ORIGINAL RESEARCH

Serum Colorectal Neoplasia Differentially Expressed Level and Prognostic Factors in Patients with RLNM of NPC

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ABSTRACT

Context • Nasopharyngeal carcinoma (NPC) is a malignant tumor with a high incidence. Regional lymph node metastasis (RLNM) affects the benefits to and prognosis of patients after treatment. Researchers speculate that CRNDE expression might have a correlation with NPC lymph node metastasis (LNM), but studies on the subject are relatively few.

Objective • The study intended to explore the predictive value of the level of serum colorectal neoplasia differentially expressed (CRNDE) for RLNM in NPC patients and to analyze the effects of RLNM on their long-term prognosis after radiotherapy and chemotherapy, to provide a reference for early prediction, diagnosis, and treatment of RLNM in NPC.

Design • The research team performed a retrospective case-control study.

Setting • The study took place at Taizhou People's Hospital in Taizhou, China.

Participants • Participants were 80 NPC patients who received treatment using radiotherapy and chemotherapy at the hospital between January 2014 and December 2017.

Groups • The research team divided participants into two groups: (1) an observation group diagnosed with RLNM, with 52 participants, and a control group that had no RLNM, with 28 participants.

Outcome Measures • The research team: (1) determined the level of colorectal neoplasia differentially expressed (CRNDE) in participants' serum to predict the risk of RLNM, (2) compared clinical total response rates (TRRs) between the groups, and (3) analyzed the five-year overall survival (OS), local relapse-free survival (LRFS), and distant metastasis-free survival (DMFS).

Results • Compared with control group, the observation group's CRNDE level was significantly higher, and its five-year OS and DMFS rates were significantly lower (all $P < .01$). No significant differences existed in the TRR and five-year LRFS rate between the observation and control groups ($P > .05$). The cut-off value for serum CRNDE was set at 3.540, the area under curve (AUC) value for the observation group was 0.805, and the 95% confidence interval (CI) was 0.715-0.889. In addition, the sensitivity was 88.5%, specificity was 57.1%, and Yoden index was 0.463. The five-year OS rates were significant lower in the observation group patients with metastatic lymph nodes > 2 in number ($P = .025$) and > 6 cm in diameter ($P = .002$) and with posterior pharyngeal LNM ($P = .049$).

Conclusions • An abnormal increase in serum CRNDE can be a basis to diagnose RLNM in NPC patients. RLNM affected the long-term prognosis of NPC patients, and the number and diameter of lymph nodes and posterior pharyngeal metastasis were the factors affecting patients' long-term. The current study's findings can provide a reference for the realization of the early diagnosis of NPC RLNM, formulating the treatment schemes and improving the long-term survival outcome of NPC patients. (*Altern Ther Health Med.* 2024;30(6):135-143).

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Nasopharyngeal carcinoma (NPC) refers to a malignant epithelial tumor that occurs in the upper and lateral walls of the nasopharynx. It possesses highly invasive characteristics and is the most common malignancy of the head and neck region in southern China, with high incidence rates in regions such as Guangdong, Guangxi, Fujian, Hainan, and Hunan. NPC accounts for approximately 30% of all malignant tumors and 70-80% of head and neck tumors. The incidence rate in males is approximately 2-3 times higher than that in females.[1] NPC has a worldwide incidence of 38% and a mortality rate of 40%.[2] Lee et al and Bossi et al found that genetics, environment, viral infections, diets, and living habits are all important factors in the occurrence of NPC.[3,4]

Middle-aged and older people have the highest NPC incidence, and the incidence for males is 2.5 times that of females.[5] According to the World Health Organization's (WHO's) pathological classification, 98% of NPCs were differentiated and undifferentiated, non-keratinized carcinomas.[6] NPC is highly metastatic and invasive.

Over 70% of nasopharyngeal carcinoma patients present with cervical lymph node involvement at the time of diagnosis, and 10%-50% of patients have had local recurrence and distant metastasis.[7,8] Head and neck lymph nodes in the lymphatic network are abundant, so the LNM rate of NPC patients is relatively high, and metastasis of posterior pharyngeal lymph nodes is the most common.[9] The posterior pharyngeal lymph nodes are adjacent to the nasopharyngeal cavity, so they have a crucial role in the clinical treatment and prognosis of NPC.

It's very important to determine the extent of NPC tumor invasion and LNM situation in the formulation of treatment methods. Due to the special concurrent location of NPC and the dense drainage of lymph nodes in a local anatomical location, 70% of NPC patients have lymph node enlargement at the initial diagnosis, thus affecting the therapeutic effects against the disease.[10]

Moreover, NPC has a unique and relatively concealed disease location in the head and neck region, specifically within the nasopharynx area. The nasopharynx is located deep within and is not easily visible or examined directly, making the early detection of NPC a complex task. Additionally, the nasopharynx is adjacent to many critical organs such as the skull base, eyes, ears, and throat. Therefore, the treatment of NPC needs to take into consideration the protection of these neighboring structures and adjacent to surrounding vital organs, so radiotherapy and chemotherapy have become the dominate treatment modes for NPC.

