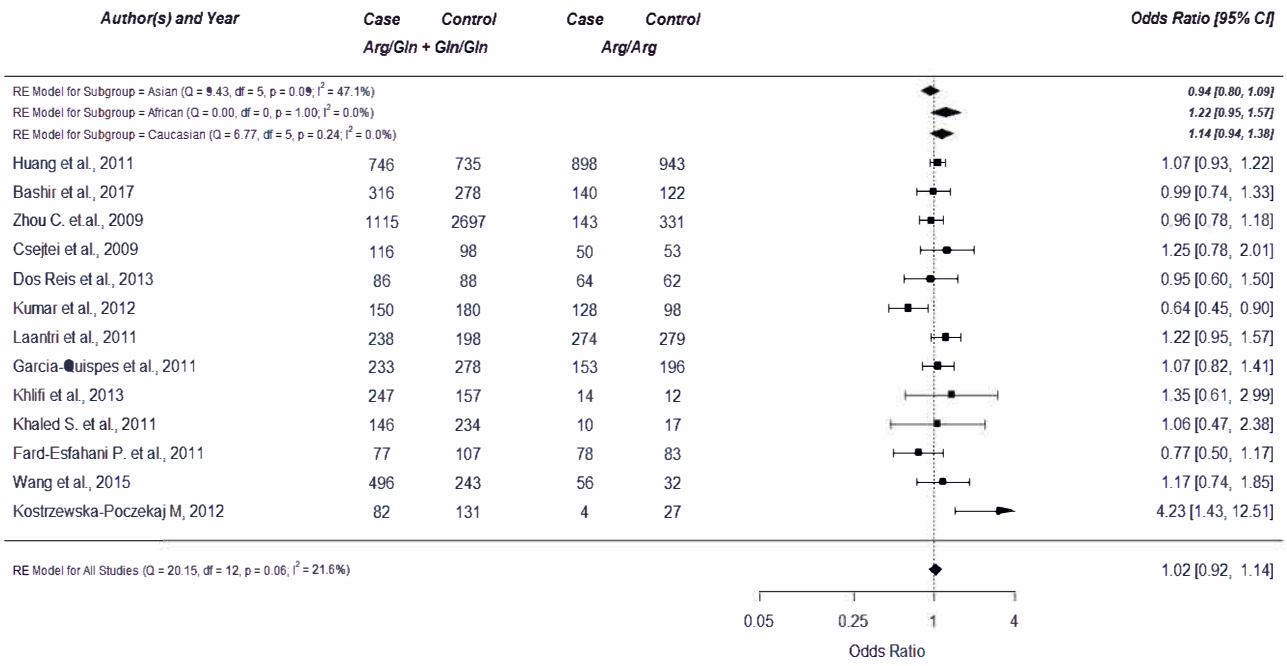

Fig. 5. Forest Plot for Dominant Model (Arg/Arg vs Gln/Gln + Arg/Gln)

XRCC1 Arg399Gln Polymorphism on Risk for Developing HNC in a Specific Population

First, the ethnicity factor was considered in subgroup analysis, according to which there was a significant correlation between Arg399Gln polymorphism and incidences of head and neck cancer among Asians while it was not observed in Caucasian populations. Further heterogeneity was not recognized in Caucasians; but it was observed in Asians while considering the various genetic models. 13 studies consisted of 6060 cases and 7679 controls and an important association was found between the said polymorphism and HNC incidences in the genetic models that were studied. Analysis of this study by using smoking as a factor, in all allelic genetic models and homozygote comparison, in all 13 studies a remarkable correlation was present. 13 studies were taken in the recessive model also and there a significant association between HNC risk and Arg399Gln genotype (Table 2) were found. All genetic models also show heterogeneity and a significant association between Arg399Gln and HNC risk was obtained in any genetic model (Fig. 6, 7 and 8). This supports the existing literature from 2009-2017, where the heterogeneity was shown in all genetic models. Under various techniques such as PCR-RFLP, TaqMan and iPLEX used in sequence analysis for genotyping of XRCC1 Arg399Gln polymorphism heterogeneity was present (Table 1).

The risk of XRCC3 Thr241Met on HNC cases in the entire population considered

Six studies were conducted consisting of 1574 controls and 1276 cases that assist us to investigate the relationship between HNC susceptibility and XRCC3 Thr241Met polymorphism (Table 3). The frequency of the XRCC3Thr241Met polymorphism between Caucasians and Asians (0% vs. 91.3%, P= 0.46) showed great differences. In this study no association between alternative genotypes of XRCC3Thr241Met polymorphism and HNC presence under homozygous comparison, and recessive model were obtained. During this study, heterogeneity in all genetic models was observed. The Forest plot of Homozygous, Heterozygous and Dominant model are given in Fig (9, 10 and 11 respectively).

