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Plasma Macrophage Inhibitory Cytokine-1 as a Complement of Epstein-Barr Virus Related Markers in Identifying Nasopharyngeal Carcinoma

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Abstract

Background: We evaluated the diagnostic value of plasma Macrophage inhibitory cytokine-1 (MIC-1) in distinguishing patients with nasopharyngeal carcinoma (NPC) and explored its complementary role with widely used Epstein-Barr virus (EBV) related markers, EBV capsid antigen-specific IgA (VCA-IgA) and EBV copy number. **Methods:** ELISA was used to analyze the plasma MIC-1 levels in 190 NPC patients, 72 VCA-IgA-positive healthy donors (VP), and 219 normal subjects with negative VCA-IgA (VN). 10 pairs of plasma samples before and after radiotherapy were also included. **Results:** The plasma MIC-1 levels were significantly higher in NPC patients (Median: 678.39 ng/mL) than those in VN and VP (310.29 and 294.59, $p < 0.001$). Receiver operating characteristic (ROC) curves of the MIC-1 concentrations revealed that the area under the ROC curve (AUC) was 0.790 (95% confidence interval [CI]: 0.748-0.832), with a sensitivity of 63.7%, and a specificity of 85.9% respectively, for distinguishing NPC patients from the healthy donors. Similarly, between NPC and VP, ROC was 0.796 (0.738-0.853) with sensitivity of 63.7%, and specificity of 88.9%. In addition, between NPC and VN, ROC was 0.788(0.744-0.832) with sensitivity of 63.7%, and specificity of 84.9%. Further, we found that MIC-1 could complement VCA-IgA and EBV DNA markers, with a negative rate of 88.9% in VCA-IgA-positive healthy controls, and a positive rate of 59.0% in EBV DNA negative NPC patients, respectively. Also, the MIC-1 plasma concentration dropped significantly after radiotherapy ($p = 0.027$). **Conclusions:** MIC-1 can complement VCA-IgA titers and EBV DNA copy number tests in NPC detection, improve identification of EBV DNA-negative NPC patients, and distinguish NPC from VCA -IgA positive healthy controls.

Keywords

MIC-1, nasopharyngeal carcinoma, VCA-IgA, EBV DNA, radiotherapy

List of abbreviations

AUC, area under the ROC curve; CI, confidence interval; EBV, Epstein-Barr virus; MIC-1, Macrophage inhibitory cytokine-1; NPC, Nasopharyngeal Carcinoma; NPV, negative predictive value; PPV, positive predictive value; ROC, Receiver operating characteristic; Sen, sensitivity; Spe, specificity; TGF-β, transforming growth factor beta; VCA-IgA, EBV capsid antigen-specific IgA; VP, VCA-IgA-positive healthy donors; VN, subjects with negative VCA-IgA.

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Introduction

Nasopharyngeal carcinoma (NPC) is largely endemic in Southeast Asia, specifically Southern China. In these areas, the annual incidence rate of the disease peaks at 50 cases per 100,000 people, almost 25 times higher compared to the rest of the world.[1] Though the etiology of NPC includes both environmental and genetic factors, Epstein-Barr virus (EBV) infection impacts strongly.[2] Therefore, elevated levels of EBV-related antibodies, such as immunoglobulin against the EBV viral capsid antigen (VCA), have been widely used to screen NPC.[3] Also, the circulating EBV DNA has been used for the diagnosis of NPC. However, in isolation, these serological tests are often insufficient in identifying NPC with confidence.[4] Despite remarkable sensitivity, the serology test for VCA is highly unsatisfactory due to a high false-positive ratio.[5] Greater than 20% of NPC patients turn out negative in early antigen (EA) tests, therefore it is challenging to preclude NPC with a negative EA serology result.[5] Several groups observed the presence of EBV DNA in the plasma of NPC patients.[6] They consistently found a low detection rate in healthy subjects, whereas observed high variability in the NPC patients.[6] Moreover, EBV DNA tests used in diagnosing early-stage or recurrence of NPC have been reported of limited use.[7] Thus, developing new NPC diagnostic markers and complementing EBV related markers are imperative.

