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RESEARCH ARTICLE

Human beta-defensin-1 rs2738047 polymorphism is associated with shisha smoking risk among Saudi population

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Abstract

Human β-defensin (HBD), a member of the antimicrobial peptides, is essential for respiratory epithelial cells' microbial defense, and is affected by cigarette smoking (CS). Its expression is upregulated by stimulation from microbes or inflammation. Genetic polymorphisms in the HBD-1 gene have been implicated in the development of various smoking-related diseases, including chronic obstructive pulmonary disease and asthma. Thus, we sought to analyze possible associations between HBD-1 single-nucleotide polymorphism (SNP) in HBD-1 gene and CS in ethnic Saudi Arabian subjects. Variants rs1047031 (C/T), rs1799946 (C/T), rs2738047 (C/T), and rs11362 (C/T) were investigated by genotyping 575 blood specimens from males and females, smokers/non-smokers: 288/287. The CT and CT+TT genotypes of rs1799946 presented an ~5-fold increased correlation with CS among the female smokers, compared with the female controls (OR = 5.473, P = 0.02003; and OR = 5.211, P = 0.02028, respectively), an observation similar to rs11362 SNP in female smokers, but with protective effects in TT genotype, compared with the CC reference allele (OR = 0.143, P = 0.04368). In shisha smokers, the heterozygous CT and the CT/TT genotype of rs2738047 polymorphism showed the same results with ~3-fold increased correlation with CS (OR = 2.788; P = 0.03448), compared with the cigarette smokers category. No significant association was shown in genotypic distributions and allelic frequencies of rs1047031. Further investigations, including large study samples, are required to investigate the effects of shisha on human beta-defensin expression and protein levels.

Keywords Smoking · Human β-defensin-1 · Polymorphism · Genotyping · Saudi · rs2738047

Introduction

Biochemical studies have uncovered mutagenic effects of several components of cigarette smoking (CS), including alterations in DNA methylation (Steenaard et al. 2015) and induction of genetic alterations of proto-oncogenes to oncogenes, tumor suppressor genes, like the critical TP53 gene (Pfeifer et al. 2002; Taghavi et al. 2010; Almutairi et al. 2021b), and genes involved in innate immunity (Kohailan et al. 2017; Kohailan et al. 2016; Almutairi et al. 2021a). Previous reports have addressed the effects of tobacco smoking throughout the respiratory tract. Also, CS deregulates factors involved in important cell functions, including growth (Alamri et al. 2015), adhesion, and migration (Semlali et al. 2011a) in fibroblasts and human gingival epithelial cells (Semlali et al. 2011a; Semlali et al. 2011b). According to the Centers for Disease Control and Prevention, every year, more than 7 million people die worldwide due to smoking-related diseases (A Report of the Surgeon General. Atlanta 2014), such as heart diseases (Kamimura et al. 2018), lung cancer (Khuder 2001), stroke (Pan et al. 2019), asthma (Cerveri et al. 2012), and gastrointestinal mucosal (Zhang et al. 2012).

The immune response to infection is comprised of two arms, an adaptive or specific immune defense and an innate

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or non-specific immune defense (Cheroutre and Huang 2013). The innate defense system includes defensins, which are a specific group of antimicrobial peptides (AMPs) (Wang et al. 2012), also called host defense peptides (Niyonsaba et al. 2016); they are short, cationic, amphipathic and rich in cysteine residues (Pazgier et al. 2006), and are highly expressed on the surfaces of epithelial tissues. AMPs are the first line of defense between living organisms and their environment (Lehrer and Ganz 2002). Immune cells could also activate the expression of these peptides (Rohrl et al. 2008). Their vital roles in the innate immune system across species, defending against viruses, fungi, and bacteria, and involvement in adaptive immunity, inflammation, wound repair, cytokine and chemokine expression, histamine production, and the enhancement of the antibody response, have been well-established (El-Baky et al. 2015).

Humans have approximately 40 defensin genes located on different chromosomes (Patil et al. 2005), that are classified into three major subfamilies, alpha (α), beta (β), and theta (θ), according to the pairing mechanism of the six conserved cysteine residues that form three disulfide bridges (Cederlund et al. 2011; Lehrer and Lu 2012). The human β-defensins (HBDs) have been detected in many protective epithelial tissues such as the skin and the mucous membranes of the respiratory tract, the prostate, and the intestines (Schneider et al. 2005). They are also involved in the proliferation and metastasis of tumor cells (Diamond and Ryan 2011).

Human beta-defensins contain ~35 residual amino acids (Schneider et al. 2005), and their structure consists of a signal sequence of between 36 and 42 amino acids at the C-terminus preceding the mature defensin peptide (Ganz 2003). Thus far, only six types of HBDs have been described and characterized in humans. HBDs 1 through 4 are present primarily in the epithelial tissues, including the epidermis, urogenital, and respiratory tracts, while HBD-5 and HBD-6 are found only in the epididymis (Niyonsaba et al. 2009; Niyonsaba et al. 2006). HBD peptides are associated with tumorigenesis and shown to overexpress in various cancer cells (Avila 2017). Defensin gene polymorphisms have been associated with susceptibility to the human immunodeficiency virus (HIV) and the development of acquired immunodeficiency syndrome (AIDS) (Mehlotra et al. 2013).

Human beta-defensin-1 is a class of the defensin family and has the ability to directly or indirectly inactivate or kill a broad spectrum of fungi and bacteria by triggering innate and adaptive immune responses (Aerts et al. 2008; Harder et al. 2007). This gene is located at 8p22-23 (Celerino Da Silva et al. 2016) and is considered to be an essential antimicrobial peptide among epithelial and salivary defenses (Polesello et al. 2015). The expression of HBD-1 has been observed in the epidermis, sebaceous glands, and sweats ducts, and might also be induced by pathogenic molecules such as peptidoglycans and lipopolysaccharides (LPSs) (Sorensen et al. 2005).

Human beta-defensin-1 plays a vital role in the microbial defense of the ocular (corneal and conjunctival) (Huang et al. 2007), oral (Mathews et al. 1999), and respiratory epithelial cells (airway mucosal) due to its moderate antimicrobial property. In the airway, this function is affected by CS (Frederic et al. 2008). Its expression can be upregulated by stimulation from microbes or inflammation (Avila 2017). Additionally, HBD-1 might have potential anticancer properties (Prado-Montes de Oca 2010; Donald et al. 2003), as a loss of HBD-1 expression has been detected in prostate and renal carcinoma cells. It could also inhibit the proliferation of human bladder cancer cells and promote apoptosis in renal cell carcinoma (Sun et al. 2006; Bullard et al. 2008). Furthermore, HBD-1 was cytotoxic to prostate cancer cell lines at later stages and may be involved in the control of tumor progression, as well as the recognition and elimination of other types of cancer (Bullard et al. 2008; Sun et al. 2006).

Several studies showed that the low expression of HBD-1 gene owing to genetic polymorphisms is associated with the susceptibility of HIV infection in two separate studies on Italian and Brazilian patients (Braida et al. 2004; Milanese et al. 2006) and/or with the pathogenesis of inflammatory bowel disease (Wilson et al. 2014). Besides, other studies have reported that polymorphisms of HBD-1 gene are related to the susceptibility of pulmonary infectious diseases, including chronic obstructive pulmonary disease (COPD) (Matsushita et al. 2002) and asthma (Levy et al. 2005).