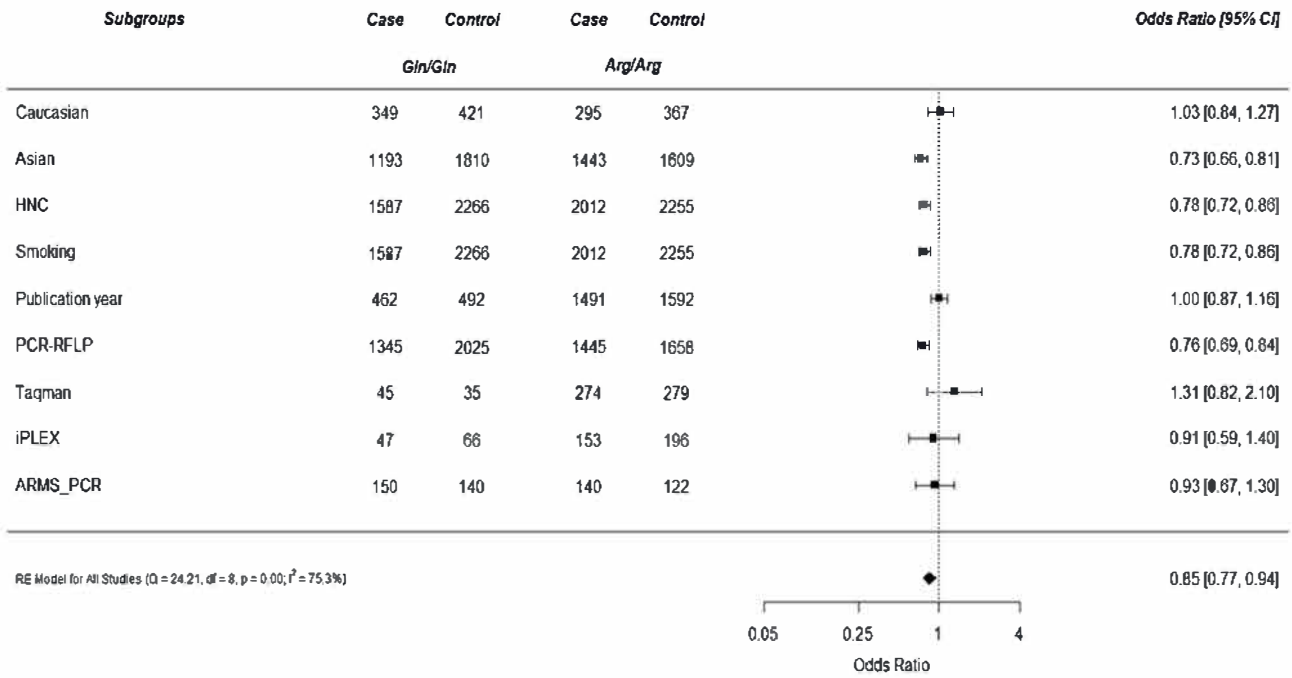

Fig. 6. Forest Plot for Homozygous (Arg/Arg vs Gln/Gln)

