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Association Between Mitochondrial DNA Copy Number and Head and Neck Squamous Cell Carcinoma: A Systematic Review and Dose-Response Meta-Analysis

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Background: The association between mitochondrial DNA (mtDNA) copy number and head and neck squamous cell carcinoma (HNSCC) risk remains unclear. Therefore, we aimed to evaluate the relationship between mtDNA copy number and HNSCC risk.


Material/Methods: We searched PubMed, Web of Science, and EMBASE until August 2020. Studies that assessed the association between mtDNA copy number and HNSCC as the outcome of interest were included. We performed a 2-class and dose-response meta-analysis to assess the association between cancer risk and mtDNA.

Results: Eight articles (2 cohort studies and 6 case-control studies) with a total of 3913 patients were included in our meta-analysis. The overall results showed that mean mtDNA copy number level from 9 studies was 0.71 higher in patients with cancer than in non-cancer controls (the standardized mean differences (SMD) 0.71, 95% CI: 0.28–1.15, $P < 0.001$). However, when 4 studies were pooled by dichotomizing mtDNA copy number at the median value into high- and low-content groups, no significant association between mtDNA content and overall cancer risk was found (odds ratio (OR)=0.87, 95% CI: 0.52–1.44, $P = 0.584$). Furthermore, we observed a non-linear association from 3 studies between increased mtDNA copy number levels (P for nonlinearity < 0.001).

Conclusions: The elevated mtDNA copy number could predict the risk of HNSCC as a biomarker. Moreover, there was non-linear relationship of risk between HNSCC and mtDNA copy number.

MeSH Keywords: **Gene Dosage • Genes, Mitochondrial • Head and Neck Neoplasms • Meta-Analysis • Review**

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Background

Head and neck cancer (HNC) mainly consists of carcinomas arising from mucosal surfaces of 4 major anatomical sites: nasal cavity/paranasal sinuses, oral cavity, pharynx, and larynx [1]. According to the latest cancer statistics in 2018, it ranked 7th among causes of cancer-related death, and there were about 700 000 new cases and 350 000 deaths around the world [2]. Studies have revealed that about 58% of patients with head and neck squamous cell carcinoma (HNSCC) are in the advanced stage of disease (stage III–IV) when diagnosed and treated. Recently, comprehensive treatment techniques including surgery, radiotherapy, and chemotherapy have significantly improved; however, there were still 30–40% of patients with distant metastasis within 5 years [1,3,4]. Therefore, it is of profound significance for the treatment of HNSCC to actively explore tumor biomarkers which can be used for molecular diagnosis, prognosis, and targeted therapy.

Mitochondria is semi-autonomous organelle with independent genetic material, which provides the energy source for organisms [5]. Compared to the nuclear genome, mitochondrial DNA (mtDNA) is prone to damage from reactive oxygen species due to lack of protective histones and introns [6–8]. Additionally, alterations of mtDNA through genetic mutations or increased/decreased mtDNA copy number contribute to multiple diseases, such as cardiovascular diseases and carcinomas [9–13]. Once mtDNA damage occurs, the level of mtDNA copy number is altered, resulting in mitochondrial dysfunction, which is considered to be an essential pathogenesis of cancer [11]. To some extent, the mtDNA copy number can reflect the level of mtDNA damage and be an indication of mitochondrial function [14,15]. Many studies have reported the association between the mtDNA copy number and HNSCC risk in peripheral blood leukocytes or tissues, but these studies have not reached consistent conclusions [16–23]. Consequently, we conducted a meta-analysis to comprehensively evaluate whether changes in the mtDNA copy number are related to HNSCC risk. This study will clarify the value of mtDNA copy number as a biomarker and ultimately provide guidance for screening the high-risk HNSCC population and achieve tertiary prevention.

Material and Methods

This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement [24].

Search strategy and study selection

A comprehensive search of the literature was carried out in PubMed, Web of Science, and EMBASE databases published up

to August 2020. The search terms were: “head and neck cancer” (e.g., “head and neck squamous cell carcinoma”, “HNSCC”, “laryngeal squamous cell carcinoma (LSCC)”, “squamous cell carcinoma of the tongue (SCCT)”, “oral squamous cell carcinoma (OSCC)”, “hypopharyngeal squamous cell carcinoma”); “mitochondrial DNA” or “mtDNA”; and “copy number” or “content”. We also searched the reference lists of extracted documents. The flow diagram detailing the database searches and included publications is illustrated in **Figure 1**.