Currently, there is no previous investigation of the effects of CS on the four HBD-1 SNPs. Therefore, the goal of the current study was to analyze possible associations between variants of HBD-1 (rs1047031, rs1799946, rs2738047, and rs11362) and CS in ethnic Saudi Arabian subjects. This could allow for the identification of sensitive genetic markers, not only for the diagnosis of diseases related to CS but also for the prevention of the potential effects of CS on healthy patients.

Materials and methods

Ethics statement and demographical information

Written ethical consent for this study was reviewed by and obtained from the Research Ethics Committee of the College of Applied Medical Sciences at King Saud University (KSU) in Riyadh, Kingdom of Saudi Arabia (KSA) (Approval Number: CAMS 13/3536). All participants of this study provided written informed consent, and the study was conducted following the principles in the Helsinki Declaration, updated in 2008. Essentially, participants who smoked cigarettes or shisha were termed smokers, while those who did not use any type of tobacco product were referred to as non-smokers. Clinical data on CS history, clinical history, including allergic symptoms, an average of cigarette sticks smoked per day, and

body mass index were filled out by each participant through a self-completed questionnaire with his/her signature.

Collection of blood samples

Blood samples were collected via direct venipuncture from a group of 288 smoking Saudi adults (patients) and a group of 287 healthy, non-smoking Saudi adults (controls) recruited from the Blood Donation Center at King Saud medical city (Riyadh, Saudi Arabia) between September 2018 and December 2019. Both groups of volunteers did not have inflammatory diseases and/or chronic respiratory diseases at the time of sample collection.

Genomic DNA extraction

As described in previous reports (Almutairi et al. 2019; Semlali et al. 2019), leukocyte genomic DNA was isolated from peripheral blood samples through the PureLink® Genomic DNA Mini Kit (Catalogue No K1820-01; Invitrogen™, Carlsbad, CA, USA), as per the manufacturer's instructions. The concentration of the extracted DNA was quantitated using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Then, the purified DNA was labelled and stored at −80°C until further analysis.

Preparation of TaqMan® SNP Genotyping Assay for PCR amplification

Genomic DNA from each blood sample was isolated for the two study groups at a final concentration of 20 ng/μL. Four tag SNPs identified in the HBD-1 gene were used for genotyping analysis. Each genotyping was carried out in a total volume of 10 μL containing 0.3 μL of 40X TaqMan® Genotyping SNP Assay (Applied Biosystems), 5.5 μL of TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 2 μL of 20 ng of isolated DNA, and 2.2 μL distilled water. Polymerase chain reaction (PCR) amplifications were performed using a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems). Details of the PCR cycling conditions have been described in previous publications of our group (Almutairi et al. 2019, Semlali et al. 2019).

Human beta-defensin-1 SNP selections

Briefly, four polymorphisms were selected in this study according to their location on the HBD-1 gene transcript, for example, 3′-UTR, or 5′-UTR, or in the exon region. Furthermore, we performed a literature review of these SNPs' association with a plethora of diseases in diverse ethnic groups (Hu et al. 2004; Matsushita et al. 2002) to further guide our selection thereof. This correlation could be explained by SNPs' ability to alter the function of their corresponding genes' which might also enhance their ability to induce diseases not yet studied. These genetic variants in HBD-1 are rs1047031 (C > T), rs1799946 (C>T), rs2738047 (C > T, L38F), and rs11362 (C > T). All details of the four HBD-1 polymorphisms are described in Table 1.

Statistical analysis

As described in previous work (Semlali et al. 2019; Almutairi et al. 2019), the deviations of the computed genotypic and allelic frequencies of each SNP were checked using a Hardy–Weinberg equilibrium assay, and genetic comparisons were performed via X^2 tests and allelic odds ratios (ORs). Confidence intervals (CIs) at 95% were measured using a two-tailed Fisher's exact test. The Statistical Package for the Social Sciences (SPSS) version 16.0 statistical software (SPSS, Chicago, USA) was used to perform all statistical calculations. A P-value of <0.05 was considered statistically significant.

Results

Clinical parameters of the study participants

The basic clinical parameters of the study inclusion among the smoking and non-smoking individuals are displayed in

Table 1 Description of the selected SNPs in *HBD-1* gene

SNP ID	SNP location	SNP type	Alleles change	Amino acids change
rs1047031	NC_000008.11:g.6870676	3′ UTR variant	C/T transition substitution	---
rs1799946	NC_000008.11:g.6877909	5′ UTR variant	C/T transition substitution	---
rs2738047	NC_000008.11:g.6870776	Missense Variant	C/T transition substitution	L38F
rs11362	NC_000008.11:g.6877877	5′ UTR variant	C/T transition substitution	---

Abbreviations: SNP: single-nucleotide polymorphism; 3′ UTR: 3-prime untranslated region; 5′ UTR: 5-prime untranslated region; L: leucine; F: phenylalanine

Table 2 Clinical and demographic data of the study participants for genotyping

Variable	Smokers, *N* (%)	Control (non-smokers), *N* (%)
Number of participants	288 (100%)	287 (100%)
Mean of age	29.76±7.09	29.13±8.83
Age of participants (years)		
≤ 29 years	155 (53.8%)	176 (61.3%)
> 29 years	133 (46.2%)	110 (38.3%)
Undetermined	0	1 (0.4%)
Gender		
Men	264 (91.7%)	203 (70.7%)
Women	24 (8.3%)	84 (29.3%)
Years of smoking		
≤ 7 years	173 (60%)	Na
> 7 years	114 (39.6%)	Na
Undetermined	1 (0.4%)	Na
Average of smoking per day		
≤ 12 times	148 (51.4%)	Na
> 12 times	139 (48.2%)	Na
Undetermined	1 (0.4%)	Na
Type of smoking		
Cigarette	216 (75%)	Na
Shisha	71 (24.6%)	Na
Undetermined	1 (0.4%)	Na

Abbreviations: *N*, number; Na, not applicable

Table 2. Both groups did not show any significant differences in their ages, genders, and any other smoking characteristics of the study. The total number of volunteers used in this study were 575 Saudi adults, comprising 288 individuals in the smoking group (91.7% men, 8.3% women), and 287 individuals of the non-smoking group (70.7% men, 29.3% women). Among the smoking group, 54% (n = 155) were aged ≤29 years and 46% (n = 133) were >29 years (mean age, 29.76 ± 7.09). Regarding the non-smoking group, ~61% were ≤29 years and 39% were ≥29 years (mean age, 29.13 ± 8.83). Regarding the length of years of smoking (duration), the percentage of participants who had engaged in smoking for ≤7 years was 60%, compared with ≥7 years of smoking (22%). Also, we categorized the smoking group based on the average of CS per day into 51.4% who had consumed CS ≤12 times, and 48.2% of those who had consumed >12 times CS. Also, we separated smoking participants depending on the smoking types into 75% consuming cigarettes, and ~25% consuming shisha.