Treatments

The preferred method for NPC's clinical treatment is radical radiotherapy, while clinicians can also use induction chemotherapy, synchronous radiotherapy, and targeted therapy for adjuvant therapy.[4,11] Using platinum-based synchronous radiotherapy and chemotherapy for locally advanced NPC patients, Zhang et al found that the three-year, local relapse-free survival (LRFS) rate was 85.3%, the three-year overall survival (OS) rate was 94.6%, and 75.5% of patients who suffered from different complications, including radiation dermatitis, mucositis, chemotherapy-induced nausea and vomiting, dysphagia, tinnitus, as well as hearing problems.[12]

Rodriguez-Galindo et al analyzed the effects of induction chemotherapy on the treatment of children with NPC and found that the five-year, event-free survival (EFS) rate and OS rate were 84.3% and 89.2%, respectively. [13] Therefore, clinicians could infer that radiotherapy and chemotherapy can improve the long-term survival rate of patients with NPC, showing obvious beneficial effects. However, NPC exhibits a high malignancy rate, rich blood flow in regional lymph nodes, and no obvious early symptoms of patients.

Regional Lymph Node Metastasis

Zhang et al found that the comprehensive treatment using mainly radiotherapy can effectively alleviate NPC's clinical symptoms, while regional LNM (RLNM), however doesn't affect the short-term curative effect of patients.[14] Short-term efficacy is an important factor in evaluating patients' prognoses. The importance of short-term efficacy lies in its direct impact on the assessment of a patient's prognosis. Short-term efficacy is typically used to evaluate whether treatment is effective and whether it can rapidly alleviate the patient's symptoms and disease progression. Achieving positive short-term results through treatment can often improve a patient's quality of life, reduce suffering, and slow down the progression of the disease. This not only has a positive impact on the patient's physical health but also on their psychological well-being. Furthermore, short-term efficacy can serve as the basis for subsequent treatment plans. If the short-term results are favorable, doctors may continue to recommend the same or similar treatment approaches. Conversely, if the short-term efficacy is suboptimal, a reevaluation of the treatment strategy may be necessary to ensure that the patient achieves the best possible treatment outcomes.[15]

RLNM does affect treatments' therapeutic effects and patients' long-term prognoses.[16,17] Therefore, it's very important to clarify the predictors related to the occurrence of LNM in NPC patients for the formulation of clinical treatment for them and improvement in their prognoses.

Prognostic Factors

Dionyssiou et al found that cancer cells, when the number of metastases exceeds lymph nodes' autoimmune-function and anti-tumor abilities, will enter the blood circulation system through lymphatic reflux, thus increasing the risk of long-term metastasis.[18]

The American Joint Committee on Cancer (AJCC) Staging manual indicates that a lymph-node diameter >6 cm is the standard for N staging.[19] Takabatake et al and Faustino et al found that location, number, and size of lymph nodes in LNM can all impact the prognosis of patients.[20,21] Schrembs et al found that the size of lymph nodes in LNM can directly or indirectly affect NPC patients' prognoses. [22] When nasopharyngeal carcinoma spreads to the lymph nodes, it adds complexity and risk to the disease, often requiring more intricate treatment strategies. Therefore, the presence of lymph node metastasis is typically considered an unfavorable prognostic factor that may impact a patient's survival rate and treatment outcomes.

Zhang et al analyzed the influence of long-term LNM distribution on the prognosis of patients with metastatic NPC after radiotherapy and found that age, N stage, number of metastatic sites, bone metastasis, and subphrenic distant LNM were all related to the OS rate of patients. [23]

Pan et al used meta-analysis to systematically evaluate the difference in long-term survival rate of patients with stage N, posterior pharyngeal LNM and found that the five-year OS rate was about 70% and the cancer-specific survival rate was

about 80%[24] Huang et al found that the OS, distant metastasis-free survival (DMFS), and progression-free survival rates for patients with posterior pharyngeal LNM were significantly lower than those for other NPC patients.[25] The retropharyngeal lymph nodes are lymph nodes located at the back of the throat. The lymphatic system is a part of the body responsible for filtering waste and resisting infections. When cancer cells from NPC enter the lymphatic system and spread to the retropharyngeal lymph nodes, it signifies that the cancer has spread to more distant parts of the body, such as distant lymph nodes, organs, or other tissues. Typically, more aggressive treatment methods like radiation therapy, chemotherapy, or surgery are needed at this stage, which can reduce a patient's long-term survival rate and prognosis. Similarly, Chen et al analyzed the prognosis of NPC patients with LNM who received hyperfractionated radiotherapy and who had lesions of the posterior pharyngeal lymph nodes and found that the long-term prognosis for survival was adverse. [26]

Yao et al analyzed a prognostic model for nonmetastatic, stage N1, NPC patients and found that the serum Epstein-Barr virus, DNA copy number, and gross target volume lymph nodes (GTVnd) were closely related to a poor prognosis and that clinicians could use it to predict long-term metastasis of NPC.[27] Lin et al analyzed the roles of parotid LNM on NPC patients' prognoses and found that parotid LNM was a negative prognostic factor for OS, progression-free survival, DMFS, and LRFS with poor survival outcomes.[28]

The posterior pharyngeal lymph nodes are adjacent to the nasopharyngeal cavity, so they have a crucial role in the clinical treatment and prognosis of NPC.

Colorectal Neoplasia Differentially Expressed

Colorectal neoplasia differentially expressed (CRNDE) is a member of the long, noncoding RNA family and has a major role in promoting oncogenes,[29] which has been confirmed in many studies.[30-32] Xie et al found that CRNDE presents a tendency to high expression in malignant tumors, is involved in the disease course, and increases the risk of a poor prognosis for patients.[33]

Other studies have found findings similar to those of Xie et al's. For instance, Zhao et al found that CRNDE expression was higher for temozolomide-resistant glioma patients than for other patients and that CRNDE knockout can reduce patients' survival rates and glioma cells' proliferation rate through the phosphoinositide 3-kinases (PI3K)/ protein kinase B (Akt)/ mammalian target of rapamycin (mTOR) pathway.[34] Those researchers indicate that clinicians can use CRNDE as a therapeutic target for temozolomide to treat glioma sensitivity.