Macrophage inhibitory cytokine-1 (MIC-1) is a divergent member of the cytokine superfamily transforming growth factor-beta (TGF-β).[8] In humans, MIC-1 is predominantly expressed in the placenta tissue. However, its expression levels can be rapidly elevated by p53, and are also strongly regulated by cytokines such as TGF-β and interleukin-1.[9] This suggests that MIC-1 could act downstream of the signaling pathways, such as the ERK signaling pathway and Akt signaling pathway, involved in the regulation of apoptosis and cell cycle arrest.[10] Interestingly, in several cancers, such as prostate, breast, and colorectal, the levels of MIC-1 are dramatically upregulated.[11-13] Therefore MIC-1 could of great interest in stratification, diagnosis, and prognosis of such diseases.[14] Nevertheless, the diagnostic significance of the blood MIC-1 level in NPC has not been explored yet. Here, we evaluate the diagnostic importance of plasma MIC-1 and assess if it may complement clinically used EBV related markers in NPC. Additionally, we explored if it can be used to monitor the patient's progress after radiotherapy.

Materials and Methods

Patients and Ethical Statement

The plasma samples from 190 pathologically confirmed NPC patients were drawn during diagnosis at the Sun Yat-sen University Cancer Center before any treatment between January 2017 and March 2018. TNM stage was established based on the 2009 Union for International Cancer Control/American Joint Committee on Cancer staging system for NPC. Patients accompanying another malignancy or skin disease were excluded. The patient parameters are collected from medical files and displayed in Table 1. Apart from that, we also recruited 72 cancer-free healthy controls with positive VCA-IgA (VP) and followed them up for 6-12 months to rule out inflammation-related diseases and cancer. 219 volunteers undergoing routine physical examinations with negative results comprised the

Table 1. Levels of MIC-1 and Clinical Characteristics in 190 Untreated NPC Patients.

Charateristics	No. of patients	MIC-1(pg /ML) Median (IQR)	p value[a]
Age (yr)			
< 48	94	525.70 (295.30-797.10)	< 0.001
≥48	96	867.30 (551.50-1448.00)	
Sex			
Female	139	699.30 (443.04-1135.28)	0.113
Male	51	549.15 (301.74-916.44)	
EBV DNA copy number			0.2317
$\leq 10^3$	78	640.86 (443.04-1135.28)	
10^3-10^4	57	748.19 (466.24-1070.67)	
10^4-10^5	37	692.92 (352.11-1395.22)	
$\geq 10^5$	16	648.60 (529.91-1595.15)	
VCA-IgA titers			0.5842
≤ 1:40	72	623.35 (350.89-1163.03)	
1:80	27	583.64 (316.95-897.67)	
1:160	73	717.52 (393.22-931.36)	
≥ 1:320	18	789.26 (424.50-1188.21)	
EA-IgA titers			0.9632
≤ 1:10	64	668.44 (404.28-1106.06)	
1:20	18	594.98 (295.62-1202.12)	
1:40	72	689.66 (320.15-966.96)	
≥ 1:80	35	692.92 (443.04-966.11)	
EBV-DNase antibody			0.1788
Negative	17	533.04 (280.26-1111.21)	
Positvie	148	700.84 (379.96-1104.14)	
Rta-IgG			< 0.001
Negative	26	317.52 (246.15-538.02)	
Positvie	125	748.45 (435.77-1185.98)	
Zta-IgG			0.0616
Negative	36	536.86 (278.16-860.03)	
Positvie	113	673.27 (361.42-1147.92)	
pT status			
pT1-3	117	603.70 (304.30-917.40)	0.276
pT4	48	700.80 (397.40-1102.00)	
pTx	25	928.60 (730.8-1318.00)	
pN status			
pN 0-1	56	553.70 (272.60-855.90)	0.066
pN 2-3	109	647.10 (390.50-1010.00)	
pNx	25	928.60 (730.80-1318.00)	
pM status			
pM 0	153	624.90 (321.40-937.30)	0.768
pM 1	12	666.00 (314.30-1072.00)	
pMx	25	928.60 (730.80-1318.00)	
Overall stage			
Stage I-III	89	647.10 (301.90-937.30)	0.818
Stage IV	76	599.60 (359.20-982.90)	
Stage X	25	928.60 (730.80-1318.00)	

[a]Kruskal-Wallis test or Mann-Whitney U test, $p < 0.05$ was considered statistically significant. MIC-1: Macrophage inhibitory cytokine-1; IQR: interquartile range

EBV VCA-IgA negative (VN) group. Samples from the VP and VN groups were collected from the health examination department at our center. The preliminary screening phase included plasma from 20 pathologically confirmed cervical carcinoma, 20 prostate carcinoma, 20 leukemia, 20 NPC, 20 gastric cancer, 20 thyroid carcinoma, 20 colorectal carcinoma patients, and 20 healthy controls. To evaluate the relationship between MIC-1 plasma levels and radiotherapy, we enrolled 10 pairs of samples from NPC patients before and after radiotherapy.