Genotypic distribution of HBD-1 gene polymorphisms among smokers and non-smokers

Through the TaqMan genotyping assay, the four SNPs were tested in 575 Saudi individuals, including 288 from the smoking group and 287 from the healthy, non-smoking group. Table 3 shows the general prevalence of genotype and allele frequencies of HBD-1 rs1047031, rs1799946, rs2738047, and rs11362 with smoking participants, compared with the healthy controls. Among the genotype and allele frequencies of these SNPs, we found no significant association with smoking habit. In rs1047031 controls, the genotypic allocations of CC, CT, and TT genotypes identified were 82%, 17%, and 1%, respectively, while in the smoking group, 84%, 15%, and 1% were identified, respectively. However, the distributions of rs1799946 SNP of CC, CT, and TT genotypes among non-smokers were 27%, 50%, and 23, respectively, whereas in smoking volunteers were 26%, 52%, and 22%, respectively, demonstrating no significant difference. Regarding the results of the rs2738047 variant, the genotype distributions were 96% CC and 4% CT in both groups studied. In contrast, HBD-1 rs11362 SNP was distributed as controls: 33% CC, 49% CT, and 18% TT and for the smoking individuals: 34%, 50%, and 16%, respectively.

Association between HBD-1 gene polymorphisms and smokers' ages

To investigate any relationship between HBD-1 gene polymorphisms and the smokers' ages, we categorized all smokers and non-smokers into two groups, as follows: those whose age was 29 years or less (group A; ≤ 29 years) and those whose ages were more than 29 years old (group B; > 29 years). The numbers of smokers and non-smokers were 155 and 176, respectively, in group A (133) and group B (110) (see Table 2). The results analysis did show any associations between HBD-1 rs1047031, rs1799946, rs2738047, and rs11362 and allelic and genotypic distributions in both group A (smokers) and group B (healthy controls) (Table 4). Among younger smokers (group A), the genotype allocations of CC, CT, and TT genotype polymorphisms were found to be 83%, 16%, and 1%, respectively, in non-smokers, while they were 81%, 19%, and 0%, respectively, in smokers in SNP rs1047031. In SNP rs1799946 of group A, the CC, TT homozygous, and CT heterozygous frequencies were 30%, 23%, and 47%, respectively, in non-smokers, compared with 26%, 19%, and 55%, respectively, in smokers. As for HBD-1 SNPs rs2738047, and rs11362, the genotype frequencies were 97%/32% CC, 3%/47% CT, and 0%/21% TT, respectively, in non-smoking controls, while they were 95%/30% CC, 5%/55% CT, and 0%/15%, respectively, in the smoking participants (Table 4). In contrast, among older smokers (group B), no significant differences were observed when compared with older non-smokers. The distributions of CC, CT, and TT genotypes of the rs1047031 and rs1799946 SNPs were found to be 82%/23%, 18%/53%, and 0%/24%, respectively, in non-smokers of group B, and 88%/26%, 11%/48%, and 1%/26%, respectively, in smokers of group B. For rs2738047 and

Table 3 General genotype distributions of *HBD1* gene polymorphisms among smokers and non-smokers (controls)

Polymorphisms	Alleles	Controls		Smokers		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**280**	**100%**	**283**	**100%**				
	CC	231	**82%**	239	84%	Ref			
	CT	47	17%	43	15%	0.88	0.563–1.389	0.29	0. 59319
	TT	2	1%	1	1%	0.48	0.044–5.366	0.37	0. 54520
	CT+TT	49	18%	44	16%	0.87	0.556–1.355	0. 39	0. 53282
	C	509	91%	521	92%	Ref			
	T	51	9%	45	8%	0. 86	0.567–1.311	0. 48	0. 48714
rs1799946	**Total**	**283**	**100%**	**285**	**100%**				
	CC	77	27%	74	26%	Ref			
	CT	141	50%	148	52%	1.092	0.737–1.619	0.19	0.66059
	TT	65	23%	63	22%	1.009	0.630–1.615	0.00	0.97183
	CT+TT	206	73%	211	74%	1.066	0.734–1.547	0.11	0.73730
	C	295	52%	296	52%	Ref			
	T	271	48%	274	48%	1.008	0.798–1.272	0.00	0.94881
rs2738047	**Total**	**287**	**100%**	**286**	**100%**				
	CC	276	96%	274	96%	Ref			
	CT	11	4%	12	4%	1.099	0.477–2.533	0.05	0.82480
	TT	0	0%	0	0%	1.007	0.020–50.945	-	1.00000
	CT+TT	11	4%	12	4%	1.099	0.477–2.533	0.05	0.82480
	C	563	98%	560	98%	Ref			
	T	11	2%	12	2%	1.097	0.480–2.506	0.05	0.82658
rs11362	**Total**	**281**	**100%**	**288**	**100%**				
	CC	92	33%	99	34%	Ref			
	CT	138	49%	143	50%	0.963	0.667–1.391	0.04	0.84060
	TT	51	18%	46	16%	0.838	0.514–1.367	0.50	0.47932
	CT+TT	189	67%	189	66%	0.929	0.656–1.316	0.17	0.67971
	C	322	57%	341	59%	Ref			
	T	240	43%	235	41%	0.925	0.730–1.170	0.42	0.51447

Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, % = percent, Ref = reference allele, OR: odds ratio

rs11362 polymorphisms, the distributions were 95%/34% CC, 5%/52% CT, and 0%/14% TT, respectively, in group B (non-smoking controls), while they were 96%/39% CC, 4%/44% CT, and 0%/17%, respectively, in group B (smoking group) (Table 4).

The relationship between HBD-1 SNPs and gender of smokers

We also examined the correlation between HBD-1 gene polymorphisms in smokers among 84 controls and 24 smokers of females, and 203 controls and 264 smokers of males. Although the number of female smokers was small, compared to the healthy females, our results supported an association between polymorphisms rs1799946 and rs1136 in the HBD-1 gene and smokers versus non-smokers. As shown in Table 5, in the female gender, the CT and CT+TT genotypes of rs1799946 presented approximately 5-fold increased correlation with CS among the female smokers compared with the female controls (OR = 5.473, $P = 0.02003$; and OR = 5.211, $P = 0.02028$, respectively). Also, as presented in Table 5, similar significant results were observed in rs1136 SNP in females, but with protective effects. TT genotype appeared to have a protective association with female smokers, compared with the CC reference allele (OR = 0.143, $P = 0.04368$).