Zhang et al showed that CRNDE expression was related to chemotherapy's sensitivity in gastric cancer and indicated that clinicians can use it as a potential prognostic marker and therapeutic target for chemotherapy for gastric cancer.[35]

Xin et al found that CRNDE expression was higher in gastric-cancer tissues and tumor-related macrophages than in normal tissues and could mediate ubiquitination of phosphatase and tension homology and then participate in gastric cancer's progression.[36]

Zhang et al analyzed the ability of CRNDE to predict survival rate and tumor recurrence for colorectal-cancer patients with liver metastasis and found that the mortality rate was higher in patients with a high CRNDE expression than in those with normal expression.[37] Those researchers also found that CRNDE expression was closely related to tumor differentiation, degree of primary invasion, LNM, number of liver metastases, and liver-metastasis grade.

Ding et al found that the expression of CRDNE was upregulated in clear renal cell carcinoma and that patients with elevated CRNDE presented the characteristics of having an advanced clinical stage, a large tumor size, LNM, distant metastasis, and a poor pathological grade.[38] Thus, the CRNDE level exhibits a negative correlation with patients' OS rates.

Therefore, researchers speculate that CRNDE expression might have a correlation with NPC LNM, but studies on the subject are relatively few.

Current Study

The current study intended to explore the predictive value of the level of serum CRNDE for RLNM in NPC patients and to analyze the effects of RLNM on their long-term prognosis after radiotherapy and chemotherapy, to provide a reference for early prediction, diagnosis, and treatment of RLNM in NPC.

METHODS

Participants

The research team performed a retrospective case-control study, which took place at Taizhou People's Hospital in Taizhou, China. Potential participants were NPC patients who received radiotherapy and chemotherapy at the hospital between January 2014 and December 2017. Participant identification was completed by selecting patients diagnosed with nasopharyngeal carcinoma from hospital records who received radiation therapy and chemotherapy within the specified time frame. The researchers did not have direct interactions with the patients, and data were collected from historical clinical records.

The study included potential participants if they: (1) met the clinical diagnostic criteria of NPC,[39] which a puncture biopsy had confirmed; (2) had primary NPC; and (3) were able to take care of themselves, had a normal mental state, and could cooperate with treatment.

The "World Health Organization Classification of Head and Neck Tumors" (Fourth Edition) clinical diagnostic criteria for NPC included[40]: (1) a minimum diameter of a cross-sectional lymph node of greater than 1 cm; (2) a central lymph node that was necrotic or showed the features of annular enhancement; (3) clustering of at least three lymph nodes with a minimum diameter of >0.8 cm in a single area; (4) boundaries of lymph-nodes that are irregularly enhanced, with the surrounding fat interval having disappeared; and (5)

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a minimum diameter of laryngeal lymph nodes of greater than 0.4 cm.

The study included potential participants if they: (1) had gastric, colon, or esophageal cancers, or other malignant tumors; (2) had heart, liver, kidney, or other important organ lesions; (3) had received radiotherapy or chemotherapy before admission to hospital, or (4) had infectious diseases. In the preliminary phase of this studyexperiment, a total of 127 participants were initially included, all of whom met the aforementioned diagnostic criteria. However, during the data collection process, it was discovered that 26 patients were subsequently affected by other diseases that could influence the results of this study, 15 patients developed drug allergies during the treatment, and 6 patients had incomplete or missing data. Consequently, data from 47 participants were excluded, and only data from 80 nasopharyngeal carcinoma patients were included for the study.

The ethical principles of the Helsinki Declaration were adhered to in this work, with the aim of safeguarding the rights and privacy of human research subjects. Comprehensive informed consent was obtained from all participants in this work, and necessary measures were taken to ensure their privacy and data security. The highest ethical standards were followed throughout the research process to ensure its legality and reliability. Prior to their involvement in the study, all individuals involved provided explicit consent in accordance with a written informed consent form. The informed consent document detailed the study's objectives, methods, risks, potential benefits, as well as the rights and privacy protection of participants. Patients were presented with an explanation of the research by qualified personnel and had their questions addressed before participating. They had sufficient time to consider whether they wished to participate in the study, and their involvement was entirely voluntary. The content of the informed consent document was written in clear and understandable language to ensure that patients fully understood their rights and the participation process. Each patient personally signed the informed consent document, and their signature represented their voluntary agreement to participate in this experiment. In cases involving minors or legally authorized representatives, the legal guardians also signed the informed consent document. The informed consent process was regarded as a vital measure to ensure research ethics compliance and the protection of patients' rights, guaranteeing transparency and legality throughout the research process.

Procedures

Imaging upon admission. All NPC patients received computerized tomography (CT) and other imaging examinations upon admission.