Published studies were included in our analysis if they met the following criteria: 1) case-control or cohort observational studies conducted on human adults; 2) studies that assessed the association between mtDNA copy number and HNSCC as the outcome of interest; 3) studies that reported the level or distribution of mtDNA copy number in both cancer cases and controls; and 4) studies that reported odds ratio (OR) with the corresponding 95% confidence intervals (95% CI) or other available data for calculating the OR and 95% CI. The exclusion criteria were: 1) letters, editorials, case reports, reviews, meta-analyses, and ecological studies; 2) non-English studies; 3) the mtDNA copy number was used to assess other cancers or without a control population. In case of duplications, the publication with the most complete information available was included in this study.

Data extraction and risk of bias assessment

Two reviewers (ZZ and YL) independently screened included publications and discrepancies were resolved by consensus or the third reviewer (HW). The following details were extracted: the name of the first author, publication year, cancer type, location of study, study design, source of sample, method used for measurement of mtDNA copy number, sample size, mtDNA level copies, mtDNA gene, nuclear DNA (nDNA) gene, age, sex, smoking, and case/control patients by mtDNA copy number level categorization.

The Newcastle-Ottawa Scale (NOS), which is commonly used to assess the quality of selection, comparability, and outcome/exposure of study participants, was used to evaluate included studies. The maximum score is 9 stars [25]. Discrepancies generated from NOS assessment by 2 independent researchers were resolved by consensus.

Statistical analysis

In 2-class meta-analysis, the mean and standard deviation (SD) of mtDNA copy number level was used to estimate the effect size in the HNSCC case group and control group. If not provided, the mean and SD of the mtDNA copy number were evaluated based on median, first quartile, third quartile, and sample size [26,27]. In addition, the pooled risk was compared

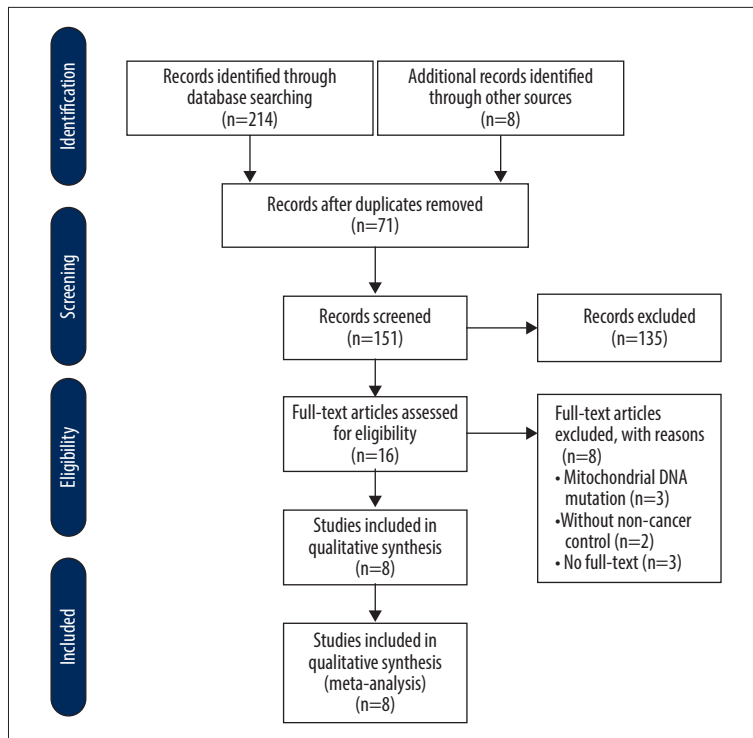


Figure 1. The review flow diagram for identification of study screening and selection.

between high versus low median mtDNA copy number level. If studies reported the association between 3 or more mtDNA copy number categories and HNSCC risk, we identified the possible non-linear association through a 2-stage, random-effects, dose-response meta-analysis. We also conducted through modeling of mtDNA copy number level and restricted cubic splines with 3 knots.

