METHYLATION OF *ECADHERIN GENE* IS CORRELATED WITH INCREASED RISK OF NASOPHARYNGEAL CARCINOMA: A META-ANALYSIS

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ABSTRACT

Background: The objective of this study was to estimate the correlation between the E-cadherin (*CDH1*) promoter methylation and the risk of nasopharyngeal cancer.

Methods: Based on previous online articles for the evaluation the hypermethylated status of *CDH1* gene at the promoter region with nasopharyngeal carcinoma, two independent reviewers selected studies through databases on PubMed, Google Scholar from 2001 to 2014. The softwared MedCalc® version 18.11 was applied for calculating pooled odd ratios (OR) with levels of data heterogeneity by the fixed and random effects models.

Results: Of a total of 99 articles, 12 studies with 508 clinical samples of nasopharyngeal carcinoma patients and 282 normal samples were selected in the systematic review for meta-analysis. Overall, the results demonstrated the highly significant association between *CDH1* promoter methylation with nasopharyngeal carcinoma under the fixed effects model (OR = 16.155, 95% CI: 8.533 - 30.585, p<0.001). The further subgroup analysis was conducted on types of samples, methods for detecting *CDH1* methylation and patient ethnicity. In particularly, the results indicated the frequency of *CDH1* promoter methylation was significant higher in nasopharyngeal cancer samples than normal samples in Asia (OR = 15.879; 95% CI: 7.28 - 34.608, p<0.001), Africa (OR = 10.667; 95% CI: 1.214 - 93.719, p<0.001) and America (OR = 3.9362; 95% CI: 0.1779 - 87.107, p>0.001).

Conclusion: This study proposed the strong association between *CDH1* promoter methylation and the risk of nasopharyngeal carcinoma in Asia and other populations. For this reason, the abnormal methylation in *CDH1* gene should be a potential hallmark of prognosis and diagnosis for nasopharyngeal carcinoma.

Keywords: DNA methylation; *Ecadherin* gene; Meta – analysis; Nasopharyngeal carcinoma.

1. Introduction

There is generally well known that nasopharyngeal carcinoma (NPC) is one of Epstein-Bar virus associated cancer (Lung *et al.*, 2014; Nasopharyngeal cancer statistics) and a head and neck cancer rare (Chou *et al.*, 2008; Lung *et al.*, 2014; Nasopharyngeal cancer statistics) which has the geographical distribution. The early cases of NPC were reported by Jackson (1901). According to WHO estimates for 2018, nasopharyngeal cancer as nasopharyngeal carcinoma (NPC) is

the 24th most common cancer worldwide. There were about 129,079 new cases and 72,987 deaths from NPC in 2018. Globally, age-standardized incidence and mortality rates were over 1 case per 100,000 men, in contrast less than 1 case per 100,000 women. NPC, although NPC is a rare malignancy in the world, was remarkable in some endemic areas of Southern China, Southeast Asia, North Africa and the Arctic. The highest incidence of NPC presented in five countries of Asia, including Malaysia, Singapore, Indonesia, Vietnam and Brunei, in 2012, China, Indonesia, Vietnam, India and Malaysia in 2018, respectively. NPC is the 6th most common cancer in Vietnam with 6212 new cases and 4232 deaths, respectively.

Das *et al*., Luczak *et al*. and Kulis & Esteller indicated DNA methylation is an epigenetic mechanism that is categorized into hypermethylation and hypomethylation of tumor suppressor genes (TSG) or proto-oncogenes. In addition, recent studies demonstrated a lot of evidence that DNA methylation profiles associated with risk of cancer diseases, typically of nasopharyngeal carcinoma. Besides eating habit with large amounts of salt-preserved fish and meats, environmental exposures as dust and smoke, family history, EBV infection and genetic factors, especially in as DNA methylation plays the critical roles in the carcinogenesis of NPC.

Among the tumor suppressor genes, E-cadherin gene (CDH1) is located in a gene cluster of the cadherin family on chromosome 16 (16p22.1) and is composed 16 exons. This gene encodes epithelial cadherin, a transmembrane glycoprotein that activates in the progress of cell adhesion, cell signal transduction, cell maturation and tissue organization. CDH1 gene was expressed mostly in the surfaces and cavities of human body.