Treatments. All NPC patients received radical radiotherapy or radiotherapy and chemotherapy, according to International Commission on Radiation Units and Measurements' (ICRU's) patient ICRU62, The reference to "patient ICRU62" relates to a set of guidelines and standards developed by the International Commission on Radiation Units and Measurements (ICRU). These guidelines provide detailed information on how to determine the treatment target volume (the areas requiring radiation therapy) and appropriate radiation dosage when treating nasopharyngeal carcinoma patients. Once the treatment target regions are identified, the hospital conducted three-dimensional conformal radiotherapy (3D-CRT) or intensity-modulated radiation therapy (IMRT) to minimize exposure to surrounding healthy tissues while delivering the treatment. which included determination of a target range and radiation dose. After outlining the treatment's target area for the patient, the hospital conducted three-dimensional chemoradiotherapy (3D-CRT) or intensity-modulated radiation therapy (IMRT).

The radiation dose in the gross tumor area was 60-70 gray (Gy) and in the clinical target area was 50-60 Gy. Patients suffering from stage I NPC might receive radiotherapy alone. Stage II, III, IVA, and IVB NPC patients received concurrent radiotherapy and chemotherapy. The hospital implemented chemotherapy as follows: 30 mg/m2 of cisplatin single for 7 weeks, once a week; 75 mg/m2 of cisplatin combined with 135 mg/m2 of paclitaxel or 75 mg/m^2 of cisplatin with 75 mg/m2 docetaxel on day 1 at 3 weeks apart. "Day 1" refers to the first day of each treatment cycle, which marks the beginning of each course of treatment. "at 3 weeks apart" means that there is a 3-week interval between different treatment cycles, indicating that a new course of treatment begins every 3 weeks. This means that every 3 weeks, on the first day, either 75 mg/m^2 of cisplatin is used in combination with 135 mg/m^2 of paclitaxel, or 75 mg/m^2 of cisplatin is used in combination with 75 mg/m^2 of docetaxel.

Groups. According to the results of the examinations upon admission, the research team divided participants into two groups: (1) an observation group diagnosed with RLNM and (2) a control group that had no RLNM.

Data collection. In this work, a retrospective case-control research design was employed. Data collection primarily relied on existing medical records and related documents, including patient histories, laboratory reports, imaging examinations, surgical reports, and other patient-related information. During the data collection process, it extracted essential information pertaining to the study's target and control variables. This encompassed patients' basic information, disease diagnoses, treatment histories, laboratory test results, surgical reports, and other data relevant to the research question. We also documented the diagnosis date, treatment regimens, treatment duration, and other crucial time points for each case to ensure data accuracy and completeness. Approval from the research ethics committee was obtained to access and analyze patients' medical records. All data access and usage adhered to pertinent ethical regulations and legal requirements to safeguard patient privacy. Data collection was carried out by research personnel who had undergone training in medical data confidentiality. These individuals possessed medical backgrounds, enabling them to accurately extract and record case information. The

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retrospective data collection covered a specific timeframe, spanning from January 2014 to December 2017. Cases within this timeframe were included in the study sample.

Karnofsky (KPS) scores. Originally developed for cancer patients, this scoring system has later found application in the assessment of other chronic diseases. The scoring is based on the extent to which patients can perform specific activities and daily functions. The key indicator is the patient's functional ability, categorized into a range of levels, from complete disability to normal activity. A score of 100 represents full normalcy, no symptoms, and no activity restrictions. A score of 10 indicates a patient is in the terminal stage, likely requiring specialized symptom palliation treatment.

Tumor, Node, Metastasis (TMS) staging. The tumor, nodes, metastasis (TMS) staging system typically includes five stages: I = early-stage cancer, usually localized at the primary site. II = locally advanced but still confined to the primary site. Although more severe than Stage I, there are still treatment opportunities. III = regional lymph node involvement stage, indicating a more severe condition. Treatment usually requires more complex strategies. IV = distant organ metastasis, the most severe stage requiring more complex treatment and having a less favorable prognosis. V = additional special circumstances, such as recurrence or secondary tumors. TMS staging helps determine treatment plans and predict disease prognosis.

Outcome measures. The research team: (1) determined the level of CRNDE in participants' serum to predict the risk of RLNM, (2) compared clinical total response rates (TRRs) between the groups, and (3) analyzed the five-year OS, LRFS, and DMFS.

Detection of serum CRNDE. The hospital: The main objective of this work was to explore the association of CRNDE levels with specific diseases or pathological conditions by analyzing existing serum samples. The experiment followed a retrospective research design, and researchers did not directly interact with patients or obtain new venous blood samples. Upon admission, patients had 5 mL of venous blood drawn, and these samples were previously collected and stored in the research institution's biospecimen repository. The advantage of this approach was the utilization of existing resources, avoiding the need for additional patient recruitment and data collection efforts. The experiment received approval from the research ethics committee to ensure the legal use of existing biospecimens and strict adherence to ethical regulations and legal requirements for the protection of patient privacy and rights. (1) on a patient's admission, obtained 5 mL of his or fasting venous blood; (2) after treating it with an anticoagulant of heparin sodium, collected serum using centrifugation; (3) extracted total RNA from the serum using the TRIzol method (Sigma-Aldrich. St. Louis, Missouri, USA); (4) detected the concentration, purity, and integrity of the extracted RNA using a microspectrophotometer (JASCO, Kyoto, 日本) and agarose gel electrophoresis (Thermo Fisher SCIENTIFIC, Waltham, Massachusetts, USA); (5) performed complementary DNA (cDNA) reverse transcription to extract RNA, strictly according to manufacturer's instructions for a reverse transcription kit, the PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) from Beijing Baori Biotechnology (Beijing, China); and (6) using cDNA as a template, determined the expression of the target gene according to the manufacturer's instructions for the real-time fluorescence quantitative assay kit, the TB Green Premix Ex Taq II (Tli RNaseH Plus) from Beijing Baori Biotechnology.