However, the results of rs1047031 and rs2738047 polymorphisms demonstrated no significant relationship with CS in the female gender. In rs1047031, the

Table 4 Comparison of genotypic allocations of *HBD-1* gene polymorphisms in smokers with entire controls at ages ≤ 29 years and > 29 years

Polymorphisms	Alleles	Controls		≤ 29 years		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**171**	**100%**	**153**	**100%**				
	CC	141	83%	124	81%	Ref			
	CT	28	16%	29	19%	1.178	0.664–2.088	0.31	0.57532
	TT	2	1%	0	0%	0.227	0.011–4.780	1.75	0.18621
	CT+TT	30	17%	29	19%	1.099	0.625–1.933	0.11	0.74261
	C	310	91%	277	91%	Ref			
	T	32	9%	29	9%	1.014	0.598–1.720	0.00	0.95821
rs1799946	**Total**	**175**	**100%**	**154**	**100%**				
	CC	52	30%	40	26%	Ref			
	CT	83	47%	85	55%	1.331	0.798–2.220	1.21	0.27209
	TT	40	23%	29	19%	0.943	0.501–1.772	0.03	0.85410
	CT+TT	123	70%	114	74%	1.205	0.742–1.956	0.57	0.45070
	C	187	53%	165	54%	Ref			
	T	163	47%	143	46%	0.994	0.731–1.352	0.00	0.97076
rs2738047	**Total**	**176**	**100%**	**155**	**100%**				
	CC	170	97%	148	95%	Ref			
	CT	6	3%	7	5%	1.340	0.441–4.076	0.27	0.60489
	TT	0	0%	0	0%	1.148	0.023–58.221	-	1.00000
	CT+TT	6	3%	7	5%	1.340	0.441–4.076	0.27	0.60489
	C	346	98%	303	98%	Ref			
	T	6	2%	7	2%	1.332	0.443–4.007	0.26	0.60853
rs11362	**Total**	**171**	**100%**	**155**	**100%**				
	CC	55	32%	47	30%	Ref			
	CT	80	47%	85	55%	1.243	0.758–2.039	0.75	0.38795
	TT	36	21%	23	15%	0.748	0.389–1.435	0.77	0.38153
	CT+TT	116	68%	108	70%	1.090	0.681–1.742	0.13	0.72031
	C	190	56%	179	58%	Ref			
	T	152	44%	131	42%	0.915	0.671–1.248	0.32	0.57376
Polymorphisms	**Alleles**	**Controls**		**> 29 years**		**OR**	**95% CI**	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**108**	**100%**	**130**	**100%**				
	CC	89	82%	115	88%	Ref			
	CT	19	18%	14	11%	0.570	0.271–1.200	2.23	0.13553
	TT	0	0%	1	1%	2.325	0.094–57.747	0.77	0.37991
	CT+TT	19	18%	15	12%	0.611	0.294–1.270	1.77	0.18390
	C	197	91%	244	94%	Ref			
	T	19	9%	16	6%	0.680	0.341–1.357	1.21	0.27147
rs1799946	**Total**	**107**	**100%**	**131**	**100%**				
	CC	25	23%	34	26%	Ref			
	CT	57	53%	63	48%	0.813	0.433–1.524	0.42	0.51752
	TT	25	24%	34	26%	1.000	0.482–2.076	0.00	1.00000
	CT+TT	82	77%	97	74%	0.870	0.480–1.576	0.21	0.64532
	C	107	50%	131	50%	Ref			
	T	107	50%	131	50%	1.000	0.697–1.435	0.00	1.00000

Table 4 (continued)

Polymorphisms	Alleles	Controls		≤ 29 years		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs2738047	**Total**	**110**	**100%**	**131**	**100%**				
	CC	105	95%	126	96%	Ref			
	CT	5	5%	5	4%	0.833	0.235–2.957	0.08	0.77754
	TT	0	0%	0	0%	0.834	0.016–42.390	-	1.00000
	CT+ TT	5	5%	5	4%	0.833	0.235–2.957	0.08	0.77754
	C	215	98%	257	98%	Ref			
	T	5	2%	5	2%	0.837	0.239–2.928	0.08	1.00003
rs11362	**Total**	**109**	**100%**	**133**	**100%**				
	CC	37	34%	52	39%	Ref			
	CT	57	52%	58	44%	0.724	0.414–1.265	1.29	0.25608
	TT	15	14%	23	17%	1.091	0.503–2.368	0.05	0.82563
	CT+ TT	72	66%	81	61%	0.800	0.472–1.357	0.68	0.40819
	C	131	60%	162	61%	Ref			
	T	87	40%	104	39%	0.967	0.670–1.394	0.03	0.85597

Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, % = percent, Ref = reference allele, OR: odds ratio

percentage of genotype distributions of CC, CT, and TT for female non-smokers was 80%, 19%, and 1%, and female smokers were 79%, 21%, and 0%, while in rs2738047, the percentage of genotype distributions of CC, CT, and TT for healthy female controls was 95%, 5%, and 0%, and for female smokers, 92%, 8%, and 0%.

Finally, in the male gender, the results of the four SNPs tested also did not display any correlation between the genotypic frequencies and CS. The genotype distributions for the CC reference allele were 83% and 25%, for heterozygous CT 16% and 52%, and for a double-mutant allele TT 1% and 23%, in healthy males in rs1047031 and rs1799946 variants, respectively. However, in male smokers, these values for CC were 85% and 28%, for CT 14% and 51%, and for TT 1% and 21%, respectively. For SNPs rs2738047 and rs11362, the male genotype distributions in in healthy controls were as follows: CC 97% and 31%, CT 3% and 54%, and TT 0% and 15%. However, in smokers, the values were as follows: CC 96% and 34%, CT 4% and 49%, and TT 0% and 17% (Table 5).

The association between SNPs in HBD-1 gene and daily CS rate

Furthermore, we classified the smoking group into two categories according to the daily CS consuming rate, as follows: moderate smokers (smoking ≤ 12 times daily), which included 148 participants, and heavy smokers (smoking >12 times daily), which included 139 participants. We carried out this classification to enable us to investigate the relationship between the genetic variations of HBD-1 gene and the daily rate of CS consumption. The analyses of this study showed no significant association between the HBD-1 SNPs (rs1047031, rs1799946, rs2738047, and rs11362) and either the moderate or the heavy smokers, compared with the non-smokers. In the moderate and heavy smoking groups, the percentage of rs1047031 genotype distributions (CC, CT, TT) in the controls of both classes was 82%, 17%, and 1%. However, in the moderate smoking group, the allocations were 88% for CC, 12% for CT, and 0% for TT, and in heavy smokers, 81% for CC, 18% for CT, and 1% for TT. In rs1799946 SNP, the genotype frequencies were distributed, as follows: control groups for both: CC 27%, CT 50%, and TT 23%; moderate smokers: CC 23%, CT 47%, and TT 30%; heavy smokers: CC 29%, CT 57%, and TT 14%. In contrast, the rs2738047 and rs11362 genotypes in healthy controls of both classes were distributed into CC 96% and 33%, CT 4% and 49%, and TT 0% and 18%. Furthermore, in rs2738047 SNP, the allocations for moderate smokers were 95% for CC, 5% for CT, and 0% for TT, and for heavy smokers, 97% for CC, 3% for CT, and 0% for TT. In rs11362 SNP, however, the distribution for moderate smokers was 41% for CC, 45% for CT, and 14% for TT, and heavy smokers, 27% for CC, 54% for CT, and 19% for TT (Table 6).