Up to dates, numerous studies demonstrated the significant correlation between the methylated status of *CDH1* gene and the risk of developing nasopharyngeal cancer, however, the methylated frequencies of *CDH1* gene promoter hypermethylation were significantly distinguishable. Particularly, the range of those values is estimated from 11% to 65%. All of those conclusions and controversies depend on a variety of clinical characteristics of samples and molecular methods for identifying or quantifying DNA methylation in nasopharyngeal carcinoma. Therefore, this study was conducted meta-analysis for evaluating in detail about the correlation between *CDH1* methylation and nasopharyngeal cancer progress.

2. Materials and Method

A systematic review was conducted by searching the primary research studies from PubMed, Google Scholar database, up to March 1, 2019. The search strategy based on the various combinations of critical keywords: "*CDH1";* "DNA methylation", "nasopharyngeal carcinoma" etc.

The selection of published studies were performed by two independent reviewers with the inclusion and exclusion criteria. The inclusive data included (1) case control studies estimated the frequencies of *CDH1* methylation in nasopharyngeal carcinoma and control samples; (2) the correctly method for *CDH1* methylation screening; (3) types of clinical samples includes tissues as NPC primary tumor biopsies and blood; (4) English publications. The exclusion data consisted of (1) cohort or review studies; (2) studies could not be calculated the original frequencies of *CDH1* methylation; (3) unpublished and incomplete studies. Figure 1 illustrated a flow chart of the process for selecting studies.

Data extraction criteria described types of studies (case control or cohort), methods for detecting *CDH1* methylation, patient ethnicity, the frequencies of *CDH1* methylation and some clinicopathological characteristics of nasopharyngeal cancer.

The user-friendly, fast and reliable software as MedCalc® version 18.11 was applied for the statistical analysis as Cochran's Q $\chi 2$ statistic for calculating the Odd ratios (OR) with 95% confidential intervals (CIs) in the fixed effects model (F) or the random effects model (R). In order to identify the suitable models, the I^2 statistic test was performed to quantify the heterogeneity of data. The higher I^2 values expressed the greater heterogeneity in the ranges from 50% to 100%, so the best model was the random effects model and vice versa (Cochrane handbook for systematic reviews of interventions).