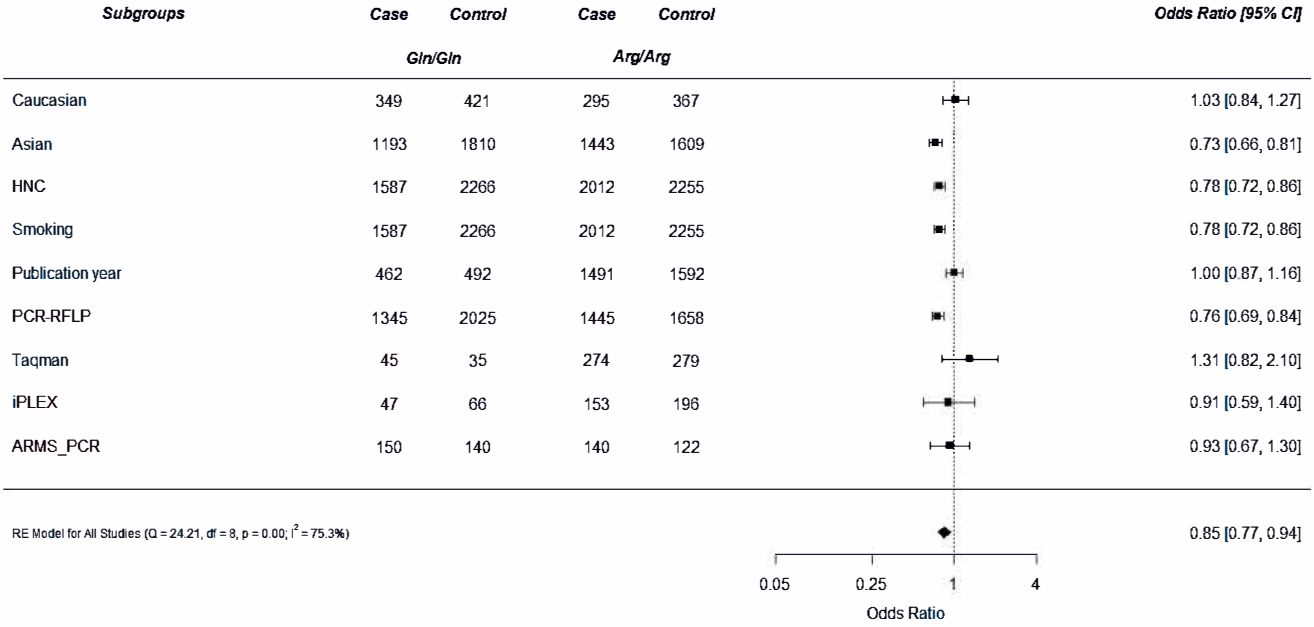

Fig. 7. Forest Plot for Heterozygous (Arg/Arg vs Arg/Gln)

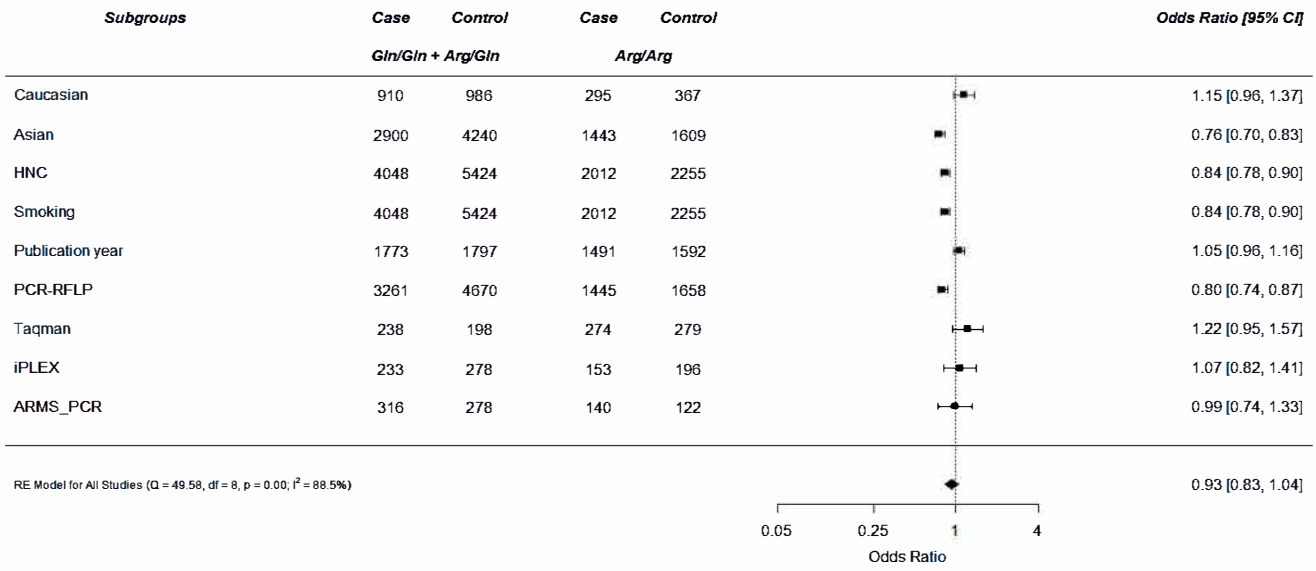

Fig. 8. Forest Plot for Dominant Model (Arg/Arg vs Gln/Gln + Arg/Gln)