Frequencies of HBD-1 SNPs according to the smoking duration

We also separated the smoking populations based on the years of smoking into 173 short-term smokers (≤ 7 years) and 114 long-term smokers (> 7 years) to test the

Table 5 Genotype and allele frequencies of polymorphisms in *HBD-1* gene in male and female smokers with controls

Polymorphisms	Alleles	Controls		Males		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**198**	**100%**	**259**	**100%**				
	CC	165	83%	220	85%	Ref			
	CT	32	16%	38	14%	0.891	0.534–1.486	0.20	0.65720
	TT	1	1%	1	1%	0.750	0.047–12.079	0.04	0.83869
	CT+TT	33	17%	39	15%	0.886	0.535–1.470	0.22	0.63995
	C	362	91%	478	92%	Ref			
	T	34	9%	40	8%	0.891	0.553–1.436	0.23	0.63520
rs1799946	**Total**	**199**	**100%**	**261**	**100%**				
	CC	50	25%	72	28%	Ref			
	CT	104	52%	133	51%	0.888	0.570–1.383	0.28	0.59921
	TT	45	23%	56	21%	0.864	0.507–1.473	0.29	0.59142
	CT+TT	149	75%	189	72%	0.881	0.579–1.340	0.35	0.55367
	C	204	51%	277	53%	Ref			
	T	194	49%	245	47%	0.930	0.716–1.208	0.30	0.58629
rs2738047	**Total**	**203**	**100%**	**262**	**100%**				
	CC	196	97%	252	96%	Ref			
	CT	7	3%	10	4%	1.111	0.415–2.972	0.04	0.83367
	TT	0	0%	0	0%	0.778	0.015–39.394	-	1.00000
	CT+TT	7	3%	10	4%	1.111	0.415–2.972	0.04	0.83367
	C	399	98%	514	98%	Ref			
	T	7	2%	10	2%	1.109	0.418–2.939	0.04	0.83520
rs11362	**Total**	**199**	**100%**	**264**	**100%**				
	CC	62	31%	89	34%	Ref			
	CT	107	54%	130	49%	0.846	0.560–1.279	0.63	0.42846
	TT	30	15%	45	17%	1.045	0.594–1.837	0.02	0.87865
	CT+TT	137	69%	175	66%	0.890	0.600–1.319	0.34	0.56133
	C	231	58%	308	58%	Ref			
	T	167	42%	220	42%	0.988	0.759–1.286	0.01	0.92866
Polymorphisms	**Alleles**	**Controls**		**Females**		**OR**	**95% CI**	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**82**	**100%**	**24**	**100%**				
	CC	66	80%	19	79%	Ref			
	CT	15	19%	5	21%	1.158	0.373–3.597	0.06	0.79977
	TT	1	1%	0	0%	1.137	0.045–29.030	0.29	0.59220
	CT+TT	16	20%	5	21%	1.086	0.352–3.348	0.02	0.88643
	C	147	90%	43	90%	Ref			
	T	17	10%	5	10%	1.005	0.351–2.883	0.00	1.00036
rs1799946	**Total**	**84**	**100%**	**24**	**100%**				
	CC	27	32%	2	8%	Ref			
	CT	37	44%	15	63%	5.473	1.154–25.956	5.41	**0.02003***
	TT	20	24%	7	29%	4.725	0.885–25.213	3.75	0.05269
	CT+TT	57	68%	22	92%	5.211	1.142–23.781	5.39	**0.02028***
	C	91	54%	19	40%	Ref			
	T	77	46%	29	60%	1.804	0.939–3.467	3.18	0.07468

Table 5 (continued)

Polymorphisms	Alleles	Controls		Males		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs2738047	**Total**	**84**	**100%**	**24**	**100%**				
	CC	80	95%	22	92%	Ref			
	CT	4	5%	2	8%	1.818	0.312–10.586	0.45	0.50054
	TT	0	0%	0	0%	3.578	0.069–185.385	-	1.00000
	CT+ TT	4	5%	2	8%	1.818	0.312–10.586	0.45	0.50054
	C	164	98%	46	96%	Ref			
	T	4	2%	2	4%	1.783	0.316–10.041	0.44	0.78072
rs11362	**Total**	**82**	**100%**	**24**	**100%**				
	CC	30	37%	10	42%	Ref			
	CT	31	38%	13	54%	1.258	0.479–3.303	0.22	0.64079
	TT	21	25%	1	4%	0.143	0.017–1.202	4.07	**0.04368***
	CT+ TT	52	63%	14	58%	0.808	0.319–2.042	0.20	0.65149
	C	91	55%	33	69%	Ref			
	T	73	45%	15	31%	0.567	0.286–1.123	2.69	0.10098

*$P < 0.05$. Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, % = percent, Ref = reference allele, OR: odds ratio

genotype differences of HBD-1 gene SNPs with the duration of CS. As shown in Table 7, the genotype and allele correlations and statistical analyses of those SNPs in both groups of smokers are described and compared with the non-smoking populations. Nevertheless, the genotype frequencies of the four selected SNPs did not correlate significantly with CS in both short-term smokers and long-term smokers in contrast with non-smokers (Table 7). The genotype distributions of rs1047031 SNP in both groups in the controls were as follows: CC 82%, CT 17%, and TT 1%, while with the short-term and long-term smokers, they were distributed as CC 84% and 85%, CT 16% and 14%, and TT 0% and 1%. In rs1799946 SNP, the percentage of genotype distributions of CC, CT, and TT in controls in both groups of years of smoking was 27%, 50%, and 23%, respectively. Among short-term smokers, these ratios were 24%, 55%, and 21%, respectively, whereas, among long-term smokers, they were 29%, 48%, and 23%, respectively. In contrast, the genotype allocations of rs2738047 SNP in both groups for the smoking duration were CC 96%, CT 4%, and TT 0%, in the healthy controls, while in short-term and long-term smokers, they were distributed as CC 95% and 97%, CT 5%, and TT 3% and 0%. Finally, in rs11362 SNP, the genotype distributions of CC, CT, and TT in controls in both groups of years of smoking were represented by 33%, 49%, and 18%, respectively. However, among short-term smokers, these ratios were 32%, 54%, and 14%, respectively, whereas, among long-term smokers, they were allocated as 38%, 43%, and 19%, respectively (Table 7).

Comparison between the effect of CS and shisha smoking on HBD-1 polymorphisms

Finally, we evaluated if the risk associated with CS would be different from another type of smoking, such as the tobacco concoction, shisha (CS vs shisha). All smokers were grouped into two categories: cigarette smokers and shisha smokers, which included 216 and 71 individuals from both groups, respectively. This study showed that the genotype frequency results of rs1047031, rs1799946, and rs11362 in both types of smoking and for rs2738047 in cigarettes did not show any significant correlation with both types of smoking, compared with the controls (Table 8). For rs1047031, the genotype distributions of CC, CT, and TT for non-smokers were 82%, 17%, and 1%, respectively, whereas with the cigarette and shisha smokers, the CC, CT, and TT were 83% and 89%, 16% and 11%, and 1% and 0%, respectively. For rs1799946, the genotype allocations of CC, CT, and TT of non-smokers were 27%, 50%, and 23%, respectively, whereas with the cigarette and shisha smokers, the CC, CT, and TT were 28% and 20% CC, 52% and 52% CT, and 20% and 28% TT, respectively. Also, the HBD-1 rs11362 results did not reveal any significant association in either the category of smoking types when compared with non-smokers. The genotype allocations of CC, CT, and TT of the controls were 33%, 49%, and 18%, respectively; however, the genotype distributions among cigarette smokers were 32%, 51%, and 17%, respectively, while among shisha smokers they were 41%, 46%, and 13%, respectively. In contrast, for HBD-1 SNP rs2738047, the genotype frequencies among smokers showed

