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Evaluation of serum granulysin as a potential biomarker for nasopharyngeal carcinoma

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ABSTRACT

Background: Granulysin (GNLY) is excreted from cytotoxic T lymphocytes and natural killer cells, and plays an important role in antitumor immunity. However, few studies have estimated serum GNLY concentrations in patients with nasopharyngeal carcinoma (NPC). We evaluated GNLY as a potential biomarker for NPC.
Methods: Serum GNLY concentrations were measured in blood samples taken from 98 NPC patients, 56 nasopharyngitis (NPT) patients, and 99 healthy subjects. The clinical relevance of GNLY in NPC was also investigated. We also assessed the association between serum GNLY and serum immunoglobulin A antibodies against the Epstein–Barr virus (EBV) viral capsid antigen (VCA-IgA) and EBV DNA.
Results: Serum GNLY levels were significantly lower in NPC patients and significantly higher in nasopharyngitis patients compared to healthy controls. Thus, serum GNLY performs well as a biomarker for distinguishing between NPC and NPT. The serum GNLY concentration is elevated with corresponding increases in clinical stage and shows a significant correlation with VCA-IgA and EBV DNA concentration.
Conclusions: Serum GNLY is closely associated with the clinical characteristics of NPC and may be a potential biomarker for NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common cancers in Southern China and Southeast Asia [1,2]. The early symptoms of NPC are not obvious, which often could not be distinguished from the symptoms of nasopharyngitis (NPT). The correct diagnosis depends on pathology and CT examination. Because NPC is associated with Epstein–Barr virus (EBV) infection, circulating EBV-related biomarkers, such as immunoglobulin A antibodies against the EBV viral capsid antigen (VCA-IgA) and EBV DNA are frequently used as NPC biomarkers for screening patients presenting with relevant symptoms [3–7]. However, more studies are required to confirm their possible limitations and new biomarkers are needed for this disease.

Granulysin (GNLY) is a cytolytic granule protein that is excreted from cytotoxic T lymphocytes (CTL) and natural killer (NK) cells [8,9] and is found in the sera as a biomarker of viral infection [10]. It functions as a cytotoxic and pro-inflammatory protein, which is involved the graft-versus-host reaction and has both antimicrobial and antitumor activity [11–14]. Although many studies have evaluated serum GNLY as a biomarker in patients with solid or haematological malignancies [15–20], few studies have measured serum GNLY concentrations in patients with NPC.

2. Patients and methods

2.1. Patients

Ninety-eight patients with NPC (68 men, 30 women; range 20–69 y), confirmed by pathological examination between October 2012 and May 2013 at Sun Yat-sen University Cancer Center (Guangzhou, China), were enrolled in the study. One hundred and fifty-five cancer-free volunteers (85 men, 70 women; range 22–81 y), including 99 healthy subjects (without infection, cancer, or other known disease) and 56 NPT patients (diagnosis was confirmed by pathology or CT plain scan. Patients merging other infections were excluded), served as a control population. Cancer TNM staging was defined according to the 2009 UICC/AJCC staging system for NPC. This study was approved by the research ethics committee of Sun Yat-sen University Cancer

Abbreviations: GNLY, Granulysin; NPC, Nasopharyngeal carcinoma; NPT, Nasopharyngitis; EBV, The Epstein–Barr virus; VCA, Viral capsid antigen; CTL, Cytotoxic T lymphocytes; NK, Natural killer.

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Center and was performed in accordance with the Declaration of Helsinki.

2.2. ELISA for GNLY in the sera

Peripheral blood samples were collected in the morning to minimize the effect of diurnal cortisol secretion. The serum samples were separated by centrifugation for 15 min at 1000 ×*g* at 2–8 °C within 10 min of collection and were stored at −80 °C until use. The samples were brought to room temperature (18–25 °C) for 30 min before use. The concentration of human GNLY was measured by a custom ELISA kit (Cusabio Biotech Co., Ltd.). The assay was performed according to the manufacturer's instructions. The detection range of the assay was 3.12 ng/ml–200 ng/ml and the minimum detectable concentration is typically less than 0.78 ng/ml.

2.3. Detection of EBV-related seromarkers

EBV DNA in serum was measured by real-time quantitative PCR as previously described [5]. The serum antibody titres of EBV VCA-IgA were detected by using a semi-quantitative ELISA method that has been reported before [3]. A titre of >1:10 was considered positive for VCA-IgA [4].