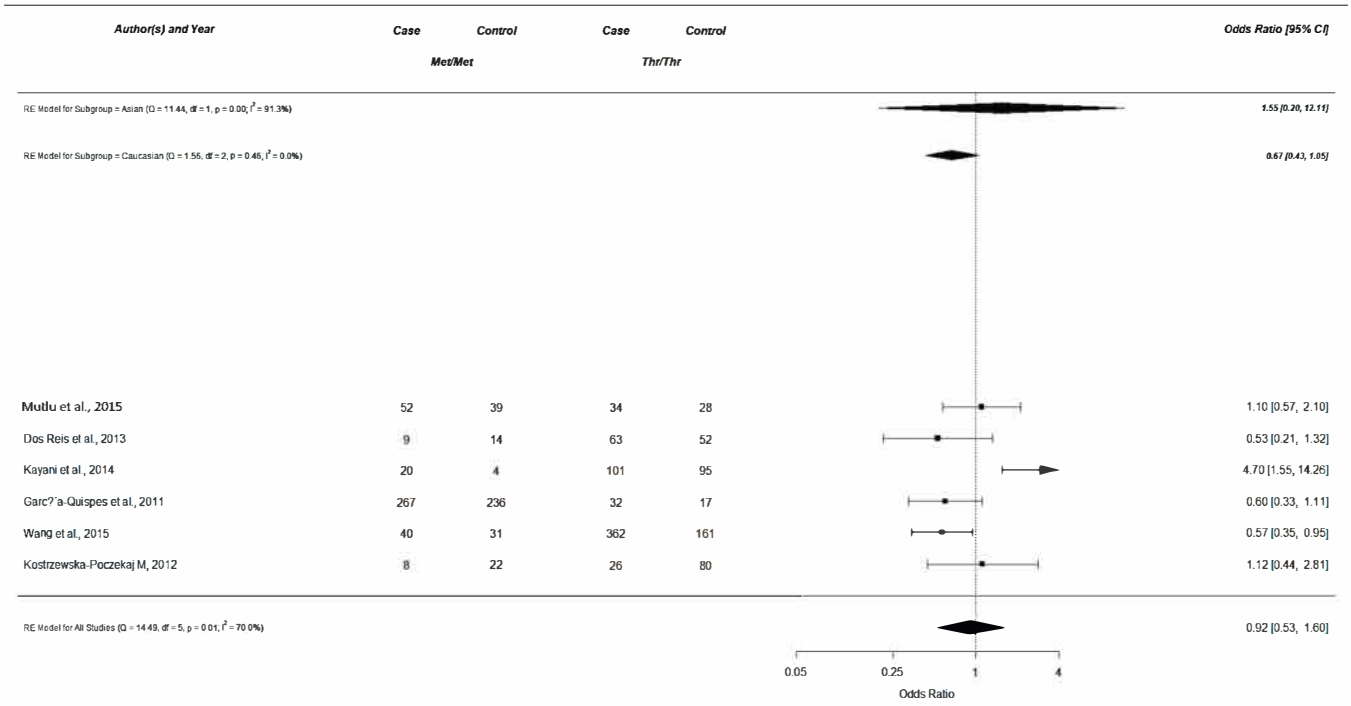

Fig. 9. Forest Plot for Homozygous (Thr/Thr vs Met/Met)

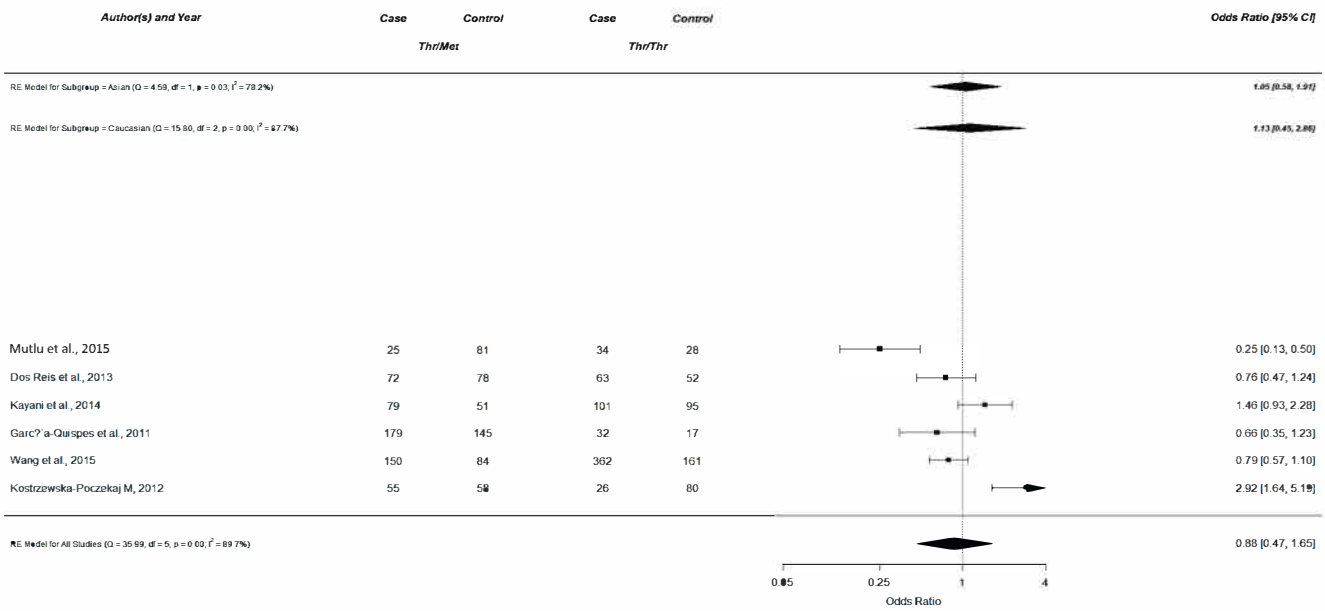

Fig. 10. Forest Plot for Heterozygous (Thr/Thr vs Thr/Met)