Table 6 Comparison of genotype frequencies of *HBD-1* gene polymorphisms in smokers who smoked ≤12 times/day and > 12 times/day

Polymorphisms	Alleles	Controls		≤12 times/day		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**280**	**100%**	**144**	**100%**				
	CC	231	82%	127	88%	Ref			
	CT	47	17%	17	12%	0.658	0.363–1.193	1.92	0.16603
	TT	2	1%	0	0%	0.363	0.017–7.622	1.10	0.29510
	CT+TT	49	18%	17	12%	0.631	0.349–1.141	2.35	0.12559
	C	509	91%	271	94%	Ref			
	T	51	9%	17	6%	0.626	0.355–1.105	2.65	0.10370
rs1799946	**Total**	**283**	**100%**	**146**	**100%**				
	CC	77	27%	34	23%	Ref			
	CT	141	50%	69	47%	1.108	0.675–1.820	0.17	0.68442
	TT	65	23%	43	30%	1.498	0.858–2.617	2.03	0.15470
	CT+TT	206	73%	112	77%	1.231	0.774–1.959	0.77	0.37961
	C	295	52%	137	47%	Ref			
	T	271	48%	155	53%	1.232	0.928–1.634	2.09	0.14871
rs2738047	**Total**	**287**	**100%**	**147**	**100%**				
	CC	276	96%	139	95%	Ref			
	CT	11	4%	8	5%	1.444	0.568–3.672	0.60	0.43801
	TT	0	0%	0	0%	1.982	0.039–100.419	-	1.00000
	CT+TT	11	4%	8	5%	1.444	0.568–3.672	0.60	0.43801
	C	563	98%	286	97%	Ref			
	T	11	2%	8	3%	1.432	0.570–3.599	0.59	0.44318
rs11362	**Total**	**281**	**100%**	**148**	**100%**				
	CC	92	33%	61	41%	Ref			
	CT	138	49%	67	45%	0.732	0.474–1.132	1.97	0.16048
	TT	51	18%	20	14%	0.591	0.321–1.089	2.88	0.08992
	CT+TT	189	67%	87	59%	0.694	0.460–1.048	3.04	0.08147
	C	322	57%	189	64%	Ref			
	T	240	43%	107	36%	0.760	0.568–1.015	3.46	0.06288
Polymorphisms	**Alleles**	**Controls**		**>12 times/day**		**OR**	**95% CI**	**X^2**	**P value**
		N	**%**	**N**	**%**				
rs1047031	**Total**	**280**	**100%**	**138**	**100%**				
	CC	231	82%	112	81%	Ref			
	CT	47	17%	25	18%	1.097	0.642–1.873	0.12	0.73429
	TT	2	1%	1	1%	1.031	0.093–11.494	0.00	0.98004
	CT+TT	49	18%	26	19%	1.094	0.646–1.853	0.11	0.73694
	C	509	91%	249	90%	Ref			
	T	51	9%	27	10%	1.082	0.663–1.767	0.10	0.75218
rs1799946	**Total**	**283**	**100%**	**138**	**100%**				
	CC	77	27%	40	29%	Ref			
	CT	141	50%	78	57%	1.065	0.664–1.707	0.07	0.79385
	TT	65	23%	20	14%	0.592	0.315–1.112	2.68	0.10170
	CT+TT	206	73%	98	71%	0.916	0.583–1.438	0.15	0.70241
	C	295	52%	158	57%	Ref			
	T	271	48%	118	43%	0.813	0.608–1.086	1.96	0.16135
rs2738047	**Total**	**287**	**100%**	**138**	**100%**				
	CC	276	96%	134	97%	Ref			
	CT	11	4%	4	3%	0.749	0.234–2.396	0.24	0.62503

Table 6 (continued)

Polymorphisms	Alleles	Controls		≤12 times/day		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
	TT	0	0%	0	0%	2.056	0.041–104.166	-	1.00000
	CT+TT	11	4%	4	3%	0.749	0.234–2.396	0.24	0.62503
	C	563	98%	272	99%	Ref			
	T	11	2%	4	1%	0.753	0.237–2.385	0.23	0.78468
rs11362	**Total**	**281**	**100%**	**139**	**100%**				
	CC	92	33%	38	27%	Ref			
	CT	138	49%	75	54%	1.316	0.821–2.107	1.31	0.25293
	TT	51	18%	26	19%	1.234	0.674–2.260	0.47	0.49496
	CT+TT	189	67%	101	73%	1.294	0.826–2.026	1.27	0.25980
	C	322	57%	151	54%	Ref			
	T	240	43%	127	46%	1.128	0.845–1.507	0.67	0.41276

Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, %= percent, Ref = reference allele, OR: odds ratio

a significant allocation when compared with controls in the shisha smokers' category. Among the shisha smokers, the heterozygous CT and the combined variants CT/TT showed the same results, with an ~3-fold increase in correlation with CS (OR= 2.788; *P*=0.03448), while in cigarette smokers, no associations were observed with the genotype frequencies for this polymorphism equaled CC, CT, and TT, 32%, 51%, and 17%, respectively, compared to the controls, which were 33%, 49%, and 18%, respectively.

Discussion

The smoke derived from a cigarette is a complex mixture of chemicals, with harmful components such as carbon monoxide (CO), nitrogen oxides, and hydrogen cyanide (HCN) gases along with liquid vapors, including formaldehyde, benzene, acrolein, and some N-nitrosamines. Besides, there are submicron-sized solid particles such as nicotine, polyaromatic hydrocarbons (PAHs), phenol, and specific tobacco-specific nitrosamines (TSNAs) contained in the smoke (2011). Consequently, the chemical complexity and the chemical toxin content make CS highly deleterious, with diverse effects on human health. In particular, HCN is likely to affect the human respiratory system by its toxic effects on the respiratory epithelial and the cilia lining triggering diseases, including lung cancer, breast cancer, colon cancer, cardiovascular, and asthma (Khuder 2001; Di Cello et al. 2013; Kytola et al. 2017; West 2017; Cerveri et al. 2012).

In Saudi population, smoking is a risk factor for cardiac diseases (Al-Sieni et al. 2013), COPD (Al Ghobain et al. 2015), benign oral mucosal lesions (Al-Attas et al. 2014), decrease in lung functions (Milaat and el-Ganai 1998), transmission of COVID-19 infection (Ahmed et al. 2020), and periodontal disease (Natto et al. 2005). Furthermore, smoking affects host innate immunity contributing to structural and functional alterations of respiratory ciliary epithelium and different types of human immune cells (Mehta et al. 2008). Remarkably, CS involves genetic variation leading to changes in gene expression (Kopa and Pawliczak 2018), which contribute to the development of several diseases (Yadav et al. 2017).