Venous blood samples (3 mL) obtained from all the participants were drawn into EDTA-K2 anticoagulant tubes, centrifuged at 3600 rpm for 8 min, and then stored at -80°C till further use.

Ethics, Consent, and Permissions

This study was reviewed and approved by the Institutional Review Board and Ethics Committee of SYSUCC(GZR2018-147). At the time of patients' admission, as a General standard procedure at our center, their written informed consent was obtained to use Clinical parameters and collected samples for further studies. The records were anonymous and de-identified before use.

ELISA Assay

Plasma MIC-1 concentrations were measured using a double-antibody sandwich ELISA at room temperature (RT) as per the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Briefly, 96-well microplates (Costar, USA) were coated with mouse anti-human MIC-1 antibody (100 μL/well, 2.0 μg/mL) overnight. After blocking, 100 μL of the standard or serum (5-fold dilution in 3% BSA) were added and incubated for longer than 1 h. Hereafter, 100 μL of the biotinylated goat anti-human MIC-1 antibody (12.5 ng/mL) was added into each well and incubated for 2 h. Then, Streptavidin-HRP (100 μL/well) diluted to 200 times was incubated for 20 min. Finally, the substrate solution (tetramethylbenzidine) was added and the reaction was stopped using 2 N H_2SO_4. Absorbance was recorded at a dual-wavelength of 450/630 nm. Each plate also contained a standard control (coefficient of variation < 12%).

Immunoenzymatic Assay of Plasma EBV VCA-IgA

Plasma EBV titers were assessed using the classic immunoenzymatic assay (IEA),[15] obtained from the Shanghai Institute of Biological Products. Two independent observers determined the degree of staining.

Real-Time Quantitative Measurement of Plasma EBV DNA

EBV DNA, extracted from the pretreated plasma, was subjected to real-time quantitative polymerase chain reaction to obtain copy number as described previously.[16] The clinically used copy number, 1000 copies/mL, was set as the cut-off level.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prizm 8.0 or SPSS 23.0 (SPSS Inc.) programs. The relationships between the plasma MIC-1 levels and the clinicopathologic parameters, as well as the comparisons of MIC-1 concentration between different groups, were analyzed using the Mann-Whitney U test. The diagnostic ability of MIC-1 was assessed using the area under the receiver operating characteristic (ROC) curve (AUC). The maximal Yuden index value was considered as the cut-off value for MIC-1. Also, sensitivity (Sen), specificity (Spe), positive predictive value (PPV), and negative predictive value (NPV) were applied to evaluate the overall diagnostic performance of MIC-1. The plasma levels of MIC-1, before and after radiotherapy in NPC patients, were compared using the paired *t*-test. All statistical tests were 2-sided, and $p < 0.05$ were considered as statistically significant.

Results

MIC-1 Levels in the Preliminary Screening Phase

Blood plasma from 7 cancer subjects was subjected to MIC-1 ELISA assay along with healthy controls. The Mann-Whitney U test revealed that MIC-1 levels were significantly elevated in prostate cancer, colorectal cancer, and NPC but not cervical cancer, gastric cancer, thyroid carcinoma, and leukemia (Figure 1A). Interestingly, MIC-1 levels were particularly high in NPC, signifying its importance in NPC detection.

Plasma MIC-1 Levels in NPC and Its Association With Clinicopathological Characteristics

We further explored the diagnostic role of MIC-1 in NPC. The plasma concentrations of MIC-1 in 3 groups (VN: n = 219, VP: n = 72 and NPC: n = 190) are presented in Figure 1B. MIC-1 levels were upregulated in NPC patients compared to both VN group ($p < 0.001$) and VP group ($p < 0.001$). However, the plasma levels of MIC-1 in VN were similar to VP ($p = 0.951$). The median plasma levels of MIC-1 were 678.39 pg/mL (IQR, 368.52 to 1083.48 pg/mL) in NPC patients, 310.29 pg/mL (IQR, 215.62 to 404.51 pg/mL) in the VP group, and 294.6 pg/mL (IQR, 202.56 to 457.86 pg/mL) in the VN group. The association between the plasma MIC-1 concentrations and the clinicopathological features are presented in Table 1. Though we found that MIC-1 levels were significantly associated with age and Rta-IgG ($p < 0.001$), the other parameters such as sex, EBV DNA copy number, VCA- IgA, EA-IgA, EBV-DNase antibody, Zta-IgG, pT, pN, pM, and overall stage ($p > 0.05$) were not related.

