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Bcl-2(Rs956572) G/A Mutation in Cigarette Smokers and receptiveness to Lung Cancer

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Abstract: *Cigarette smokingis the root cause of cancer and death from lung cancer. Singlenucleotidepolymorphisms(SNPs) are the salient location in an individual genome which influences the diseases. Since cigarette smoke contain large number of oxidants which may induce mutations. Bcl-2 SNPs have been found to be associated with predisposition of different cancers. In present study we examine the association of bcl-2 variant rs956572 with the cigarette smokers to predict the risk of smoking related cancers in smokers. Among the smokers, 42% were found with GG genotype, 43% were heterozygous GA and 15% were AA,respectively. Homozygous AA genotype was recorded (15%) more when compared to non-smokers (12%) whereas; heterozygous GA genotype was recorded (36%) less when compared to smokers (43%). There was a significant difference among GG, GA and AA genotypes with Chi–Square value = 6.05, p<0.05. However, GG vs GA and GG vs GA+AA genotype showed statistically significant with OR: 0.67 [0.48 – 0.96], p< 0.05 and the recessive model. Recent findings, suggest that bcl-2 G/A (rs956572) variation is associated with decreased expression of Bcl-2 proteins. Our finding gives a clue that the transition mutation of G/A (rs956572) may leads to the lung diseases including cancer in smokers. However, there will be a need of more extensive and elaborated study to establish bcl-2(rs956572) G/T as a prognostic marker for the risk of lung cancer in smokers.*
Keywords*: Bcl-2, (rs956572) G, smokers, lung cancer, Single nucleotide polymorphisms*

I. Introduction

Seventeen types of cancers are reported among smokers and 90 out of 100 peoples were hard hit of lung cancers [1]. Unlike other cancers, lung cancer kills the patients inmany ways,including bronchitis and pneumonia. The number of deaths of lungcancers has increased from 26 to 30%in last few years [2]. Only in US, the total death recorded more than 48,000 due to the tobacco usage[3].For cancer patients and survivors, those smoke are likely to develop secondary primary cancer. Smoking increases the risk of dozens other cancers including COPD (Chronic Obstructive Pulmonary Diseases), reproductive effect in women, oral cavity cancer and Asthma etc.[4].In recent years the survival rate for lung cancer patients achieved from 37 % to 43 %, because of secured improvements in medical instrumentation advancement and surgical technique [4].The most common type geneticvariation in Singlenucleotidepolymorphisms (SNPs) have proved to be an important in the study of individual health.Recently there is great interest and attention to investigate the association of cancer with SNPs asMdm2, bcl2 or p53. Scientists have studied the correlation of some genes with the disease;for example P53 coded gene from one of the polymorphismact astumor suppressor protein. That it regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer, because of its role in conserving stability by preventing genome mutation [5]. Proclivity of disorder with the association of different SNPs had been worked out and still there is a challenge for the researchers. Bcl-2 is one of the regulatory proteins and identified geneas a cause of cancers [6]. Among the contemporary studies on bcl-2, It is well documented the relationship of cancers. For example, a SNP rs1801018, Thr7Thr of bcl-2 is associated with differentiated thyroid cancer known as PTC (PapillaryThyroidCancer)published in Korean Population study[7] (Eun et al, 2011). In one of the study of Chinese population, it was claimed that bcl-2 (-938C - A) polymorphism is associated with breast cancer susceptibility [8] (Zhang et al, 2001)**.** As far as the lung cancer is concerned, rs1462129 and rs255102 of bcl-2 have been found associated with the disease in an American population [9]. A study on bcl-2 polymorphism reveals that -938CC genotype shows significantly worse survival condition of the lung cancer patients [10].In addition of that, polymorphisms in bcl-2 (–938 AA-AC) may be linked with endurance of small cell lung cancer patients. An improvement sign were observed who were treating of chemotherapy.[11]

The Complex of genetic predisposition to biological systems extendstomodulate a combination of SNP and polymorphism and association with lung cancer with environmentalcarcinogenic exposure[12; 13]. This is also an important factor to develop lungcancers in the smoking genes [14].Hence, so far no study has comprehensively investigated of bcl-2 (rs956572) (C>T) with the cigarette smokers to predict the risk of corresponding cancers in smokers.