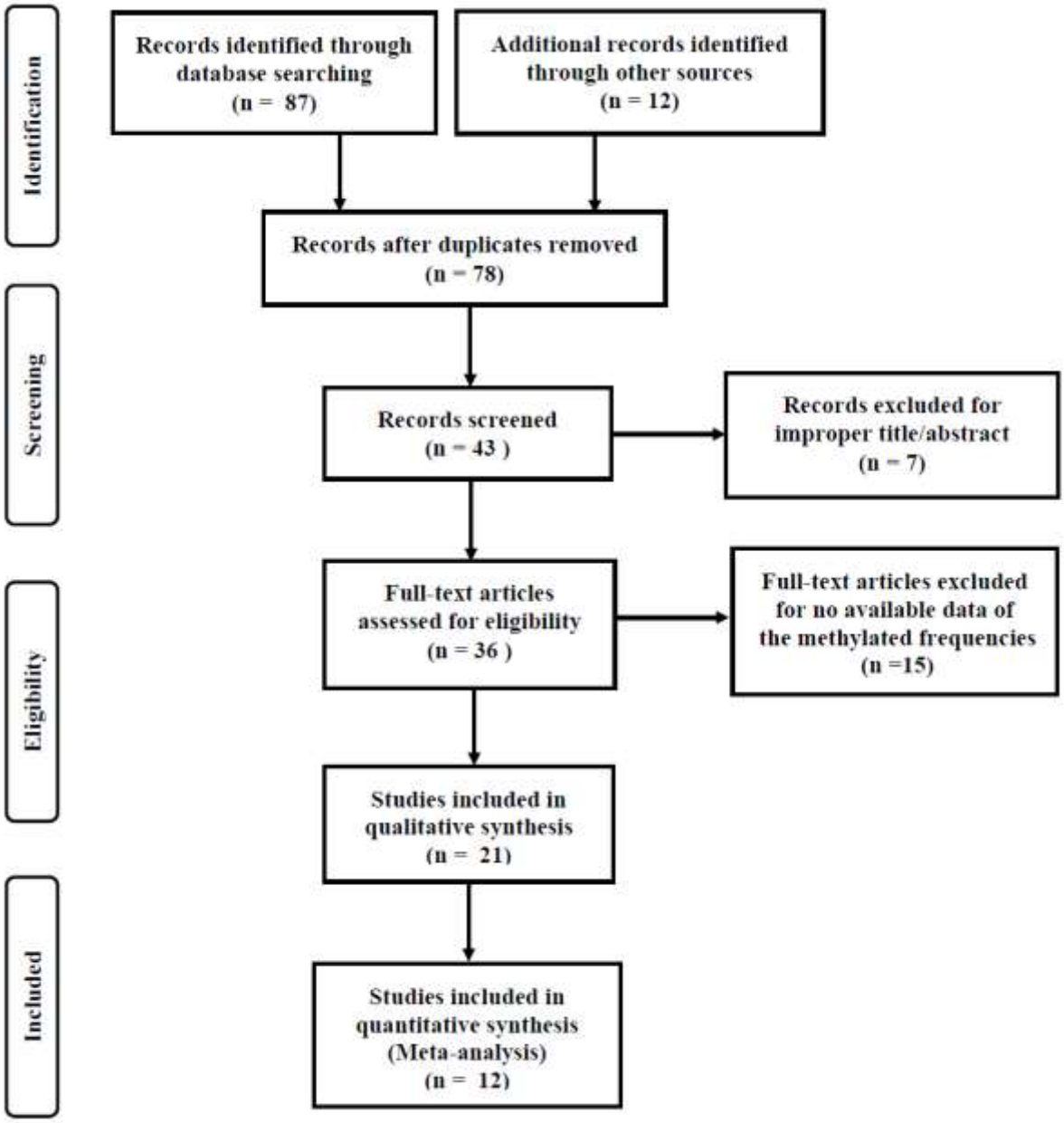

Figure 1. The flow chart of systematic review process

3. Results

Study characteristics

As shown in Figure 1 and Table 1, a total of 12 case control studies, including 508 nasopharyngeal carcinoma samples and 282 normal samples were evaluated the status of *CDH1* methylation. The subjects were conducted in three populations in Asia, Africa and America from 2001 to 2015. The number of studies analyzed the status of CDH1 methylation in tissues samples as tumor biopsies, blood samples and others (MT: Mouth and throat rinsing fluid; BC: Buffy coat) were 11, 2 and 1, respectively (Table 1).

Table 1

The eligibilily studies in systematic review for the correlation between *CDH1* methylation and NPC risk

Studies (First author, Years)	Nation	Ethnicity	Method	Sample Types	Case		Control	
					Events	Total	Events	Total
Asokan, 2014	India	Asian	MSP	Tissue	6	10	0	5
Challouf, 2012	Tunisia	African	MSP	Tissue	4	36	0	19
Ran, 2011	China	Asian	MSP	Tissue	13	20	0	5
Niemhom, 2008	Japan	Asian	MSP	Tissue	28	66	0	15
Ayadi, 2008	Tunisia	African	MSP	Tissue	35	44	0	3
Sun, 2006	China	Asian	MSP	Tissue	52	58	0	10
Wong, 2004	China	Asian	QMSP	Tissue	19	41	4	43
Wong, 2003	China	Asian	MSP	Blood	15	30	0	5
Tsao, 2003	China	Asian	MSP	Tissue	15	29	1	15
Chang, 2003	China	Asian	MSP	Tissue	16	30	0	6
Chang, 2003	China	Asian	MSP	Blood	2	30	0	43
Chang, 2003	China	Asian	MSP	(MT)	8	30	0	37
Chang, 2003	China	Asian	MSP	(BC)	13	30	0	43
Corn, 2001	USA	American	NMSP	Tissue	26	31	6	31
Nakayama, 2001	USA	American	MSP	Tissue	18	23	0	2

Note: (MT): Mouth and throat rinsing fluid; (BC): Buffy coat;

CDH1 methylation and NPC risk

The key results for Chi-square statistic and heterogeneity test are illustrated in Figure 2 and Figure 3. With the exclusion of the same article of Chang *et al.*[6], a pooled odds ratio (OR) as the overall index was calculated at 16.155 (OR = 16.155, 95% CI: 8.533 – 30.585, $p<0.001$) in the fixed effects model, in which the number of clinical samples of NPC and normal samples were 418 and 159, respectively. It is clearly shown that the frequency of *CDH1* methylation in NPC samples was twelve times higher than in controls, in other words, the increasing of NPC risk was associated with *CDH1* hypermethylation.