Heterogeneity between studies was analyzed using the I^2 , where an I^2 greater than 50 indicates substantial heterogeneity [28,29]. A random-effects model was performed if a fixed random-effects model was unavailable [28]. When possible, subgroup analysis was used to investigate potential sources of heterogeneity. Furthermore, publication bias was examined by using funnel plots and Egger's and Begg's test.

The data were collected and integrated in Excel 2016 (Microsoft Office, Redmond, USA). All statistical analysis was conducted using STATA version 15 (StataCorp LLC, College Station, TX, USA), and a statistical significance was set at P value <0.05 .

Results

According to corresponding search terms and strategies, we applied this literature search in PubMed, Web of Science, and EMBASE, and identified 92, 28, and 94 studies, respectively. For 16 additional articles included from relevant references, a total of 222 references were identified and screened for

eligibility. Of these, 71 articles were excluded for duplications. After screening the title and abstract, 135 articles were further excluded. After reading the full texts, we excluded 3 for mtDNA mutation, 2 for lack of without non-cancer control, and 3 for no full text. Finally, only 8 articles on 9 studies were included for quantitative synthesis (Figure 1).

Characteristics of studies and patients

Table 1 and Supplementary Table 1 summarized the main characteristics of the patients and studies included in this comprehensive analysis. Among the 8 included articles, 3 studies were from the USA, 2 were from India, and 3 were from China. DNA was extracted from peripheral blood lymphocytes (PBL), tissues, or saliva, then quantitative reverse transcription PCR amplification was performed for the mtDNA gene and the nuclear gene. All studies were observational, including 6 case-control studies and 2 cohort studies. A total of 3913 patients (1288 cancer cases and 2625 non-cancer controls) were included in the studies. In the case group, 73.3% (934/1274) were male, 57.1% (727/1274) were ever-smoking, and the mean age was 59.6 years. Except for Kim 2004, all studies were considered high-quality (NOS score ≥ 6). Out of 8 eligible articles, 1 paper (Jiang 2005) evaluated 2 mtDNA genes and were analyzed as 2 studies for level of mtDNA copy number.

Table 1. The baseline characteristics of included studies.

Author	Year	Cancer type	Region	Study Design	Samples	Method for mtDNA
Kim	2004	Head and neck squamous cell carcinoma	America	Case-control	Saliva	qRT-PCR
Jiang	2005	Head and neck squamous cell carcinoma	America	Case-control	Saliva	qRT-PCR
Mondal	2013	Oral squamous cell carcinoma	India	Case-control	Tissues	qRT-PCR
Cheau-Feng Lin	2014	Oral/oropharyngeal/hypopharyngeal/nasopharyngeal/laryngeal/nasal cancer	Taiwan, China	Retrospective cohort	PBL	qRT-PCR
Dang	2014	Laryngeal cancer	Xi'an, China	Cohort	Tissues	qRT-PCR
Ghosh	2014	Nasopharyngeal carcinoma	India	Case-control	PBL	qRT-PCR
He	2014	Oral premalignant lesions	America	Case-control	PBL	qRT-PCR
Wang	2018	Oral cavity/Oropharynx/Larynx/nasal sinuses/parotid/salivary gland	Nanjing, China	Case-control	PBL	qRT-PCR

Author	N of patients		mtDNA copy number level		mtDNA gene	nDNA gene	NOS score
	Case/control	Case	Case	Control			
Kim	14/655	0.0773 (0.0307, 0.2693)*	0.0257 (0.0134, 0.0584)*	Cox I	β-actin	4	
Jiang	94/656	0.076**	0.054**	Cox I	β-actin	6	
		0.055**	0.046**	Cox II	β-actin		
Mondal	124/140	0.22*	0.89*	D-loop	GAPDH	6	
Cheau-Feng Lin	75/80	8.22 (4.41, 17.55)*	4.84*	tRNA-leu	18s	6	
Dang	204/40	11.91±4.35**	4.72±0.70**	MT-ND1	β-actin	6	
Ghosh	64/100	1.98*	4.11*	D-loop	GAPDH	6	
He	143/357	1.36±0.74**	1.11±0.32**	MT-ND1	HGB	7	
Wang	570/597	4.33 (1.38, 29.85)*	3.5 (1.93, 11.22)*	MT-ND1	HGB	8	

* Median (first quartile, third quartile) mtDNA copy number level; ** mean±sd mtDNA copy number level. N – number; NOS – Newcastle-Ottawa Quality Assessment Scale; PBL – peripheral blood lymphocytes; qRT-PCR – real-time quantitative PCR; Cox – cytochrome c oxidase.