Diagnostic Ability of Plasma MIC-1 Levels in NPC Patients

To validate the discriminatory competence of plasma MIC-1 levels, we utilized plasma from a total of 481 participants, including 190 NPC, 72 VP, and 219 VN samples. ROC curves analyses illustrated that the MIC-1 plasma levels strikingly distinguished the subjects with or without NPC, with an AUC value of 0.790

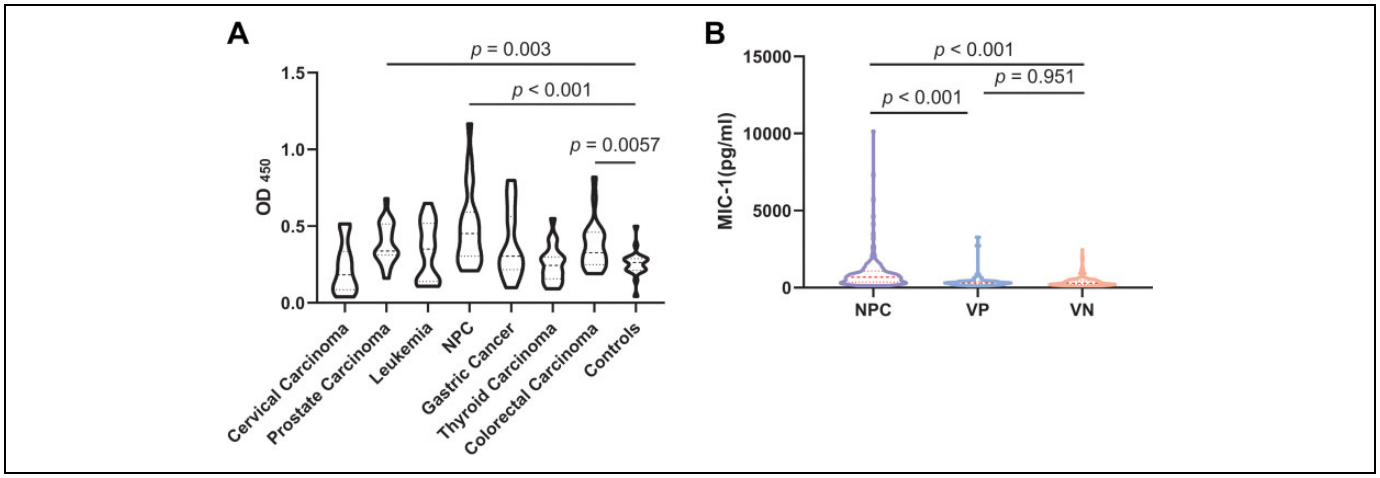

Figure 1. The plasma concentration of MIC-1 in study subjects. (A) Plasma levels of MIC-1 were measured in patients from 7 cancers (NPC, prostate cancer, cervical cancer, colorectal cancer, gastric cancer, thyroid carcinoma, and leukemia) and healthy controls; (B) plasma levels of MIC-1 were measured in NPC patients, viral capsid antigen (VCA-IgA) negative (VN) cohort, and VCA-IgA positive (VP) cohort. *p*-value was obtained from the Mann-Whitney U test. MIC-1: Macrophage inhibitory cytokine-1; NPC: nasopharyngeal carcinoma.