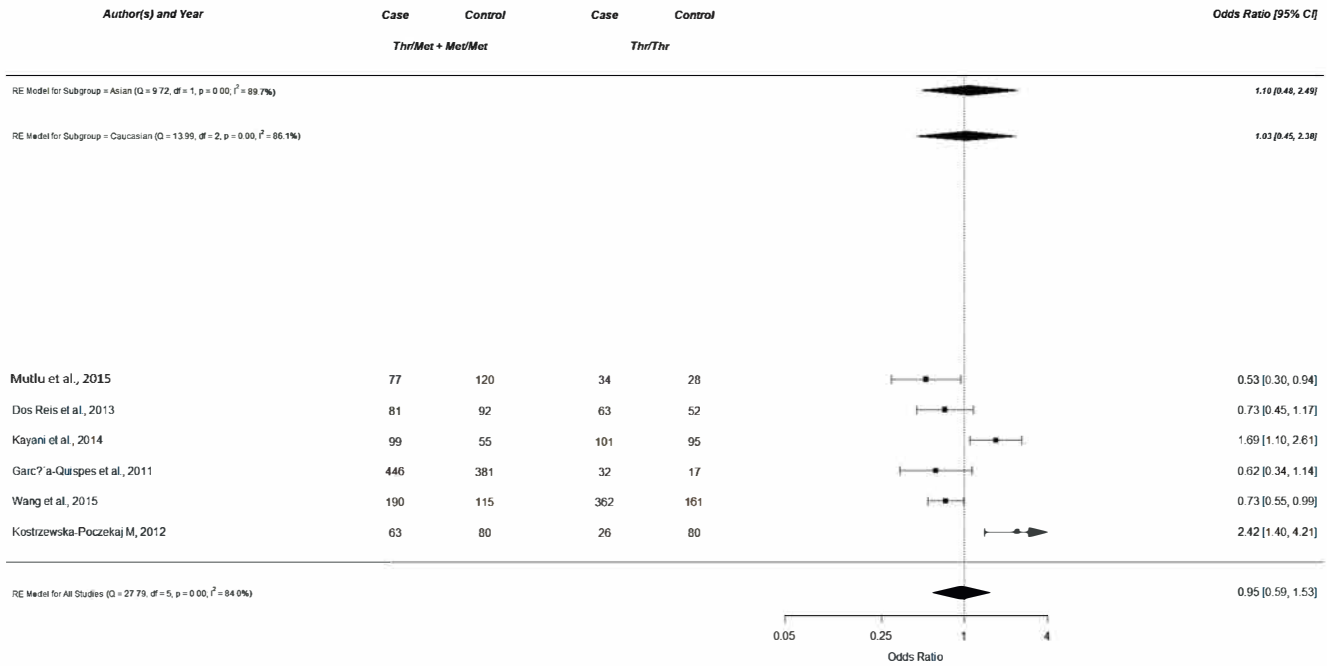

Fig. 11. Forest Plot for Dominant Model (Thr/Thr vs Met/Met + Thr/Met)

XRCC3 Thr241Met Polymorphism on HNC Risk in a Specific Population

Ethnicity in subgroup analysis was taken into consideration, according to which there was a significant association between XRCC3 Thr241Met polymorphism and HNC risk in Asians but in Caucasians it was absent. Further heterogeneity was not recognized in Caucasians; whereas the importance of heterogeneity was detected in Asians under all genetic models. 6 studies consisting of 1574 cases and 1276 controls were studied on HNC population, and there was an important association between XRCC3 Thr241Met polymorphism and HNC susceptibility (Fig. 10). Heterogeneity was present in all genetic models. During analysis of this study by using smoking as a factor, in all allelic genetic models and homozygote comparison, 6 studies were included and significant association was present. All the studies were taken in the recessive model also and no significant association between HNC risk and Thr241Met genotype were found. All genetic models also show heterogeneity. In this study a significant association between Thr241Met and HNC risk under dominant genetic model was observed (Fig. 12, 13 and 14). After review of publications from 2011-2017, the heterogeneity was shown in dominant genetic model. Under various techniques such as PCR-RFLP, TaqMan and iPLEX used in sequence analysis for genotyping of XRCC3 Thr241Met polymorphism heterogeneity was present (Table 3). There was significant association between Thr241Met and HNC risk in analysis under different genetic model.