Meta-analysis of the average level of mtDNA copy number

The first 2-class meta-analysis was performed for comparison of the mtDNA copy number level. In **Figure 2**, we pooled data from 9 studies to demonstrate the mtDNA copy number level between HNSCC cases and the control group [16–23]. There was a significantly higher level of mtDNA copy number among HNSCC cases (SMD=0.71, 95% CI: 0.28–1.15, $P<0.001$) based on the random-effects model and a substantial level of heterogeneity was observed ($I^2=96.8\%$, $P<0.001$).

Funnel plots are shown in **Supplementary Figure 1**. An unexpected shape was observed, indicating evidence of significant bias. However, there was no evidence of bias in the Egger’s

test and Begg’s test results ($P=0.227$ and 0.348 , respectively). Results of subgrouping are presented in **Table 2**. There was a low level of heterogeneity in subgrouping according to region, mtDNA gene, and NOS score.

Meta-Analysis of mtDNA copy number level and HNSCC risks

We conducted the second 2-class meta-analysis to assess the potential association between mtDNA content level and HNSCC. When 4 studies were pooled by dichotomizing mtDNA copy number at the median value into high- and low-content groups, including a total of 2047 participants and 404 with high contents in the case group, no significant association between

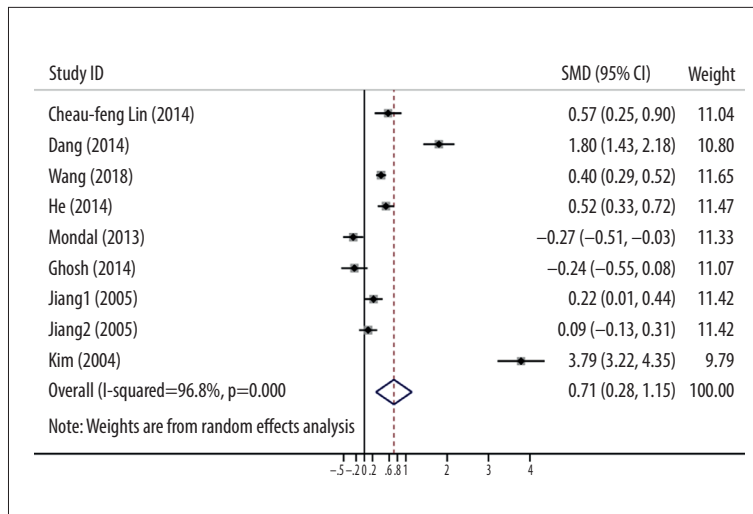


Figure 2. Forest plots of SMD with 95% CI for the levels of mtDNA copy number in head and neck squamous cell carcinoma (HNSCC) cases versus controls.

Table 2. Subgroup analyses for the two class meta-analysis.

Studies	N. of Studies	SMD (95% CI)	Heterogeneity (I ² ; P value)	N. of studies	OR (95% CI)	Heterogeneity (I ² ; P value)
All studies	9	0.71 (0.28–1.15)	96.8%; <0.001	4	0.87 (0.52–1.44)	82.5%; 0.001
Study design						
Cohort	2	1.18 (-0.02,2.38)	95.8%; <0.001	–		
Case-control	7	0.58 (0.11,1.04)	96.8%; <0.001	4	0.87 (0.52–1.44)	82.5%; 0.001
Sample						
PBL	4	0.33 (0.06–0.60)	83.7%; <0.001	3	1.03 (0.61–1.73)	79.9%; 0.007
Tissues	2	0.76 (-1.27,2.79)	98.8%; <0.001	1	0.52 (0.30–0.87)	–
Saliva	3	1.33 (-0.06,2.72)	98.6%; <0.001	–		
Region						
China	3	0.91 (0.16–1.66)	95.9%; <0.001	1	1.06 (0.84–1.34)	–
India	2	-0.26 (-0.45 – -0.07)	0%; 0.884	2	0.51 (0.34–0.77)	0%; 0.938
America	4	1.10 (0.23,1.97)	98.0%; <0.001	1	1.69 (1.14–2.51)	–
mtDNA gene						
ND1	3	0.88 (0.30–1.46)	95.9%; <0.001	2	1.30 (0.83–2.05)	74.9%; 0.046
D-loop	2	-0.26 (-0.45 – -0.07)	0%; 0.884	2	0.51 (0.34–0.77)	0%; 0.938
Cox I/Cox II	3	1.33 (-0.06–2.72)	98.6%; <0.001	–		
tRNA-leu	1	0.57 (0.25–0.90)	–	–		
NOS score						
<6	1	3.79 (3.22–4.35)	–	–		
6	6	0.35 (-0.13–0.84)	94.9%; <0.001	2	0.51 (0.34–0.77)	0%; 0.938
>6	2	0.44 (0.33–0.54)	2.4%; 0.311	2	1.30 (0.83–2.05)	74.9%; 0.046