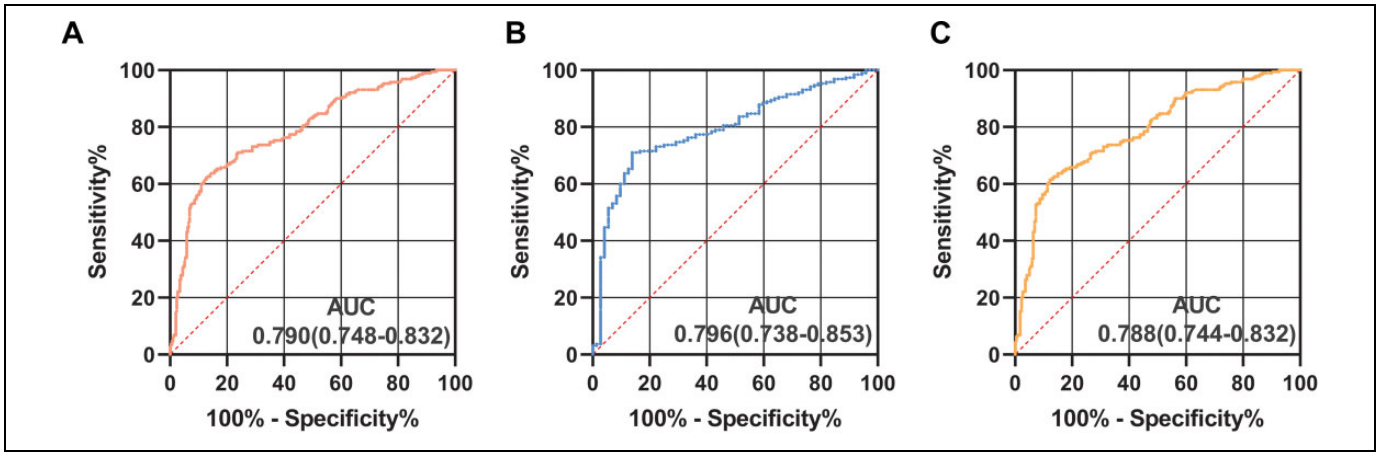

Figure 2. Diagnostic performance of plasma MIC-1 levels in NPC. (A) ROC curves of NPC patients versus all controls. (B) ROC curves of NPC patients versus VCA-IgA positive cohort; (C) ROC curves of NPC patients versus VCA-IgA negative cohort. MIC-1: Macrophage inhibitory cytokine-1; NPC: nasopharyngeal carcinoma; VP: VCA-IgA positive; VN: VCA-IgA negative.

(95% CI, 0.748-0.832, Figure 2A). When the cutoff value was set to the optimal point (528.27 pg/mL), the sensitivity, specificity, PPV, and NPV were 63.7%, 85.9%, 74.7%, and 78.4% (Table 2), respectively. For discriminating the NPC patients from the VP cohort, the AUC value of plasma MIC-1 was 0.796 (95% CI, 0.738 to 0.853, Figure 2B), with 63.7% sensitivity, 88.9% specificity, a striking 93.8% PPV and 48.1% NPV. Also, the AUC of MIC-1 for differentiating NPC patients from the VN cohort was 0.788 (95% CI, 0.744-0.832, Figure 2C), the sensitivity was the same whereas the specificity was 84.9% (Table 2).

Table 2. Results for Measurement of Plasma MIC-1 in the Diagnosis of NPC.

	NPC vs VP + VN	NPC vs VP	NPC vs VN
Sensitivity	63.70%	63.70%	63.70%
Specificity	85.90%	88.90%	84.90%
PPV	74.70%	93.80%	78.60%
NPV	78.40%	48.10%	72.90%
AUC (95%CI)	0.790 (0.748-0.832)	0.796 (0.738-0.853)	0.788 (0.744-0.832)

MIC-1: Macrophage inhibitory cytokine-1; NPC: nasopharyngeal carcinoma; VP: VCA-IgA positive; VN: VCA-IgA negative;

The Complement Role of MIC-1 for EBV-Related Markers in the Diagnosis of NPC

We further explored the relationship between plasma MIC-1 concentrations and EBV-related markers, like VCA-IgA titer and EBV DNA content. To assess the association between MIC-1 concentrations and VCA-IgA titer, the NPC patients were divided into 4 groups, based on VCA-IgA titers: $\leq$ 1:40 (n = 72), 1:80 (n = 27), 1:160 (n = 73), and $\geq$ 1:320 (n = 18).