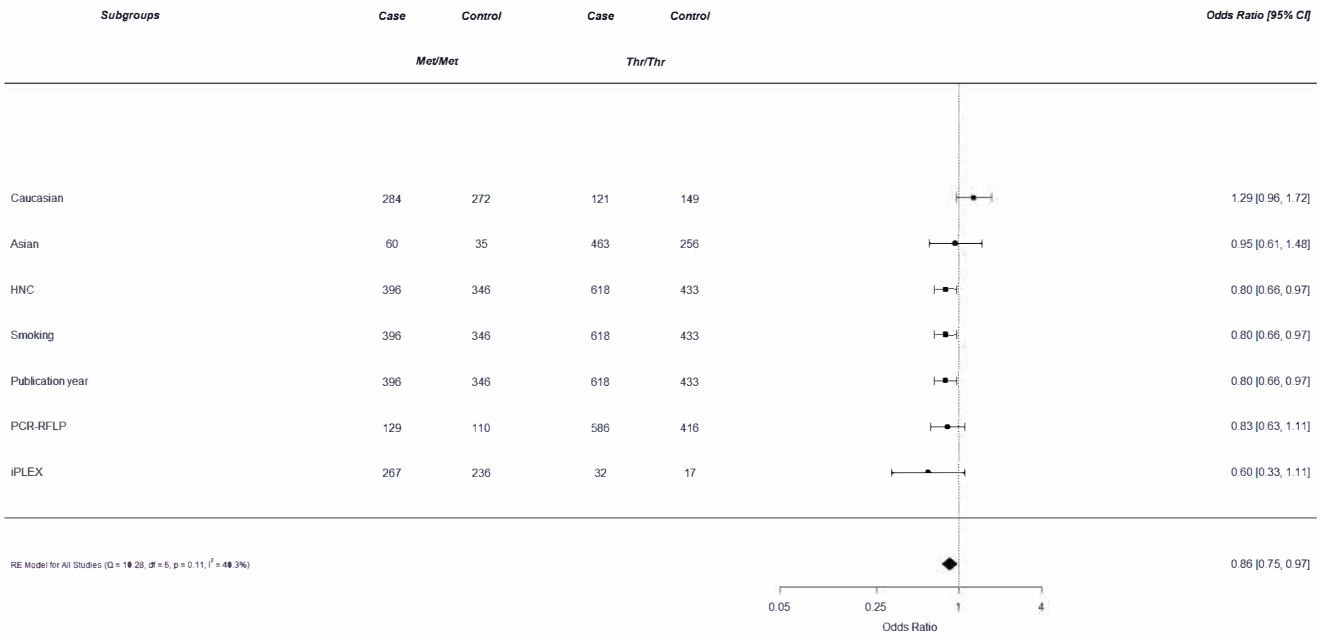

Fig. 12. Forest Plot for Homozygous (Thr/Thrvs Met/Met)