The reaction system included the following reagents: (1) 6.5 μL of a TB Green Premix Ex Taq II (Tli RNaseH Plus) from Baoxin Yuanbai Biotechnology Co., LTD., Xinjiang, China), (2) 0.5 μL of an upstream primer, CRNDE_F (Thermo Fisher SCIENTIFIC, Waltham, Massachusetts, USA), (3) 0.5 μL of a downstream primer, CRNDE_R (Thermo Fisher SCIENTIFIC, Waltham, Massachusetts, USA), (4) 1.0 μL of cDNA template (Thermo Fisher SCIENTIFIC, Waltham, Massachusetts, USA), and (5) 4 μL of sterilized water.

The reaction procedure was set as follows: 30 s at 95°C and 60°C for 40 cycles, respectively, and 10 min at 4°C. The research team used glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference gene: (F) 5 '-TGTGGGCATCAATGGATTTGG-3'; (R) 5 '-ACACCATGTATTCCGGGTCAAT-3'. The team calculated the relative expression level of CRNDE using the 2-CT method: (F) 5'-CGATCGCGCTATTGTCATGG-3'; (R) 5'-TCCGCCTCGCTTAGACATTG-3'.

Treatment effects and follow-up evaluation. Based on Response Evaluation Criteria in Solid Tumors,[41] the hospital evaluated the treatments' effects on tumor regression using nasopharyngeal-enhanced magnetic resonance imaging (MRI) and other imaging examinations.

For the responses: (1) complete response (CR) = all lesions had disappeared and serum-oncology marker levels had been normal for 4 weeks; (2) partial response (PR) = 30% or more reduction in the primary lesion's diameter compared to the time of admission and maintained for 4 weeks; (3) stable disease (SD) = the primary lesion's diameter had decreased by 0-30% or increased by 0-20% compared with the time of admission, but no new lesions had occurred; and (4) disease progression (PD) = an increase of 20% or more in the primary lesion's diameter compared to the time of admission or a new lesion.

ROC analysis. The research team created an ROC area for the CRNDE expression related to NPC RLNM prediction. The data used in the experiment were obtained from a selected sample of nasopharyngeal carcinoma (NPC) patients from the research institute. These samples included known positive (those with cervical lymph node metastasis, RLNM) and negative (those without RLNM) cases. The samples were sourced from clinical records and examinations of the cases. The samples were derived from the analysis of 5 ml venous blood collected from patients upon admission, as documented in the clinical records and examinations. The study measured

the expression levels of the CRNDE gene in each sample using standard experimental methods to extract RNA and perform quantitative PCR analysis. The RLNM status of each sample was determined based on clinical records and imaging examination data. Positive samples indicated the presence of RLNM, while negative samples indicated the absence of RLNM. Proper processing and standardization of CRNDE expression data were carried out to ensure data consistency and comparability. This included data cleaning and the removal of outliers, among other processing steps.

Follow-up. The research team followed-up with all participants at regular intervals, with a median follow-up time of 36 months. The team analyzed the five-year OS, LRFS, and DMFS.

Outcome Measures

Serum CRNDE expression. The research team examined the differences in serum CRNDE expression levels between NPC patients with and without RLNM.

Diagnostic efficacy. The research team set 3.540 as the cut-off value for the serum CRNDE level. That level allowed the research team to diagnose NPC RLNM. To determine an appropriate cutoff value, we utilized statistical analysis methods by creating ROC curves based on a combination of previous research and clinical data, with diagnostic test performance measured by the AUC. The chosen cutoff value was 3.540. This value was selected because it strikes a balance between sensitivity and specificity, minimizing misclassification to the greatest extent. If a patient's CRNDE level is higher than 3.540, we diagnose them as RLNM positive; if it is lower than 3.540, we diagnose them as RLNM negative. This process allows us to make RLNM status diagnoses based on CRNDE levels.

Short-term efficacy. Three weeks after the start of treatment, a short-term efficacy assessment was conducted for the patients, with the following evaluation criteria. Complete remission (CR): After short-term treatment, the tumor completely disappeared or significantly decreased, there were no RLNM, no new lesions appeared, and the serum tumor marker levels returned to normal for at least 4 weeks. Partial remission (PR): After short-term treatment, the tumor exhibited significant shrinkage (15%), with no RLNM and no new lesions for at least 4 weeks. Stable disease (SD): The patient's tumor did not significantly increase in size after short-term treatment, but also did not exhibit significant shrinkage, with tumor diameter changes within 15%. Disease progression (PD): The patient's tumor showed a significant increase in size after short-term treatment, typically indicated by a tumor diameter increase of over 15% or the appearance of new lesions.

Long-term prognosis. To assess the long-term prognosis of non-metastatic NPC patients, we plotted cumulative survival curves, with a specific focus on five-year overall survival (OS), distant metastasis-free survival (DMFS), and local recurrence-free survival (LRFS) for non-metastatic patients with RLNM. This work included non-metastatic NPC patients categorized into RLNM and non-RLNM groups. The long-term follow-up data for these two groups were collected, including data on five-year OS, DMFS, and LRFS. Subsequently, the survival analysis methods were employed to create cumulative survival curves to depict the survival status over five years between these groups. High OS indicated better survival, high DMFS reflected a lower risk of distant metastasis, and high LRFS signified a lower risk of local recurrence. This aided in the understanding of treatment effectiveness and prognosis.