II. Material And Methods

Experimental setup

Three hundred healthy human volunteers were included in the present study with age≥ 18 yearswith mean 33 and S.D. 3.5 years smokers(SM) and three hundred age matched non-smoker(NSM) volunteers with the same age group. In the present study we have used structured questionnaire and the Ethical clearance from Riyadh, Saudi Arabia.All the study participants wereselected from the central region of the KSA.

Sample collection and DNA Extraction:

Genomic DNA was wrenching outcarried using predesigned TaqMan® assaysas determined accordingly [15]. Analysis of Genotype frequency deviations were analyzed by $\chi 2$ test using the SPSS version in previous study[15].

III. Results

In this present study, we determined the polymorphic status of bcl-2 (rs956572) (G>A) by using TaqMan based Real Time PCR. We determined for the first time an association between bcl2 gene polymorphism and smokers(SM) among the Saudi population.

BCL-2 Polymorphism

Genotypic dispensationas shown (Table-I) wasrecordedas 42% for the GG, 43% for the heterozygous GA status, and 15% for the AA, respectively. The homozygous AA genotype was observed in higher percentage (15%) in SM volunteers when compared to NSM volunteers (12%) whereas; heterozygous GG genotype was seen in 42 % only in SM volunteers when compared to NSM volunteers (52%).

There was a marginal significant difference among GG, GA and AA genotypes ($\chi 2 = 5.15$, $p<0.05$). Also, an important alliance was noted between allelomorph andsmokers ($\chi 2 = 5.422$, $p<0.05$) (Table-II). Hence, these groups were combined before further statistical analysis (Table-III). Data showed that GG vs. AA and GA vs. AA genotypes did not exhibited a considerable variance I in boththe type of volunteers,SM and NSM OR: 0.646 [95% CI: 0.393 – 1.062] withp>0.05, OR: 0.956 [95% CI: 0.575 – 1.587], p>0.05 respectively. But GG vs GA genotype were considerable variance among SM and NSMOR: 0.676 [95% CI: 0.478 – 0.957], p<0.05 volunteers. Dominant model GG vs GA+AA was also associated significantly with the volunteer SM OR: 0.66 [95% CI: 0.484-0.923], p<0.05. While A allele was found as significant independent risk factor OR: 0.746 [95% CI: 0.586-0.949],p<0.05 (Table-III).

Frequency of G allele was 0.63 and 0.70 among SM and NSM volunteers. Whereas, for A allele it was 0.36 and 0.30 among and volunteer (NSM). A considerable alliance of allele was noted with volunteer (SM). However a higher percentage of GG genotype in NSM suggests that transition mutation of G allele to A favors the smoking behavior. In other words cigarette smoking seems to be a factor for G to A transition at rs956572 in bcl-2 gene.

IV. Discussion

Cigarette smoking (CS) can cause cancer in many organs system and responsible for the deterioration of overall health. It causescancer, larynx, asthma related disease, bone decreasing density,periodic risk of pneumonia and uterine cervix. It alsoincreases in adenocarcinomas and impairs immune system [16].Conventionally, it smoke divided into gaseous phase (92 %) and Particulate matter (8%). There is certainly a threat of cancer in smokers due to the inhaling of free radicals, expected more than 1015active free radicals in one puff only as reported by PryorandStone[17].

It is well known fact that,cigarettesmogis a complex mixture of highly carcinogens chemicals. Over 7000 chemicals in tobacco are well known.Among them, more than 250 have beenaffirm more harmful including ammonia, N-Nitrosamines,-NH_2 containing compounds, PAHs, HCN and NO gases etc. that have been evaluated by National Toxicology Program[18]. Free radicals are the main cause of cellular damage and DNA, including key gene which is the common path for lung cancer and variety of cancers. It is well documented that active free radicals are capable to oxidize the biomolecules as well, and may reorient the gene expression and cancer too [16].