Study (First author, Years)	Nasopharyngeal cancer		Non-cancer		Weight (%)	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total		
Asokan, 2014	6	10	0	5	4.09	15.89 [0.691-365.156]
Challouf, 2012	4	36	0	19	4.54	5.4 [0.276-105.810]
Ran, 2011	13	20	0	5	4.37	19.8 [0.9-409.639]
Niemhom, 2008	28	66	0	15	4.92	22.948 [1.317-399.729]
Ayadi, 2008	35	44	0	3	4.32	26.158 [1.241-551.509]
Sun, 2006	52	58	0	10	27.97	169.615 [8.861-3246.575]
Wong, 2004	19	41	4	43	4.52	8.42 [2.541-27.907]
Wong, 2003	15	30	0	5	8.64	11 [0.559-216.456]
Tsao, 2003	15	29	1	15	4.58	15 [1.738-129.493]
Chang, 2003	16	30	0	6	4.61	14.793 [0.765-285.978]
Paul, 2001	26	31	6	31	23.48	21.667 [5.860-80.114]
Nakayama, 2001	18	23	0	2	3.97	16.818 [0.698-405.286]
Total (95% CI)		**418**		**159**	**100**	**16.155 [8.533-30.585]**
Total events	**247**		**11**			
Heterogeneity: Chi^2=4.5321; df= 11 (P = 0.9517); I^2= 0.00%						
Test for overall effect: Z=8.543 (P<0.001)						

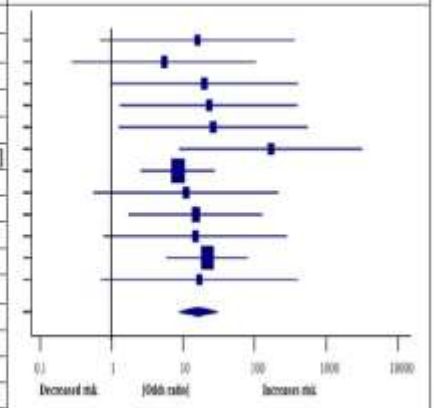

Figure 2. Forest plot of the correlation between *CDH1* methylation and NPC risk

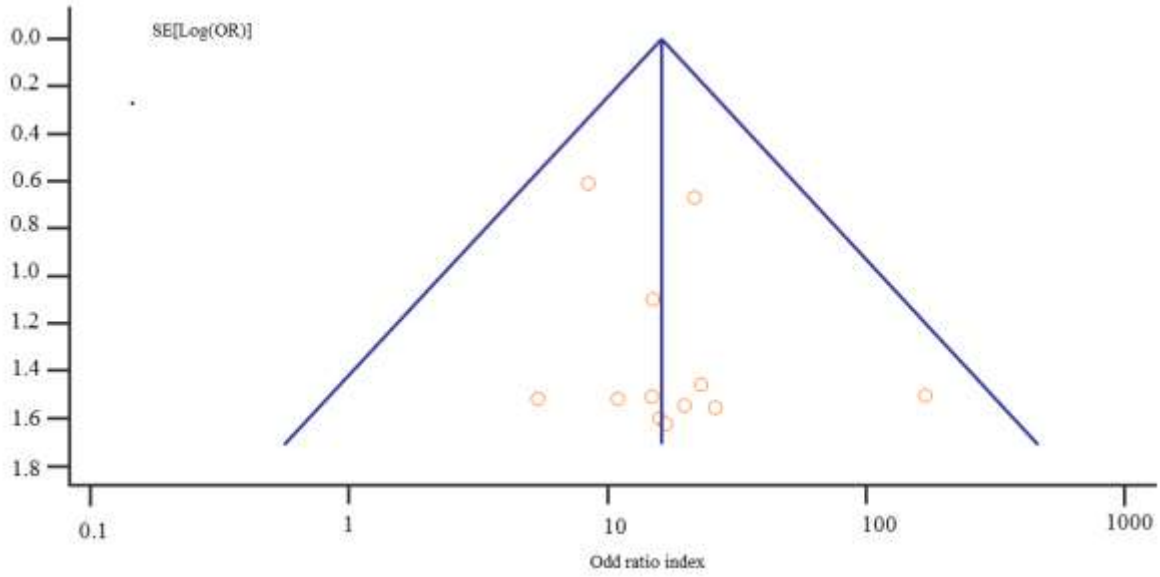

Figure 3. Funnel plot for the evaluating publication bias for the correlation between *CDH1* methylation and NPC risk