N – number; SMD – standardized mean differences; OR – odds ratio; CI – confidence interval; PBL – peripheral blood lymphocytes; Cox – cytochrome c oxidase; NOS – Newcastle-Ottawa Quality Assessment Scale.

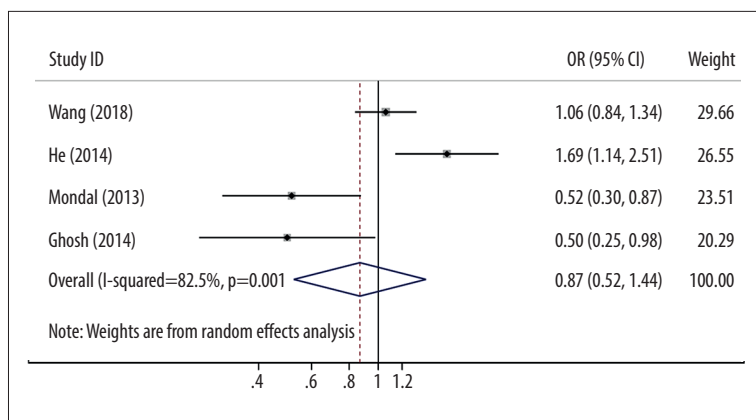


Figure 3. Forest plot presenting OR for head and neck squamous cell carcinoma (HNSCC) in the high versus the low category of mtDNA copy number by dichotomizing at the median value.

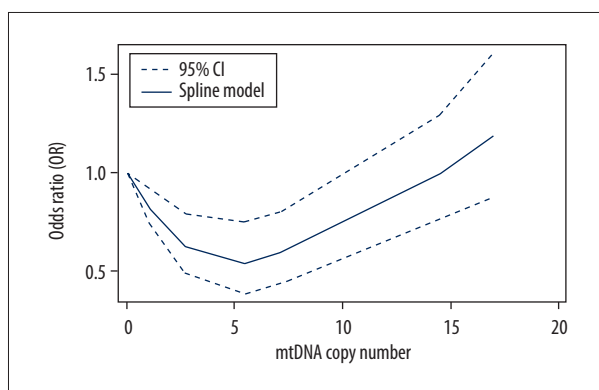


Figure 4. Dose-response association between the mtDNA copy number and head and neck squamous cell carcinoma (HNSCC) risk. mtDNA copy number was modeled with restricted cubic splines in a random-effects dose-response model. Solid line indicates the spline model; dashed lines represent 95% confidence intervals.

mtDNA content and overall cancer risk was found (pooled effect size comparing high and low contents; OR=0.87, 95% CI: 0.52–1.44, $P=0.584$) with substantial heterogeneity ($I^2=82.5%$, $P=0.001$) (Figure 3) [18–20,23]. Similarly, funnel plots appeared symmetrical, indicating no overt evidence of significant bias (all $P>0.50$) (Supplementary Figure 1). Furthermore, subgroup analysis showed a low level of heterogeneity in subgrouping according to region, mtDNA gene, and NOS score (Table 2).