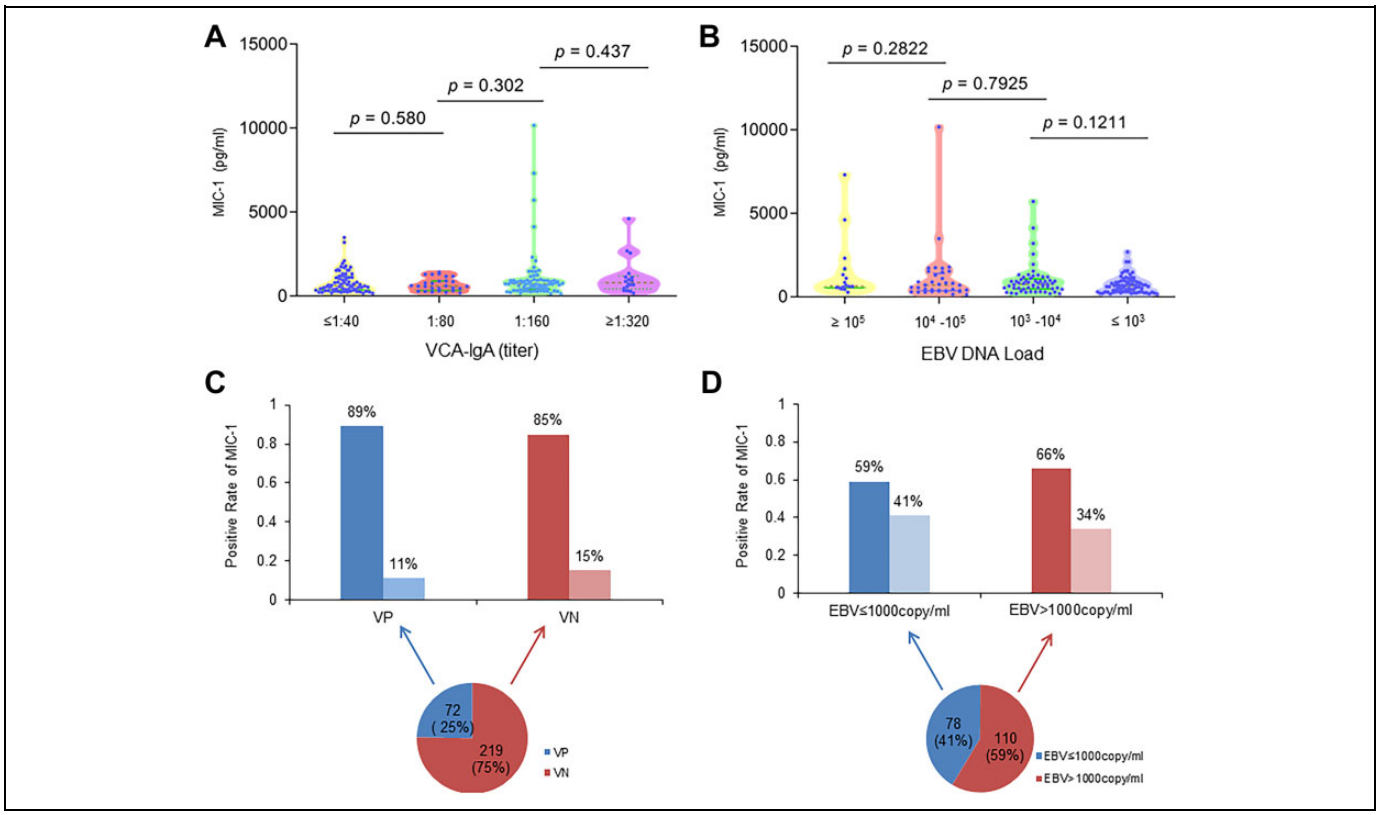

Figure 3. The ability of plasma MIC-1 in reducing false diagnosis in VCA- IgA positive healthy controls and EBV DNA negative NPC patients. (A) The relationship between plasma MIC-1 levels and VCA-IgA titers; (B) The relationship between plasma MIC-1 levels and EBV DNA content; (C) The negative rate of MIC-1 in healthy controls; (D) The positive rate of MIC-1 in NPC patients. MIC-1: Macrophage inhibitory cytokine-1; NPC: nasopharyngeal carcinoma; VP: VCA-IgA positive; VN: VCA-IgA negative.

We found no significant differences in plasma MIC-1 concentrations among these 4 groups ($p > 0.05$) (Figure 3A). Moreover, similar results were observed when we separated the NPC patients into 4 groups according to the plasma EBV DNA copy number: $\geq 10^5$ (n = 16), 10^4 -10^5 (n = 37), 10^3-10^4 (n = 57), and $\leq 10^3$ (n = 78). The results suggest that MIC-1 concentrations are unlikely to be directly related to VCA-IgA titer and EBV DNA copy numbers (Figure 3B).

We further explored whether plasma MIC-1 can supplement VCA-IgA in distinguishing healthy controls. Figure 3C shows the percentages of healthy controls stratified by 528.27 pg/mL cutoff values of plasma MIC-1. In VP, the rate of negative results for plasma MIC-1 was 88.9%, which is slightly higher than VN (84.9%).