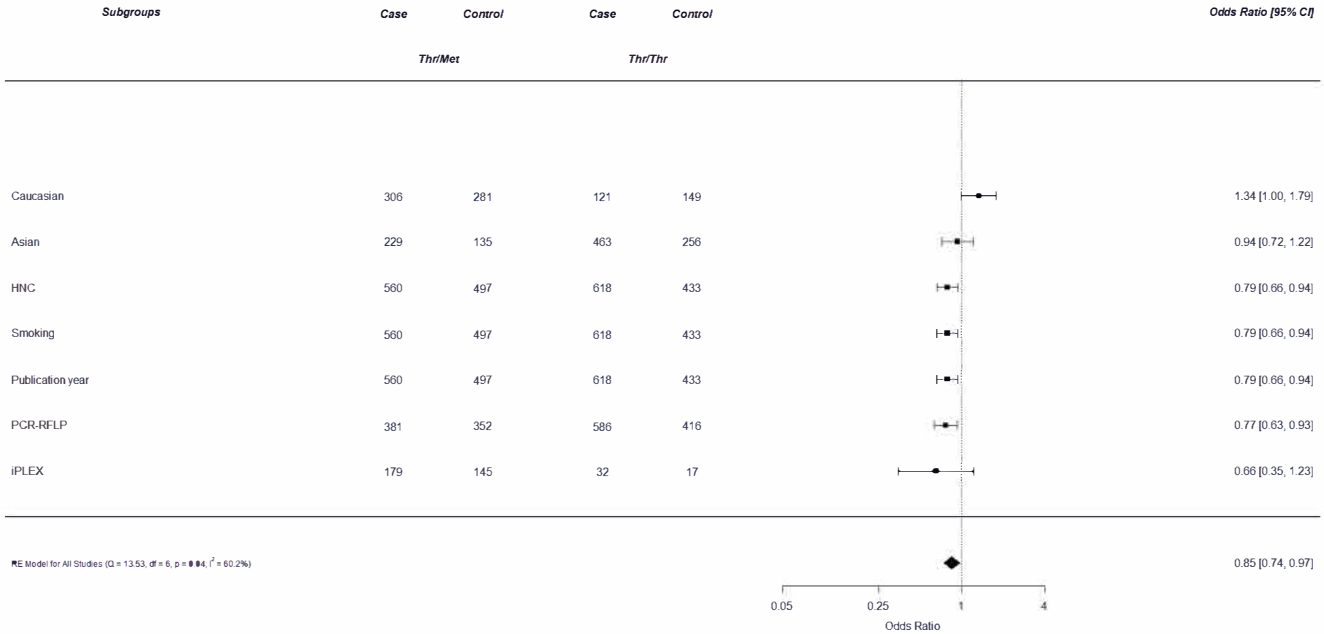

Fig. 13. Forest Plot for Heterozygous (Thr/ThrvsThr/Met)

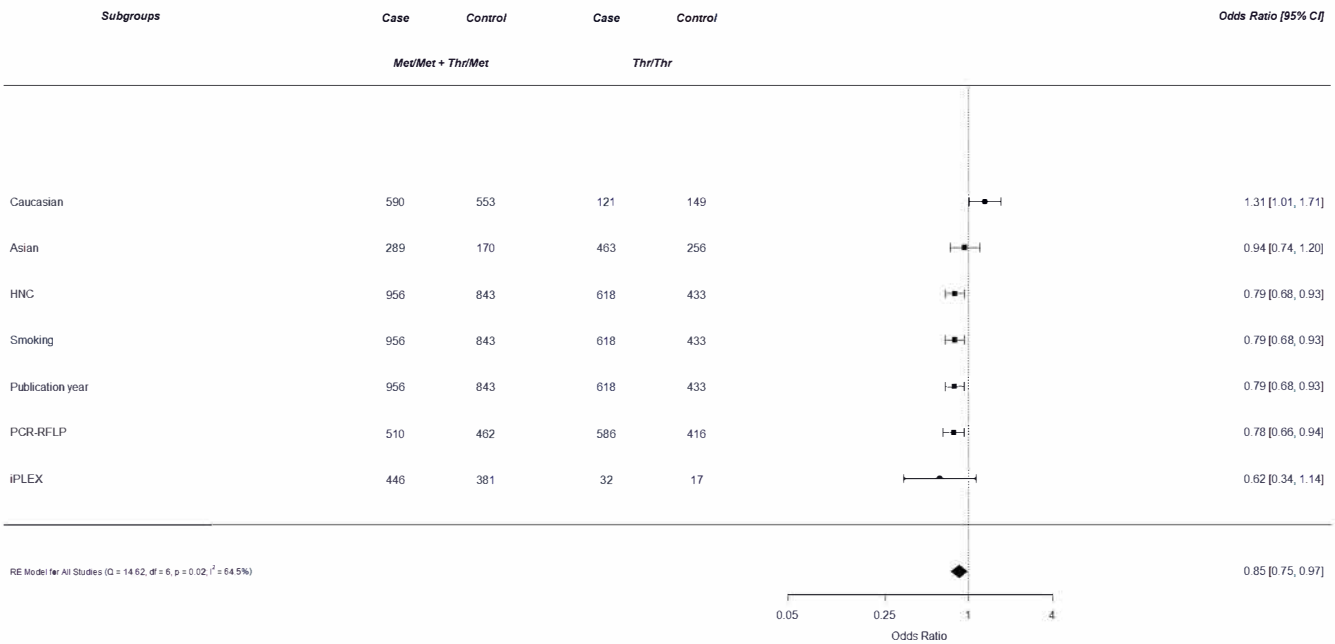

Fig. 14. Forest Plot for Dominant Model (Thr/Thr vs Met/Met + Thr/Met)

Cells are protected from nucleic acid damage and over all genomic integrity are maintained because of the DNA repair machinery. Different biomarkers are present such as disease stage, tumour size, family history, radiation exposure and many more for HNC pathophysiology. Various polymorphisms in genes involved in DNA repair mechanisms are well known which helps in diagnosis and even identify the area required to be treated. Due to these reasons, mutations in XRCC1 and XRCC3, as these genes are important for genomic homeostasis and even help in prevention of carcinogenesis were taken into consideration.