Linear and non-linear dose-response analysis

Three of 8 publications on the association between mtDNA copy number level and HNSCC risk were included for dose-response meta-analysis [19,20,23]. We found a significant non-linear association ($P<0.001$ for nonlinearity; Figure 4). As shown in Figure 4, there was a reduction in cancer risk between the level of 0-5 mtDNA and after that there was an increase in risk of cancer.

Discussion

We assessed available data from 8 observational studies that reported the association between mtDNA copy number level and HNSCC risk in adults. We found a significant association between higher mtDNA copy number and higher risk of cancer. In addition, there was non-linear relationship of risk between HNSCC and mtDNA copy number.

In the present meta-analysis, we performed 3 pooled evaluations: 1) to evaluate the mtDNA copy number level between HNSCC case and control groups; 2) to evaluate the risk of HNSCC for cases in the high versus the low category of mtDNA copy number by dichotomizing at the median value; and 3) to evaluate the dose-response association between mtDNA copy number and the risk of HNSCC. In the first 2-class meta-analysis, the pooled SMD was 0.71 (95% CI: 0.28–1.15), indicating that the mtDNA copy number was higher in HNSCC patients than in the controls. There was substantial heterogeneity across studies ($I^2=96.8%$, $P<0.001$), and we conducted subgroup analysis to investigate the potential sources. The results showed that region and mtDNA gene explained this heterogeneity. Human mtDNA is a matrilineal genetic genome with a circular double-stranded loop and encodes 37 genes: 13 for tRNAs, 22 for rRNAs, and 2 for polypeptides of the respiratory chain [30]. It also has a non-coding region, the D-loop, which accounts for about 6% and regulates the transcription and replication of mtDNA [31]. Moreover, the mitochondrial D-loop is where mutations occur most frequently, and it is also part of the human mitochondrial genome [25]. A growing body of evidence suggests that mitochondrial functional defects can lead to the occurrence and development of cancer [32–35]. To some extent, mtDNA mutation and copy number alteration could reflect mitochondrial functional defects, so they could be used as molecular markers for tumor screening and detection [35]. Surprisingly, region and mtDNA gene groups were included in the same article.

Adjusted for potential confounding covariance, He et al. [18], using the median mtDNA copy number as a cutoff, found that individuals with a high mtDNA copy number had significantly higher risk of having oral premalignant lesions than those with a low mtDNA copy number. Wang et al. [19] found a U-shaped association between the mtDNA copy number and HNSCC risk. Interestingly, there was no significant association between mtDNA content and overall HNSCC risk in our second 2-class meta-analysis.

It seems that the possible reason for these differences was the dose-dependent association between mtDNA copy number level and HNSCC risk. The present study found that the relationship between mtDNA copy number level and the risk of HNSCC is a non-linear association (P for nonlinearity <0.001) and there is a reduction in cancer risk between the level of 0–5 mtDNA, and after that there is an increase in risk of cancer. It further highlights the dose-dependent relationship between mtDNA copy number and risk factors. Moreover, patients with HNSCC had 0.71 higher mtDNA copy number compared with non-cancer controls. Our findings provide an explanation for the prior conflicting evidence on mtDNA copy number levels in HNSCC.

Although previous studies have demonstrated an association between mtDNA copy number and HNSCC risk, to the best of our knowledge, our systematic review is the first comprehensive quantitative analysis from 3 pooled evaluations [14,15,36]. One of the studies aimed to interpret the relationship between mtDNA copy number and cancer prognosis [15], and others aimed to illustrate the association between mtDNA copy number and cancer risk [14,36]. These 3 studies did not take into account the relationship between dose-dependent mtDNA copy number and HNSCC.

The present study has certain limitations that should be considered. First, we only included a small number of studies, only 3 of 8 publications performing dose-response meta-analysis were included, and our comprehensive analysis could not assess the potential temporal association between mtDNA copy number and HNSCC risk. Second, pathogenic factors for HNSCC include alcohol and tobacco consumption, as well as human papilloma virus infection. However, lack of sufficient information limited our further evaluation of behavioral and clinicopathological characteristics of cancer by mtDNA copy number. In addition, not all studies provided mean and SD for evaluating the mtDNA copy number level between HNSCC case and control groups, and this led a possible bias risk, which limits interpretation of the results. Furthermore, all of the included studies were observational research, which limited the causal inference. Further long-term prospective studies should be performed for determining the mtDNA copy number as a biomarker for predicting HNSCC.