Cigarette smoking has been shown to attenuate HBD-1 expression and secretion markedly in the lung epithelium. HBD-1, a member of the critical antimicrobial peptides, has been implicated in local defense of airway homeostasis (Wang et al. 2015). Also, the HBD-1 peptide has been shown to play an essential role in an innate immune response against human microbes, and its dysregulation has been involved in cancer progression (Marzani et al. 2012). The majority of Saudi individuals is non-smokers and is of different ages. The current smoking prevalence is much lower in females than in males; however, cigarette and shish smoking are potential threats and affect all general populations (Bassiony 2009). In this investigation, we aimed to examine the present hypothesis: CS might contribute to nucleotide changes in the HBD-1 gene, which might lead to the initiation of multiple smoking-related diseases (Wang et al. 2015; Slebioda et al. 2021; Loo et al. 2012; Chen et al. 2019) such as chronic periodontitis (Zupin et al. 2017), and chronic obstructive pulmonary disease (Matsushita et al. 2002). Consequently, we aimed to identify the possible associations between HBD-1 polymorphisms rs1047031, rs1799946, rs2738047, and rs11362 and smoking by investigating the polymorphic distributions and effects on different parameters between smokers and non-smokers control among the Saudi population.

Our results indicated that there were no significant differences in genetics distributions of HBD-1 genetic variants

Table 7 Comparison of genotype frequencies of HBD-1 gene polymorphisms in participants who smoked for ≤ 7 years and > 7 years, and the entire non-smoking controls

Polymorphisms	Alleles	Controls		≤ 7 Years		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**280**	**100%**	**170**	**100%**				
	CC	231	82%	143	84%	Ref			
	CT	47	17%	27	16%	0.928	0.553–1.557	0.08	0.77698
	TT	2	1%	0	0%	0.323	0.015–6.769	1.23	0.26662
	CT+ TT	49	18%	27	16%	0.890	0.532–1.488	0.20	0.65699
	C	509	91%	313	92%	Ref			
	T	51	9%	27	8%	0.861	0.529–1.401	0.36	0.54666
rs1799946	**Total**	**283**	**100%**	**172**	**100%**				
	CC	77	27%	41	24%	Ref			
	CT	141	50%	95	55%	1.265	0.799–2.004	1.01	0.31516
	TT	65	23%	36	21%	1.040	0.596–1.814	0.02	0.88968
	CT+ TT	206	73%	131	76%	1.194	0.771–1.850	0.63	0.42626
	C	295	52%	177	51%	Ref			
	T	271	48%	167	49%	1.027	0.785–1.343	0.04	0.84526
rs2738047	**Total**	**287**	**100%**	**173**	**100%**				
	CC	276	96%	164	95%	Ref			
	CT	11	4%	9	5%	1.377	0.559–3.393	0.49	0.48535
	TT	0	0%	0	0%	1.681	0.033–85.113	-	1.00000
	CT+ TT	11	4%	9	5%	1.377	0.559–3.393	0.49	0.48535
	C	563	98%	337	97%	Ref			
	T	11	2%	9	3%	1.367	0.561–3.333	0.48	0.49024
rs11362	**Total**	**281**	**100%**	**173**	**100%**				
	CC	92	33%	55	32%	Ref			
	CT	138	49%	94	54%	1.139	0.745–1.742	0.36	0.54685
	TT	51	18%	24	14%	0.787	0.437–1.419	0.64	0.42543
	CT+ TT	189	67%	118	68%	1.044	0.696–1.567	0.04	0.83389
	C	322	57%	204	59%	Ref			
	T	240	43%	142	41%	0.934	0.712–1.225	0.24	0.62179
Polymorphisms	**Alleles**	**Controls**		**> 7 years**		**OR**	**95% CI**	**X^2**	**P value**
		N	**%**	**N**	**%**				
rs1047031	**Total**	**280**	**100%**	**112**	**100%**				
	CC	231	82%	95	85%	Ref			
	CT	47	17%	16	14%	0.828	0.447–1.532	0.36	0.54686
	TT	2	1%	1	1%	1.216	0.109–13.568	0.03	0.87367
	CT+ TT	49	18%	17	15%	0.844	0.462–1.539	0.31	0.57897
	C	509	91%	206	92%	Ref			
	T	51	9%	18	8%	0.872	0.498–1.529	0.23	0.63239
rs1799946	**Total**	**283**	**100%**	**112**	**100%**				
	CC	77	27%	33	29%	Ref			
	CT	141	50%	53	48%	0.877	0.524–1.469	0.25	0.61806
	TT	65	23%	26	23%	0.933	0.507–1.719	0.05	0.82479
	CT+ TT	206	73%	79	71%	0.895	0.552–1.451	0.20	0.65213
	C	295	52%	119	53%	Ref			

Table 7 (continued)

Polymorphisms	Alleles	Controls		≤ 7 Years		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
	T	271	48%	105	47%	0.960	0.705–1.309	0.06	0.79881
rs2738047	**Total**	**287**	**100%**	**112**	**100%**				
	CC	276	96%	109	97%	Ref			
	CT	11	4%	3	3%	0.691	0.189–2.523	0.32	0.57343
	TT	0	0%	0	0%	2.525	0.050–128.055	-	1.00000
	CT+ TT	11	4%	3	3%	0.691	0.189–2.523	0.32	0.57343
	C	563	98%	221	99%	Ref			
	T	11	2%	3	1%	0.695	0.192–2.514	0.31	0.76938
rs11362	**Total**	**281**	**100%**	**114**	**100%**				
	CC	92	33%	43	38%	Ref			
	CT	138	49%	49	43%	0.760	0.467–1.237	1.23	0.26823
	TT	51	18%	22	19%	0.923	0.498–1.711	0.06	0.79898
	CT+ TT	189	67%	71	62%	0.804	0.511–1.265	0.89	0.34447
	C	322	57%	135	59%	Ref			
	T	240	43%	93	41%	0.924	0.676–1.263	0.24	0.62135

Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, % = percent, Ref = reference allele, OR: odds ratio

rs1047031, rs1799946, rs2738047, and rs11362 in smokers compared to non-smokers, regarding age, years of smoking, and the average amount of either CS or shisha smoking per day, suggesting that CS might not interfere with the allelic difference of HBD-1 in a population. These results are in agreement with another recent study, which showed that CS did not have any effect between smokers and non-smokers in the same population, but rather, a different immunity gene such as TDG in SNP rs4135050 (Almutairi et al. 2019) and IL-7R SNPs rs12516866 and rs1053496 (Semlali et al. 2019). Furthermore, there are no significant differences between the pathologies of the oral cavity and the genotype of rs11362 and rs1799946 (Slebioda et al. 2021). However, significant associations were found between rs11362 and rs1800972 SNPs HBD-1 gene and chronic periodontitis development in North-East Italy (Zupin et al. 2017).

In gender comparison, although the percentage of female smokers was ~8% compared to the 92% of male smokers, it was intriguing to find a significant difference of genotypic distribution of HBD-1 rs1799946 and rs11362 in female smokers, compared with the female non-smokers, suggesting a possible interference of CS in disease development among women, as reported previously that a compromise of HBD-1 SNP activity is associated with poor outcomes of acute respiratory distress syndrome (Feng et al. 2019) and human papillomavirus infection in Brazilian females (Segat et al. 2014), as well as asthma progression among European American females (Levy et al. 2005). Additionally, it has been reported that females are more susceptible to develop colon cancer in contrast to males (Anderson et al. 2011), and this observation is likely to relate to CS, causing HBD-1 polymorphism and dysregulation of its expression (Wang et al. 2015), with the subsequent disease pathogenesis (Wiechula et al. 2010). This effect could be due to the CS effect on sex hormones. Different studies explained that smokers have higher progesterone levels (Duskova et al. 2012), and lower estrogen levels (Gu et al. 2013). Also, a previous study in Saudi pregnant smokers showed that CS could cause fetus damage, as well as different types of DNA damage in both pregnant women smokers and fetuses, which reveals that components from smoking consumption could spread effectively throughout the body (Demarini 2004).