Note: The standard error of log [OR] of each study was plotted against its log [OR]

For investigating the publication bias of studies included in this meta-analysis, the funnel plot was performed (Figure 3), but the publication bias test was not carried out by Begg's funnel plot and Egger's test. The results indicated the publication bias could present because of the asymmetrical funnel plot (Figure 3). Additionally, the inconsistency results due to the specific data which were mentioned in the materials and method section and Figure 2. Consequently, subgroup analyses were carried out for further investigation in the relation between *CDH1* methylation and those critical categories, including *CDH1* methylation analysis methods, the population of NPC patients some histopathological characteristics of NPC. The results are shown in Table 2.

Table 2

Overall and subgroup analyses for the correlation between *CDH1* methylation and NPC risk in case-control studies

		Test of associations			Test of heterogeneity		
Variables	N	OR (95%CI)	Z	P value	Mode	P_H	I^2(%)
Total	**12**	**16.155 [8.533 - 30.585]**	**8.543**	**<0.001**	**F**	**0.9517**	**0**
Methods							
MSP	**10**	**19.258 [7.742 - 47.904]**	**6.362**	**<0.001**	**F**	**0.9610**	**0**
Other	2	12.606 [5.204 - 30.535]	5.614	<0.001	F	0.2954	8.65
Ethnicity							
Asian	**8**	**15.879 [7.286 – 34.608]**	**6.956**	**<0.001**	**F**	**0.8139**	**0**
African	2	10.667 [1.214 - 93.719]	2.135	0.033	F	0.4651	0
American	1	3.9362 [0.1779 - 87.107]	0.867	0.3858	Na	Na	Na
Sample types							
Tissue	**10**	**19.258 [7.742 - 47.904]**	**6.362**	**<0.001**	**F**	**0.9610**	**0**
Others	1	29.592 [5.690 - 153.891]	4.027	<0.001	F	0.5888	0
Pathological characteristics							
Age	5	1.487 [0.653 - 3.386]	0.945	0.345	F	0.6979	0
Stage	4	1.86 [0.856 - 4.041]	1.567	0.117	F	0.3020	17.78

Note: Na (Non analysis).

Ethnicity-based subgroup analyses (Table 2) showed that significantly higher frequency of *CDH1* methylations in NPC cases than controls in both Asian (OR = 15.879; 95% CI: 7.286 - 34.608, p<0.001) by the fixed effects models with between-study homogeneity in 8 independence studies (Figure 4) and African (OR = 10.667; 95% CI: 1.214 - 93.719, p<0.001) by the fixed effects models with between-study homogeneity in 2 studies, while in American (OR = 3.9362; 95% CI: 0.1779 - 87.107, p>0.001) in only one study, respectively.

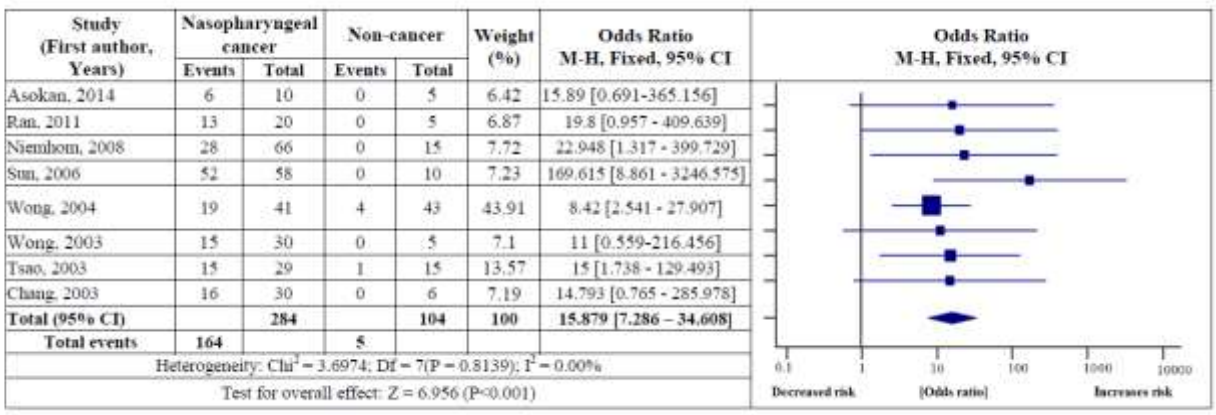

Figure 4: Forest plot of the correlation between *CDH1* methylation and NPC risk in Asian