Dramatically, a larger proportion of NPC patients were positive for plasma MIC-1 (63.7%) than the EBV DNA (58.5%). We also explored the complementary effect of MIC-1 to EBV DNA. 78/188 (59.0%) of NPC patients were EBV DNA-negative. Among them, 46 out of 78 (59.0%) EBV DNA-negative NPC patients were positive for MIC-1.

The Effect of Plasma MIC-1 in NPC Surveillance

Plasma samples were collected from 10 patients after radiotherapy. The mean plasma MIC-1 concentration before radiotherapy was 778.75 pg/mL, which afterward dropped to 511.26 pg/mL, $p = 0.027$ (Figure 4) except 1 patient.

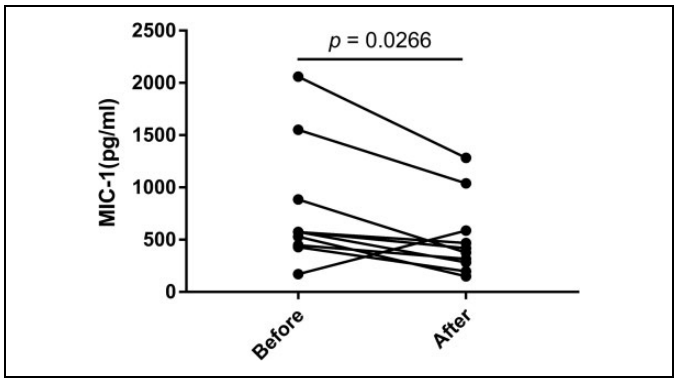

Figure 4. MIC-1 plasma concentrations before and after radiotherapy of the NPC patients. MIC-1: Macrophage inhibitory cytokine-1; NPC: nasopharyngeal carcinoma.

Discussion

Our results suggest that irrespective of VCA-IgA and EBV DNA status, detecting plasma MIC-1 level is of robust diagnostic value for NPC. MIC-1, a growth factor, is known to play regulatory roles in apoptosis, cell cycle, tumor metastasis,

invasiveness, and immunosuppression.[17,18] Apart from its high levels in placenta and activated macrophages, MIC-1 levels also get dramatically elevated in a myriad of tumors. By using Oncomine datasets, which allows differential expression analysis of a gene across all available microarray datasets at once, we found that in 57 studies of 14 cancers, levels of MIC-1 were significantly upregulated while only in 5 studies displayed a downregulation in cancers.

To be a superior diagnostic marker, secreted protein must be enriched in blood circulation to ease detection and rarely expressed in adult normal tissues, even if expressed during the embryonic stage. Luckily, MIC-1 meets both of these criteria.[19] In humans, it is well-secreted and barely detectable in normal tissues except for the placenta and embryonic tissues. The absence of MIC-1 was established in normal prostate epithelium using immunohistochemistry,[13] also it is rarely expressed in normal colonic mucosa.[12] Although MIC-1 has been associated with several tumors, its relationship with NPC is rarely reported. However, a research group in Taiwan showed the role of MIC-1 in radioresistance of NPC cell lines, where MIC-1 gene knockdown enhanced the radiosensitivity.[20] Primarily, upon testing in multiple cancers, we found that plasma MIC-1 levels were remarkably higher in NPC than other tumors. Therefore, we explored the diagnostic value of this widely described potential tumor marker in NPC.