2.4. Statistical analysis

Comparison between groups was performed with the Mann–Whitney U test, Kruskal–Wallis H test, or Wilcoxon t test. Correlations between the groups were analysed using the Spearman's rank correlation test. A $P < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic value of the parameters. Pairwise comparisons of ROC curves were performed using the Z-test. All statistical analyses were performed using SPSS 16.0 and MedCalc 9.6.

3. Results

3.1. Serum GNLY levels in NPC patients and controls

There was no correlation between serum GNLY levels and age in NPC patients or in controls (Fig. 1A). GNLY levels were significantly higher in male NPC patients than in female NPC patients ($P = 0.0048$). There were no statistically significant differences between genders in the controls (Fig. 1B). Serum GNLY levels in NPC patients were significantly lower ($P = 0.0043$) than in healthy controls. In contrast, the levels in NPT controls were significant higher ($P < 0.001$) than in healthy controls (Fig. 2). The mean GNLY levels were 14.46 ng/ml in healthy controls, 21.68 ng/ml in NPT patients, and 12.17 ng/ml in NPC patients (Fig. 2).

3.2. Clinical relevance of GNLY in NPC patients

To verify the diagnostic value of GNLY for NPC, three cohorts were established. A cohort consisted of NPC patients, NPT controls, and healthy controls. B cohort consisted of NPC patients and healthy controls. C cohort consisted of NPC patients and NPT controls. ROC curve analysis was applied to visualize the diagnostic performance of GNLY in these cohorts. As shown in Fig. 3, the ROC area under the curve (AUC) for GNLY in C cohort (AUC: 0.833) was significantly greater than in A cohort (AUC: 0.696) or in B cohort (AUC: 0.618). When the criterion of 14.46 ng/ml was applied, which represented the mean serum GNLY concentration of the healthy controls, the specificity in C cohort was as high as 78%, though the sensitivity (77.6%) did not improve.

In NPC patients, the serum GNLY concentration was elevated in correspondence with increasing clinical stages (Fig. 4). The mean GNLY concentration in patients with stage IV NPC was significantly higher

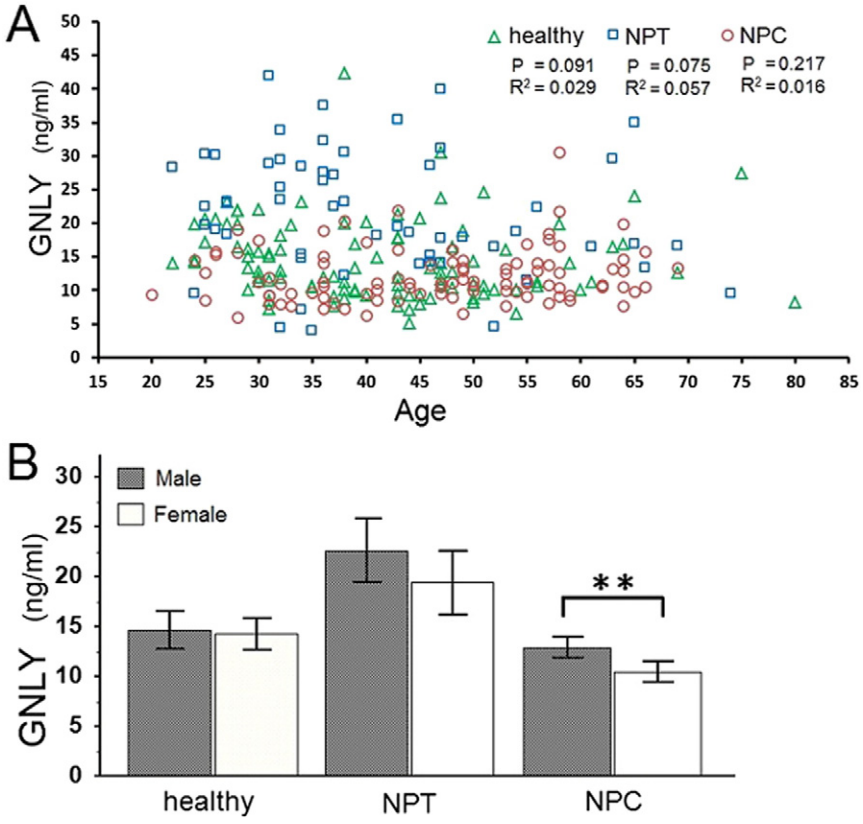

Fig. 1. (A) Serum GNLY concentrations according to age in healthy controls, NPT patients and NPC patients. (B) Serum GNLY concentrations according to sex in healthy controls, NPT patients and NPC patients. All data are presented as mean ± SEM. ** indicates a significant difference at $P < 0.01$.