Study (First author, Years)	Nasopharyngeal cancer		Non-cancer		Weight (%)	Odds Ratio M-H, Fixed, 95% CI
	Events	Total	Events	Total		
Asokan, 2014	6	10	0	5	8.42	15.89 [0.691-365.156]
Challouf, 2012	4	36	0	19	9.34	5.4 [0.276-105.810]
Ran, 2011	13	20	0	5	9.01	19.8 [0.9-409.639]
Niemhom, 2008	28	66	0	15	10.13	22.948 [1.317-399.729]
Ayadi, 2008	35	44	0	3	8.9	26.158 [1.241-551.509]
Sun, 2006	52	58	0	10	9.49	169.615 [8.861-3246.575]
Wong, 2003	15	30	0	5	9.32	11 [0.559-216.456]
Tsao, 2003	15	29	1	15	17.8	15 [1.738-129.493]
Chang, 2003	16	30	0	6	9.43	14.793 [0.765-285.978]
Nakayama, 2001	18	23	0	2	8.17	16.818 [0.698-405.286]
Total (95% CI)		**346**		**85**	**100**	**19.258 [7.742 - 47.904]**
Total events	**202**		**1**			
Heterogeneity: Chi^2=3.0813; df= 9 (P = 0.9610); I^2= 0.00%						
Test for overall effect: Z=6.362 (P<0.001)						

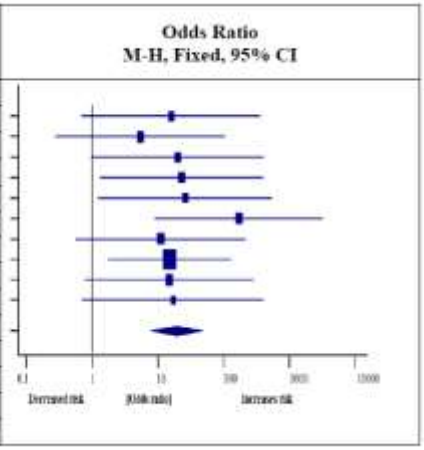

Figure 5. Forest plot of the correlation between *CDH1* methylation and NPC risk in MSP/NMSP or tissues based subgroup analysis

CDH1 methylation methods-based subgroup analysis was performed by the categories of MSP (Methylated specific PCR) and NMSP (Nested-MSP) in 10 studies and others including qMSP (Quantitative methylation-specific PCR) and QRT (Real-time quantitative polymerase chain reaction) in 2 studies, respectively. Hence, the results indicate MSP and Nested-MSP are common methods for analyzing the status of *CDH1* methylation. Eventually, by using MSP or NMSP, *CDH1* methylation frequency in NPC patients were calculated over 19 times higher than in healthy human volunteers (OR = 19.258, 95% CI: 7.742 - 47.904 , p<0.001) by the fixed effects models, data were shown in Table 2 & Figure 5.

Results of the sample types - based subgroup analyses (Table 2; Figure 5) implied the significant difference (p<0.05) in *CDH1* hypermethylation level between cancer samples and normal samples. OR value was calculated by the fixed effects models in tissues (OR = 19.258, 95% CI: 7.742 - 47.904) and others samples, including blood, mouth and throat rinsing fluid and buffy coat (OR = 29.592, 95% CI: 5.690 - 153.891) in the study of Chang *et al*., respectively.

In the clinicopathological characteristics of nasopharyngeal cancer, a total of seven and four studies were selected for the evaluation of *CDH1* methylation in late age versus early age and late - stage versus early - stage, respectively. The pooled analysis showed that there was no significant association between *CDH1* methylation and age of NPC patients or tumor-stage by the fixed effects models (Table 2).