Interestingly, our data showed that shisha usage could cause a difference in the genotype distribution of the HBD-1 polymorphism rs2738047. Despite the limited shisha smokers, we noticed the percentage of CT and CT/TT appearance significantly increased among shisha smokers than cigarette smokers. This could explain that shisha smoking might lead to the development of serious diseases, consistent with a report that showed that smoking of shisha has an association with the incidence of bladder cancer (Goerlitz et al. 2014). Also, a recent study proved the significant effect of waterpipe tobacco smoking on choline acetyltransferase gene polymorphism (Khabour et al. 2020). Therefore, waterpipe tobacco smoking could impact gene variation and result in disease progression (Kudhair et al. 2020).

Table 8 Comparison of genotype frequencies of *HBD-1* gene polymorphisms in smokers who smoked cigarettes and those who smoked shisha

Polymorphisms	Alleles	Controls		Cigarettes		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs1047031	**Total**	**280**	**100%**	**211**	**100%**				
	CC	231	82%	175	83%	Ref			
	CT	47	17%	35	16%	0.983	0.608–1.588	0.00	0.94407
	TT	2	1%	1	1%	0.660	0.059–7.337	0.12	0.73346
	CT+TT	49	18%	36	17%	0.970	0.604–1.556	0.02	0.89886
	C	509	91%	385	91%	Ref			
	T	51	9%	37	9%	0.959	0.616–1.494	0.03	0.85376
rs1799946	**Total**	**283**	**100%**	**213**	**100%**				
	CC	77	27%	60	28%	Ref			
	CT	141	50%	110	52%	1.001	0.658–1.523	0.00	0.99560
	TT	65	23%	43	20%	0.849	0.509–1.417	0.39	0.53085
	CT+TT	206	73%	153	72%	0.953	0.641–1.418	0.06	0.81279
	C	295	52%	230	54%	Ref			
	T	271	48%	196	46%	0.928	0.721–1.193	0.34	0.55907
rs2738047	**Total**	**287**	**100%**	**215**	**100%**				
	CC	276	96%	210	98%	Ref			
	CT	11	4%	5	2%	0.597	0.204–1.746	0.90	0.34148
	TT	0	0%	0	0%	1.314	0.026–66.470	-	1.00000
	CT+TT	11	4%	5	2%	0.597	0.204–1.746	0.90	0.34148
	C	563	98%	425	99%	Ref			
	T	11	2%	5	1%	0.602	0.208–1.746	0.89	0.34541
rs11362	**Total**	**281**	**100%**	**216**	**100%**				
	CC	92	33%	69	32%	Ref			
	CT	138	49%	110	51%	1.063	0.712–1.586	0.09	0.76548
	TT	51	18%	37	17%	0.967	0.572–1.637	0.02	0.90145
	CT+TT	189	67%	147	68%	1.037	0.710–1.515	0.04	0.85094
	C	322	57%	248	57%	Ref			
	T	240	43%	184	43%	0.995	0.772–1.283	0.00	0.97176
Polymorphisms	**Alleles**	**Controls N**	**%**	**Shisha N**	**%**	**OR**	**95% CI**	**X^2**	**P value**
rs1047031	**Total**	**280**	**100%**	**71**	**100%**				
	CC	231	82%	63	89%	Ref			
	CT	47	17%	8	11%	0.624	0.281–1.389	1.35	0.24448
	TT	2	1%	0	0%	0.729	.035–15.381	0.54	0.46059
	CT+TT	49	18%	8	11%	0.599	0.270–1.329	1.62	0.20346
	C	509	91%	134	94%	Ref			
	T	51	9%	8	6%	0.596	0.276–1.286	1.78	0.18274
rs1799946	**Total**	**283**	**100%**	**71**	**100%**				
	CC	77	27%	14	20%	Ref			
	CT	141	50%	37	52%	1.443	0.735–2.834	1.14	0.28489
	TT	65	23%	20	28%	1.692	0.793–3.614	1.87	0.17141
	CT+TT	206	73%	57	80%	1.522	0.802–2.888	1.67	0.19661
	C	295	52%	65	46%	Ref			
	T	271	48%	77	54%	1.290	0.892–1.865	1.83	0.17626

Table 8 (continued)

Polymorphisms	Alleles	Controls		Cigarettes		OR	95% CI	X^2	*P* value
		N	%	*N*	%				
rs2738047	**Total**	**287**	**100%**	**70**	**100%**				
	CC	276	96%	63	90%	Ref			
	CT	11	4%	7	10%	2.788	1.040–7.476	4.47	**0.03448***
	TT	0	0%	0	0%	4.354	0.086–221.534	-	1.00000
	CT+ TT	11	4%	7	10%	2.788	1.040–7.476	4.47	**0.03448***
	C	563	98%	133	95%	Ref			
	T	11	2%	7	5%	2.694	1.025–7.080	4.35	0.06791
rs11362	**Total**	**281**	**100%**	**71**	**100%**				
	CC	92	33%	29	41%	Ref			
	CT	138	49%	33	46%	0.759	0.431–1.334	0.92	0.33656
	TT	51	18%	9	13%	0.560	0.246–1.274	1.94	0.16319
	CT+ TT	189	67%	42	59%	0.705	0.413–1.203	1.65	0.19890
	C	322	57%	91	64%	Ref			
	T	240	43%	51	36%	0.752	0.513–1.101	2.15	0.14214

*$P < 0.05$. Abbreviations: HBD-1 = human beta-defensin-1, *N* = number, % = percent, Ref = reference allele, OR: odds ratio

Finally, this work has many advantages. Firstly, we determined polymorphisms in the HBD-1 gene in three categories of SNP location (3′ UTR, 5′ UTR, and exon variants) in the HBD-1 genome. Secondly, all samples collected were restricted from the same region of Riyadh, not from different regions in Saudi Arabia, and they were well monitored, along with proper storage protocols. However, our study has a limitation, since we were unable to have adequate female smokers as volunteers to participate due to the social traditions in our community.

Conclusion

Our findings showed significant alternation of HBD-1 polymorphisms rs1799946 and rs11362 among females, as well as a high percentage of rs2738047 in shisha smokers. The functional role of the HBD-1 polymorphisms in smoking is still uncertain. Therefore, further investigations, including large study samples, are required to investigate the effects of shisha on human beta-defensin expression and protein levels

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate A written ethical consent was approved by the Research Ethics Committee of the Applied Medical Sciences College at King Saud University (Riyadh, Saudi Arabia) and the reference number of the ethical approval is CAMS 13/3536. Each participant of both smoker and non-smoker groups signed consent of written informed document for participating in the current study. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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