Factors influencing long-term prognosis. According to the European Society for Radiation Oncology and Imaging Data's standards set, the research team classified the observation group's clinical data into mandibular lymph nodes, upper neck lymph nodes, middle neck lymph nodes, lower neck lymph nodes, posterior neck lymph nodes, anterior neck lymph nodes, and posterior pharyngeal lymph nodes. The research team analyzed the affecting factors for the observation group's five-year OS rate of NPC patients. The team divided the group into a survival group and a mortality group. By identifying the impact of involvement in different lymph node locations, it can determine which affected lymph nodes are significantly associated with patient survival rates. This provided doctors with more detailed information, aiding in a more accurate assessment of patient prognosis and the development of treatment strategies. If it is discovered that specific lymph node locations are correlated with lower survival rates, this may inspire further research to improve treatment strategies. Based on the analysis of these influencing factors, we can better implement personalized treatment strategies to meet the individual needs of different patients.

Statistical Analysis

The research team analyzed the data using the Statistical Package for Social Science (SPSS) 19.0 (IBM, Armonk, NY, USA). The team: (1) expressed categorical data as numbers (N) and percentages (%) and compared differences between the groups using the Chi-square (χ^2) test, (2) expressed continuous data as means ± standard deviations (SDs) and used the *t* test to compare differences between groups, and (3) collected participants' demographic and clinical data and selected COX regression analysis on the factors influencing the five-year OS rate of NPC patients.

The team plotted the receiver operating curve (ROC) and computed the area under the curve (AUC), based on which predictive value of serum CRNDE level to LNM risk in NPC patients that the team was evaluating: (1) when AUC>0.9, the predicted performance was excellent; (2) when the AUC ranged from 0.7 to 0.9, the predicted performance was normal; and (3) when the AUC was between 0.5 and 0.7, the predicted performance was bad.

The team drew a Kaplan-Meier curve to evaluate the prognosis of NPC patients suffering from RLNM, and used the Log-Rank to compare the differences between groups. $P < .05$ indicated statistically significant differences.

RESULTS

Participants

The research team included and analyzed the data of 80 participants, with 52 participants in the observation group and 28 participants in the control group (Table 1).

The observation group included 29 males (55.77%) and 23 females (44.23%), with the mean age of 56.71 ± 3.82 years and on admission, the group's KPS score was 66.09 ± 3.51. For the TNM stages, the observation group had 7, 14, 13, and 12 participants in stages I (13.46%), II (26.92%), III (25.00%), and IV (23.08%), respectively.

The control group included 16 males (57.14%) and 12 females (42.86%), with the mean age of 55.04 ± 3.54 years, and on admission, the group's KPS score was 67.13 ± 3.82. For the TNM stages, the control group had 8 in participants stage I (28.57%), 5 in stage II (17.86%), 5 in stage III (17.86%), and 5 in stage IV (17.86%).

No significant differences existed in gender, age, KPS score on admission, and TNM stage ($P > .05$).

The observation group's serum CNRDE expression, at 3.26±1.40, was significantly higher than that of the control group, at 3.73 ± 1.21 ($P < .01$).

Serum CRNDE Expression

The serum CRNDE levels in the observation group and control group were 3.73 ± 1.21 and 3.26 ± 1.40, respectively (Figure 1). The observation group's CRNDE expression was significantly lower than that of the control group ($P < .01$).

Diagnostic Efficacy

Figure 2 shows the ROC area for the CRNDE expression for NPC RLNM prediction: (1) the AUC value was 0.805; (2) the 95% CI was 0.715-0.889; (3) the negative and positive predictive values were 72.7% and 79.3%, respectively; (4) the Yoden index was 0.463 and its accuracy was 77.5%; and (5) the diagnostic sensitivity and specificity were 88.5% and 57.1%, respectively (data not shown). These results suggested that elevated serum CRNDE levels can be a basis to predict RLNM risk in NPC patients. The research team was able to use the serum CNRDE level to diagnose NPC RLNM for 58 participants in the observation group and 22 in the control group.

Short-term Efficacy

Figure 3 shows the differences in the short-term efficacy of radiotherapy and chemotherapy between the observation and control groups (data not shown). For observation group, 30 participants had a CR (57.7%), 21 had a PR (40.4%), one had a SD (1.9%), and no participants had PD (0.0%). In the control group, 17 participants had a CR (60.7%), 11 had a PR (39.3%), no participants had SD (0.0%) or PD (0.0%). No significant differences existed between the groups in the treatment's efficacy related to a CR, PR, SD, or PD ($P > .05$).