A strong correlation between NPC and EBV infection has been already established. VCA, strongly immunogenic capsid antigen of EBV, is released shortly after EBV infection and has a long half-life. More than 90% of patients with NPC are VCA-IgA positive. Hence, it is frequently used for the screening of the disease in symptomatic patients or living in high incidence areas. However, most positive results are not truly NPC. In several large population screens in the NPC-high incidence areas, such as Taiwan,[21] Hong Kong,[22] Wu Zhou, and the other southern China areas,[23] the positive ratio of VCA-IgA was only 3-10%. However, a long period follow-up of these revealed that only 1-5% of them developed into pathologically-confirmed NPC, suggesting the positive predictive value (PPV) of VCA-IgA is rather low. Due to this, several subjects, even in the absence of NPC, suffered unnecessary psychological burden of nasopharyngoscopy, radiological examinations, or aggressive biopsy, along with loss of money and time. Subsequently, EBV DNA levels in the circulation found to be more reliable in the detection of NPC. Though the detection rates in healthy control were low, several studies reported a high variability of the test among the NPC patients. For instance, Mutirangura et al. reported that in a group of 42 NPC patients, only 14 patients were positive for EBV DNA in the circulation.[24] Similarly, Hsiao JR et al. also reported a positive rate of merely 38.9%.[25] However, Lo et al. found that EBV DNA could be detected in the blood of 96% of NPC patients. Overall, this suggests that EBV DNA detection does not have standardized methods and techniques as of yet, and lacks the sensitivity.[6] In our study, 58.5% of NPC patients were EBV DNA positive, and this is consistent with Shotelersuk K, et al.[26] Interestingly, EBV DNA could also be detected in patients suffering from several other diseases,[27,28] such as infectious mononucleosis, gastric cancer, and Hodgkin's disease. Therefore, a new diagnostic marker that can detect and monitor NPC, complementing the widely used EBV markers, is of great value.

Here, we showed that plasma MIC-1 concentrations in the NPC patients were significantly higher than those in VN and VP healthy controls. Also, levels of MIC-1 were markedly higher in NPC compared to gastric cancer and leukemia, suggesting high specificity in discriminating NPC from the other EBV DNA-positive cancers. Additionally, excluding age and Rta-IgG ($p < 0.001$), MIC-1 levels showed no association with patient clinical characteristics. Notably, we observed no significant difference in the plasma MIC-1 levels between the early-stage and advanced-stage NPC patients, suggesting its applicability in both the scenarios. Also, no significant difference was noticed among the NPC patients having different VCA-IgA titer and EBV copy number, suggesting that MIC-1 is a non-EBV related marker. Additionally, ROC curve analysis revealed the diagnostic accuracy of plasma MIC-1 in distinguishing NPC from healthy controls with VCA-IgA positive or negative with similar efficiency. Compared with VCA-IgA titer, plasma MIC-1 level exhibited higher PPV and specificity. Overall these results signify that Plasma MIC-1 level alone could be a better diagnostic marker of NPC. Moreover, it can aid the diagnosis with EBV-related markers in EBV DNA negative individuals and VCA-IgA positive healthy controls. Most VP were MIC-1 negative, therefore, healthy individuals with VCA-IgA positive can be easily distinguished from the NPC patients. On the contrary, many NPC patients were positive for MIC-1 while being negative for EBV DNA. Therefore, combined testing for plasma MIC-1 and EBV DNA copy numbers could improve the diagnostic results. Additionally, this test can be utilized for population studies in high-risk areas.

The decreased plasma MIC-1 levels after radiotherapy suggests that it can also be used in assessing the radiotherapeutic response in NPC patients. However, notably in 1 sample, the MIC-1 protein level was increased after the radiotherapy. Interestingly, upon following up, we found that the particular patient was not sensitive to radiotherapy. As the previous finding showed that MIC-1 was involved in radioresistance of NPC cell lines,[20] perhaps evaluated MIC-1 level could be a reflection of radioresistance in the NPC patients. However, such a conclusion needs further verification due to the limitation of the sample size in our study.

Although our results are promising, there were few limitations. Larger and multicenter samples could rule out selection bias, control bias, and the incidence of NPC bias in the following research. Also, the oncological and biological significance of MIC-1 must be investigated further.

Conclusions

In conclusion, the current study reveals that plasma MIC-1 levels are significantly increased in NPC, which easily distinguished the NPC patients from the healthy controls. Also, a combination of MIC-1 and EBV-related markers could aid the

diagnosis. MIC-1 is an easy-to-access and non-invasive marker that could help clinicians. We will continue to verify the performance of MIC-1 in NPC screening and surveillance in samples from larger and multicenter patient cohorts.

Authors' Note

SX and LL carried out the main work, draft the manuscript and contributed equally. YP carried out the Immunoenzymatic assays. GO and NX designed and guided the study. TZ and QH helped collect the sample and the clinical parameters. All authors read and approved the final manuscript. Shan Xing and Huilan Li contributed equally to this work. The study was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was reviewed and approved by the Institutional Review Board and Ethics Committee of Sun Yat-sen University Cancer Center (Ref: GZR 2018-147). The consent has been obtained from the participants, for the use of clinical parameters and collected samples for further studies at the time of patients' admission. The records were anonymous and de-identified before use. The consent we obtained from study participants was written.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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