Around 1.35 million cases are registered worldwideevery year for the leading lung cancer of deaths in UK whereas, survival rate of lung cancer patients are 43% in USA. Recently Genomic study have identified (Genomic wise association studies- GWAS) that SNPs are the soft target of human genetic variation, and they may influence one's susceptibility to genetic damage [19].One of the candidates, bcl-2 play a direct role in regulation of the mitochondrial pathway of apoptosis.Bcl-2 (rs956572) and have been studied for their association with many diseases. For example, bcl-2 rs956572 polymorphism is associated with increased anterior cingulate cortical glutamate in euthymic bipolar I disorder [20]and intracellular calcium homeostasis in bipolar I disorder [21]. Moreover, rs956572 G-allele is associated with significantly higher bcl-2 protein

expression and diminished cellular sensitivity to stress-induced apoptosis and the A allele is also associated with smoking behavior [22]. Thebcl-2variant (rs956572) G/Aexperimented for the first time in Saudi Arabian volunteer (SM). Results indicated bcl-2 (rs956572) G/A singlenucleotide polymorphism was found significantly associated with SM ($p<0.05$). Recently, bcl-2 rs956572 G/A has been studied in euthymic bipolar I disorder patients and reported that reverse mutation G/A mutation is associated with low level of the Bcl-2 protein and associated with the high incidence of the disease[20]. Uemura and coworkers [21]reported thatrs956572 G/A variant are associates with disrupted intracellular calcium homeostasis inbipolar-I disorder and the carriers of A allele have a decreased expression of Bcl-2 protein. Hence there is an increased risk of intracellular calcium homeostasis in bipolar I disorder due to the presence of A allele. As a matter of fact, smokers are at the risk of developing variety of diseases including lung cancer and our finding suggest a higher percentage of AA genotype (15%) in SM volunteers when compared to NSM volunteers (12%). There had not been much study on bcl-2 variant G/A (rs956572) and the relation with different disease including lung cancers. Although, our results are in agreement with that of the recent previous works [20,21 and 22]. There are no more articles or study available on the variations of bcl-2 (rs956572) G/A and their association with the lung cancer and smoking behavior jeopardizes the supports to our finding.In this summary, our study advocates the association of AA genotype with bcl-2 variant G/A (rs956572) with smokers but not with non-smoker. Our findings are very much useful to study about on bcl-2 SNPs in the smokers in different population in different diseases to the young forthcoming researchers.

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Table I.Measurement of bcl-2 (rs956572) GenotypesandAllelic Frequencies.

	Genotypes					
	GG (n)	%	GA (n)	%	AA (n)	%
SM n (%)	126	42	129	43	45	15
NSM n (%)	156	52	108	36	36	12
χ2(2df.) = 6.052, p=0.049 for genotypes;						

Table II.Measurements of bcl-2 (rs956572) allelotypeand AllelicFrequencies

	Allelotypes			
	G	AF	A	AF
SM (n)	381	0.63	219	0.36
NSM (n)	420	0.7	180	0.30
χ2(1d f.) = 5.422, p=0.02 for allelic frequency; AF=allelic frequency				

Table III.Measurements ofOddSRatiowith95% CI of bcl-2 (rs956572) Gene among SMVolunteers

Genotypes	Odds Ratio	95% CI	p-value
GG vs GA	0.676	0.478-0.957	0.027
GG vs AA	0.646	0.393-1.062	0.084
GA vs AA	0.956	0.575-1.587	0.861
Dominant Model			
GG vs GA+AA	0.668	0.484-0.923	0.014
Recessive Model			
AA vs GA+AA	1.034	0.633-1.690	0.892
Allelotypes			
G/A	0.746	0.586-0.949	0.017