Long-term Prognosis

For the observation group, 38 participants showed OS (73.1%), 33 showed DMFS (63.5%), and 45 showed LRFS

Table 1. Participants' Demographic and Clinical Characteristics

Characteristics	Non-LMN Group n = 28 Mean ± SD n (%)	RLMN Group n = 52 Mean ± SD n (%)	χ^2/t	*P* value
Age, y	55.04 ± 3.54	56.71 ± 3.82	1.324	.781
Gender			4.554	.824
Male	17 (60.71)	29 (55.77)		
Female	11 (39.29)	23 (44.23)		
KPS Scores	67.13 ± 3.82	66.09 ± 3.51	3.376	.923
TNM Stages			3.120	.848
Stage I	6(21.43)	9(17.31)		
Stage II	8 (28.57)	15 (28.85)		
Stage III	10 (35.71)	19 (36.54)		
Stage IV	2 (7.14)	6 (11.54)		
Stage V	2(7.14)	3 (5.77)		
CRNDE	66.73	65.35	4.743	1.331

Abbreviations: CRNDE, colorectal neoplasia differentially expressed; KPS, Karnofsky; LMN, lymph node metastasis; RLNM, regional LMN; TNM, tumor, node, metastasis

Table 2. Effects of RLNM on Five-year OS Rate of the RLMN Group (N = 80)

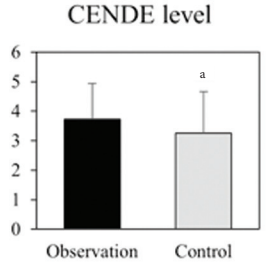
Factors		Survival Group n = 63 n (%)	Mortality Group n = 17 n (%)	χ^2	*P* value
Lymph Node's Site	Unilateral	38 (60.32)	10 (58.82)	0.439	.411
	Bilateral	25 (39.68)	7 (41.18)		
Diameter of Metastasized Lymph Nodes	0-6 cm	48 (76.19)	8 (47.06)	7.890	.002[b]
	>6 cm	15 (23.81)	9 (52.94)		
Number of Metastasized Lymph Nodes	0-1	39 (61.91)	6 (35.29)	5.823	.025[a]
	>2	24 (38.09)	11 (64.71)		
LNM Jaw	Yes	27 (42.86)	8 (47.06)	1.937	.083
	No	36 (57.14)	9 (52.94)		
LNM of the Upper Neck	Yes	27 (42.86)	10 (58.82)	0.545	.408
	No	36 (57.14)	7 (41.18)		
Mid-neck LNM	Yes	36 (57.14)	10 (58.82)	0.202	.533
	No	27 (42.86)	7 (41.18)		
LNM of Lower Neck	Yes	28 (44.44)	9 (52.94)	0.138	.674
	No	35 (55.56)	8 (47.06)		
Posterior Cervical LNM	Yes	30 (47.62)	7 (41.18)	0.145	.660
	No	33 (52.38)	10 (58.82)		
Anterior Cervical LNM	Yes	35 (55.56)	9 (52.94)	0.159	.643
	No	35 (44.44)	8 (47.06)		
Posterior Pharyngeal LNM	Yes	24 (38.10)	9 (52.94)	3.516	.049[a]
	No	39 (61.90)	8 (47.06)		

[a]$P < .05$, indicating that the mortality group's number of metastasized lymph nodes and rate of posterior pharyngeal LNM were significantly higher than those of the survival group

[b]$P < .01$, indicating that the mortality group's diameter of lymph nodes was significantly higher than that of the survival group

Abbreviations: OS, overall survival; RLNM, regional lymph node metastasis.

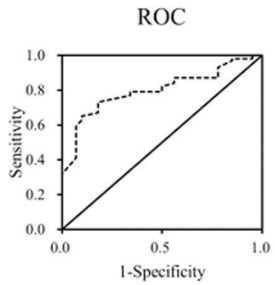
Figure 1. Comparison of Serum CRNDE Levels of the observation group (n = 52) and control group (n = 28) Groups



[a]$P < .01$, indicating that observation group's CRNDE expression was significantly lower than that of the control group

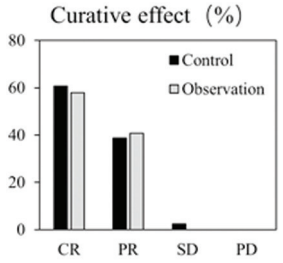
Abbreviations: CRNDE, colorectal neoplasia differentially expressed; RLNM, regional LMN.

Figure 2. ROC Curve of Serum CRNDE in Predicting Risk of Regional Lymph Node Metastasis.



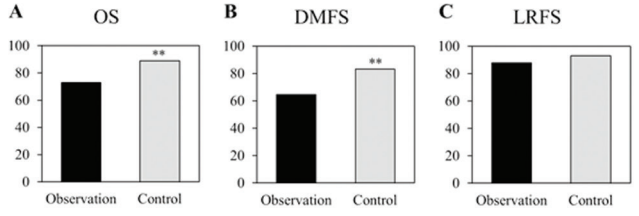
Abbreviations: CRNDE, colorectal neoplasia differentially expressed; RLNM, regional lymph node metastasis; ROC, receiver operating curve

Figure 3. Comparison of Short-term Efficacy of the observation group (n = 52) and control group (n = 28) Groups After Radiotherapy and Chemotherapy



Abbreviations: CR, complete response; PD, disease progression; PR, partial response; RLNM, regional lymph node metastasis; SD, stable disease

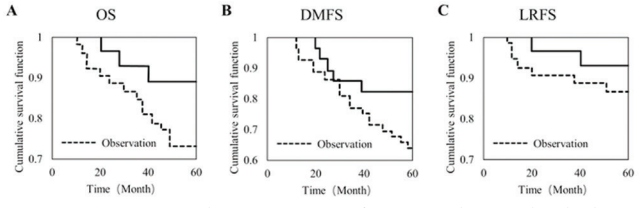
Figure 4. Comparison of the OS, DMFS, and LRFS of the observation group (n = 52) and control group (n = 28) at Five Years Posttreatment. Figure 4A-4C show the five-year OS, DMFS, and LRFS rate, respectively.