4. Discussions

Despite a meta-analysis of Wu et al. was carried out for evaluating the association between E-cadherin gene promoter methylation and the risk of NPC with ten published studies, our meta-analysis was conducted by the combination of twelve independent studies in order to reveal the the correlation between the aberrant DNA methylation in the CDH1 gene promoter region and the risk of NPC. Particularly, the overall OR of CDH1 methylation in NPC samples versus normal samples was 16.155 (OR = 16.155, 95% CI: 8.533 - 30.585, p<0.001). This results implied the a strong correlation between *CDH1* hypermethylation and nasopharyngeal cancer.

In addition, all of the subgroup analyses indicated significant evidences of the relation between NPC clinical samples and the risk of NPC. Remarkably, subgroup meta-analysis on geographical populations as ethnicity showed that *CDH1* hypermethylation was a significant risk factor of NPC patients by Odds ratio in

decreasing order for Asian, African, and American population (OR = 15.879; OR = 10.667; OR = 3.9362, respectively). In a way, a meta-analysis of Wu *et al.* inferred from previous data that was the relatively more frequency of *CDH1* methylation in NPC population in Asia than Africa (OR =16.98 vs OR = 10.67). Likewise, data of WHO, Salehiniya and Wu *et al.* showed that NPC has the characterized geographical distribution with the more frequency of new cases that occurred in Asia than Africa (81% vs 9%). Thus, the aberrant DNA methylation of *CDH1* gene might become a potential biomarker for prediction, prognosis and early detection of NPC in Asia, especially in Vietnam.

Besides, subgroup meta-analysis on sample types illustrated OR of NPC samples in comparison with normal samples, in detail, OR value was higher in tissues than other types (OR =11.442 vs OR = 12.606). However, there was the higher OR (29.592, 95% CI: 5.690 – 153.891) in blood, mouth and throat rinsing fluid and buffy coat which were only used in the research of Chang *et al*. In contrast, tissues and blood were analyzed in 11 studies and 2 studies, respectively. Thereby, those evidence showed that tissue or blood should be the common, efficient clinical samples in testing the CHD1 methylation for diagnosis of NPC.

Previously, Wu *et al.* showed that no significant association between the status of *CDH1* methylation and some clinicopathological characteristics in NPC, for examples, sex or age, EBV infection, pathological types, tumor sizes, lymph node, metastatic status, and clinical stage in patients with NPC. Definitely, our analyses have suggested the same evidence of no significant relationship between *CDH1* methylation and age or tumor stage of NPC. In fact, the reasonable explanation should be due to a lack of data and *CDH1* methylation may be an early molecular event without age-dependent in the progress of NPC which are closely related to EBV infection.

This meta-analysis had a few of these limitations that should be considered: (1) because most of those included studies written in English, publication bias exited by languages selection; (2) a total of twelve articles were case control studies; (3) the heterogeneity of the databases may express due to the differences in NPC population, methods of detecting DNA methylation, types of cancer or normal samples; (4) there was the lack of sufficient data for evaluating the associations between the *CDH1* methylation and the confounding factors of NPC, for example, lifestyle, eating habit or diet and the main clinicopathological characteristics of NPC, including EBV infection, pathological types, tumor sizes, lymph node, metastatic status; (6) there was less than ten studies in subgroup meta-analyses, so should not have sufficient statistical power for evaluating the association of the *CDH1* methylation and the risk of NPC in each criteria.

Otherwise, this meta-analysis was performed with database updates in the process of systematic review. In addition, subgroup analysis was conducted for estimating the relation of the *CDH1* methylation and NPC risk that were featured in ethnicity of NPC patients, sample types, methods of DNA methylation analysis. Eventually, the current study found the most useful evidence for the analysis of association between *CDH1* methylation and NPC.

5. Conclusions

In conclusion, the results reveal a strong association between *CDH1* promoter methylation and nasopharyngeal cancer risk. Furthermore, it is obvious that *CDH1* promoter is proved to be a promising potential biomarker for the risk prediction, prognosis and diagnosis of NPC as the hallmark of poor overall survival of this types of cancer. The study had limitations that were no statistically significant differences in the status of *CDH1* methylation between subgroups of ages and tumor-stages in

NPC patient population. As a result, a meta-analysis can be conducted in the future studies which have a large scale of studies as well as the increasing number of case and controls samples and full data of the clinicopathological characteristics■

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