The XRCC1 is one of the most important BER pathway repair protein and helps in repairing of single strand breaks together with three other DNA repair genes i.e. PARP, DNA ligase III and DNA polymerase (Hu *et al*., 2005). It is present on chromosome 19q13.2-13 with 22 exons and forms nearly 2.2 kb transcript. The XRCC1 gene polymorphisms interfere in interaction of proteins of the BER pathway which leads into lowering of DNA repair capacity and boosting of carcinogenic process. The genotype variants of XRCC1 on which genetic effect was investigated in earlier studies were Arg399Gln (rs25487), Arg280His (rs25489) and Arg194Trp (rs1799782) polymorphisms on HNC vulnerability.

XRCC3 gene is one of the most important genes present in Homologous Recombination Repair pathway and also plays important role in repairing of DNA double-strand breaks (DSBs). It is structurally and functionally related to RAD51 (Krupa *et al*., 2011). Several studies were done to understand the correlation between XRCC3 Thr241Met polymorphisms with onset of various types of cancers (Mao *et al.*, 2014; Wang *et al.*, 2015(a)). The studies related to above mentioned polymorphisms and HNC patients were very few in number.

In this study, meta-analysis was performed to discuss the association between XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms and the HNC risk (Table 1). The result showed a significant association between Arg399Gln and Thr241Met polymorphism and HNC risk. The damaging effect of Arg399Gln SNP was shown under co dominant model in total population (Gln/Gln versus Arg/Arg, OR =1.02, 95% CI: 1.08 to 1.28, P = 0.001, Table 2) and the deleterious nature of Thr241Met polymorphism was shown under recessive model in total population (Thr/Thr vs Thr/Met + Met/Met, OR=1.21, 95% CI: 1.0290 to 1.4322, P = .0215 Table 3) (Yang *et al.*, 2012).

The correlation between HNC risk and XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphism might be affected by ethnicity, smoking and various techniques used, so to detect these effects, subgroup analysis were performed. The results of subgroup analysis showed significant association between XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphism and the risk of HNC in any genetic model under smoking and different techniques used (Fig. 4 and 11). When ethnicity was taken into consideration in subgroup analysis, there was a significant association between XRCC3 Thr241Met polymorphism and HNC risk in Asians but in Caucasians it was absent and there was opposite result of XRCC1 Arg399Gln polymorphism, as it shows its association in Caucasians and not in Asians (Fig. 7 and 12).

In previous years, various meta-analysis were conducted and in one such study conducted by Lou *et al*., 2013 consisted of twenty-nine studies where 6,719 cases and 9,627 controls were analysed. No association what so ever to the onset risk for HNC and XRCC1 Arg399Gln polymorphisms was found. Some of the meta- analysis studies conducted showed the effect of XRCC1 Arg399Gln SNP on childhood lymphoblastic leukaemia in Asians. In 2015 (b), Wang *et al*., found the association between XRCC3 241 (Thr>Met) polymorphism with Thyroid carcinoma risk and there were two more preceding studies which shows related conclusions (Bastos *et al*., 2009; Fayaz *et al*., 2014). In 2014, Fayaz *et al*. found the relationship between XRCC3 241 (Thr>Met) polymorphism and risk of differentiated thyroid carcinoma. In spite of all these studies, Yu *et al*., in 2004 reported non-finding of any association between XRCC3 241 (Thr>Met) and reduced risk of Thyroid carcinoma. In consequence, further studies were carried out which are useful for confirming our findings.

In the current study, two polymorphisms in 7634 cases and 8955 controls in total with HNC were reviewed. XRCC1 Arg399Gln, Gln/Gln genotype and Gln allele were risk factor for HNC, and even Arg/Arg genotype and Arg allele were causing harm in HNC cases. In XRCC3 Thr241Met polymorphism the homozygote variants (Thr/Thr and Met/Met) were responsible for HNC risk. In Turkey, a similar study conducted by Avci *et al*., 2017 reported that cigarette smoking causes DNA damage. The damage could lead to formation of adducts, may break strands of DNA or cross-link them in such manner that cannot be repaired via different repairing mechanisms. Overall, the evidence of significant association was observed between XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms and the risk of HNC in heterozygous genetic models.

Conclusion

In conclusion, this meta-analysis study suggested that the presence of polymorphisms namely, Arg399Gln in XRCC1 and Thr241Met in XRCC3 might have an association with onset of head and neck cancer incidences. Intensive investigation is required to understand the gene

to gene and gene to environment interactions in onset of cancer incidences in different populations across the globe.

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