** $P < .01$, indicating observation group's five-year OS and DMFS were significantly lower than those of control group

Abbreviations: DMFS, distant metastasis-free survival; LRFS, local relapse-free survival; NPC, nasopharyngeal carcinoma; OS, overall survival; RLNM, regional lymph node metastasis

Figure 5. Five-year Cumulative Survival Curves of NPC Patients With OS, DMFS, and LRFS. Figures 5A-5C show the five-year OS, DMFS, and LRFS rates, respectively.



Abbreviations: DMFS, distant metastasis-free survival; LRFS, local relapse-free survival; NPC, nasopharyngeal carcinoma; OS, overall survival, RLNM, regional lymph node metastasis

(86.5%). For the control group, 25 participants showed OS (89.3%), 23 showed DMFS (82.1%), and 26 showed LRFS (92.9%). The observation group's five-year OS and DMFS were significantly lower than those of the control group ($P < .01$). No significant differences existed between the groups the in five-year LRFS ($P > .05$) Figure 4C.

Figure 5 shows the five-year OS, DMFS, and LRFS rates. The observation group OS and DMFS rate between control group and patients ($P < .05$). No significant difference existed between the groups in the five-year LRFS rate ($P > .05$).

Factors Influencing Long-term Prognosis

In the mortality subgroup of observation group, Table 2 indicated that seven participants had lymph node diameters ranging from 0 to 6 centimeters and experienced metastasis (constituting 47.06%) and nine had lymph nodes with diameters of >6 cm (52.94%). In the survival subgroup, 48 participants had lymph nodes with diameters of 0-6 cm (76.19%) and 15 had lymph nodes with diameters of >6 cm (23.81%).The survival subgroup's rate for lymph nodes with a diameter of >6 cm was significantly higher than that of the survival subgroup ($P = .002$).

In the mortality subgroup, six participants had 0-1 lymph nodes with metastasis (35.29%) and 11 had >2 lymph nodes with metastasis (64.71%). In the survival subgroup, 39 participants had 0-1 lymph nodes with metastasis (61.91%) and 24 had >2 lymph nodes with metastasis (38.09%). The survival subgroup's rate of lymph nodes with metastasis was significantly higher than that of the survival subgroup ($P = .025$).

In the mortality subgroup, rate of posterior pharyngeal LNM, at 52.94% for nine participants was significantly higher than that of the survival subgroup, at 38.10% for 24 participants ($P = .049$).

No significant differences existed between the groups in the five-year OS rate for the lymph-node's site and for the rates of jaw LNM, upper-neck LNM, middle-neck LNM, lower-neck LNM, posterior-neck LNM, and anterior-neck LNM ($P > .05$).

DISCUSSION

This work aimed to investigate the predictive value of serum CRNDE levels for regional lymph node metastasis in NPC patients and analyze the impact of RLNM on the long-term prognosis of patients after radiotherapy and chemotherapy.

Serum CRNDE is a non-coding RNA initially discovered in colorectal cancer tissues. It has been found to play a role in the proliferation, invasion, and metastasis of tumor cells. Additionally, CRNDE can influence cancer development through various mechanisms, such as regulating gene expression, participating in cell cycle control, and modulating stem cells. In this study, serum CRNDE is used as a potential biomarker to assess its levels in the blood of cancer patients. Elevated serum CRNDE levels are often associated with the presence or progression of cancer. Therefore, measuring CRNDE levels in the blood may aid in early cancer screening,

diagnosis, and prognosis assessment. This work found a significant increase in serum CRNDE levels in RLNM patients, indicating the potential diagnostic value of CRNDE in predicting RLNM. This discovery is consistent with previous research by Solís-Fernández, further emphasizing the significance of CRNDE in the field of oncology.[42]

Regarding long-term prognosis for patients, it was found that RLNM has a significantly negative impact on the five-year OS and DMFS of NPC patients. This result emphasizes once again the importance of RLNM in the treatment of nasopharyngeal cancer. Patients with multiple lymph node metastases, larger lymph node metastases, or metastases in the retropharyngeal lymph nodes have a worse long-term prognosis. These findings help clinical physicians better assess the prognosis of patients and may necessitate more aggressive treatment strategies for patients with RLNM.

This work was subjected to several limitations. Firstly, the sample size was relatively small, which may limit the generalizability of the study's findings. Further large-scale multicenter studies were needed to validate our results. Secondly, this work was retrospective in nature, which carried the risk of information and selection bias. Therefore, more prospective studies were required to confirm our findings. Finally, although this work suggested the potential utility of CRNDE in predicting RLNM, further biological research is needed to elucidate the specific mechanisms by which CRNDE functions in NPC.

In summary, the results of this work emphasized the potential importance of CRNDE as a predictive marker for RLNM and provided valuable insights into the long-term prognosis of NPC patients with RLNM. Nevertheless, further research is needed to validate these findings and elucidate the biological mechanisms involving CRNDE.

CONCLUSIONS

An abnormal increase in serum CRNDE can be a basis to diagnose RLNM in NPC patients. RLNM affected the long-term prognosis of NPC patients, and the number and diameter of lymph nodes and posterior pharyngeal metastasis were the factors affecting patients' long-term. The current study's findings can provide a reference for the realization of the early diagnosis of NPC RLNM, formulating the treatment schemes and improving the long-term survival outcome of NPC patients.

AUTHORS' DISCLOSURE STATEMENT

The authors have no potential conflicts of interest to report relevant to the study.

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