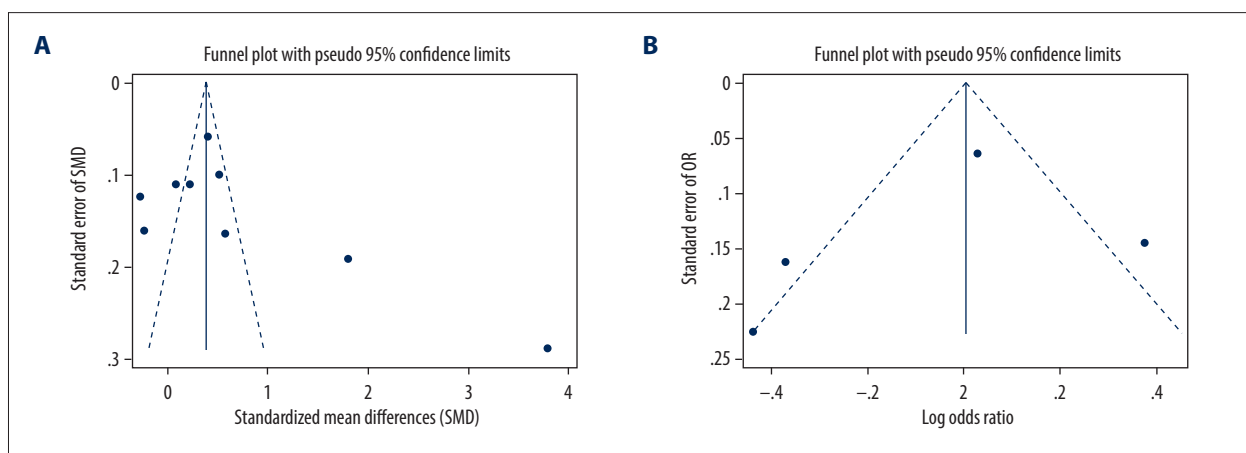
Conclusions

In conclusion, the elevated mtDNA copy number could predict the risk of HNSCC as a biomarker. Moreover, there was a non-linear relation of risk between HNSCC and mtDNA copy number.

Conflict of interest

None.

Supplementary Data



Supplementary Figure 1. Funnel plot (A) of studies reporting the level of mtDNA copy number with cancer or non-cancer; (B) of studies reporting the risk of cancer among the mtDNA copy number higher than median level.

Supplementary Table 1. Supplementary information of included studies.

Study	Age (years)	Gender (% Male)	Smoking (% ever)	Drinking (% ever)	Categorization	Number	
	Case/control	Case/control	Case/control	Case/control		Case	Control
Kim 2004	62.4/38.8	–	–	–	No		
Jiang 2005	56.8/61.8	71 (75.5) /413 (63.3)	52 (79.1) /405 (62.0)	–	No		
Mondal 2013	58/56	98 (79.0) /101 (72.1)	89 (71.7) /75 (53.4)	–	By quartile		
					≤0.1	58	43
					>0.1–1	35	42
					>1–10	19	29
					>10	12	26
Cheau-Feng Lin 2014	56±11.9/–	75 (100)/–	61 (81.3)/–	50 (66.7)/–	No		
Dang 2014	60.75±9.64/–	197 (96.6)/–	141 (69.1)/–	–	No		
Ghosh 2014	–	49 (76.6) /79 (79.0)	43 (67.2) /27 (27.0)	44 (68.8) /49 (49.0)	By quartile		
					≤0.2	31	29
					>0.2–2	15	27
					>2–12	11	25
					>12	7	19
He 2014	57.3±12.4 /58.5±11.4	87 (60.8) /215 (60.2)	83 (58.0) /175 (49.0)	85 (59.4) /108 (30.3)	By median		
					<1.08	56	186
					≥1.08	87	171
Wang 2018	61.07±10.88 /59.62±9.35	357 (62.6) /374 (62.7)	258 (45.3) /253 (42.4)	252 (44.2) /192 (32.2)	By quartile		
					<1.93	165	150
					1.93–3.50	89	149
					3.50–11.22	119	149
					>11.22	197	149

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