

Plasma progastrin levels in patients with oral squamous cell carcinoma – a cross-sectional study

Niveaux plasmatiques de progastrine chez les patients atteints de carcinome épidermoïde oral – une étude transversale

Madan Kumar P D¹, Anu V², Srividya Kumaravel³, Ranganathan Kannan⁴, Aswath Narayanan MB⁵

1. Professor and Head, Ragas Dental College and Hospital, Department of Public Health Dentistry, Chennai, India
2. Professor, Sathyabama University Dental College and Hospital, Department of Public Health Dentistry, Chennai, India
3. Reader, Sathyabama University Dental College and Hospital, Department of Biochemistry, Chennai, India
4. Professor and Head, Ragas Dental College and Hospital, Department of Oral and Maxillofacial Pathology, Chennai, India
5. Professor and Head, Tamil Nadu Government Dental college and Hospital, Department of Public Health Dentistry, Chennai, India

ABSTRACT

Introduction: Progastrin, is over-expressed in human colorectal tumor cells and also directly targets the β -catenin/Tcf4 oncogenic pathway, The association between Wnt/ β -catenin pathway and cancer is not merely restricted to colorectal cancer as disruption of this pathway results in epithelial mesenchymal transition in oral carcinogenesis. As the role of progastrin in subjects with Oral cancer (OC) is less explored, this study was contemplated to assess the role of plasma progastrin levels in subjects with various stages of oral squamous cell carcinoma.

Methods: The present study was a cross-sectional study done among 60 subjects who were divided into two study groups: Group OC (n=30) who have histologically confirmed oral squamous cell carcinoma and Group C (n=30) who were healthy controls. 10ml of blood samples were drawn from the study subjects and plasma was analysed for progastrin peptide.

Results: The present study demonstrated that the mean plasma progastrin levels (hPG80) were significantly elevated in Group OC (20.51 pM/L) when compared to the control (9.9 pM/L). No significant difference in Plasma level of progastrin between well, moderate and poorly differentiated OC and with increasing staging of OC.

Conclusion: The present study showed increased levels of plasma progastrin in subject with oral squamous cell carcinoma when compared to the control, highlighting the potential of using plasma progastrin levels as a biomarker for early detection of oral malignancies

Keywords: Oral squamous cell carcinoma, Human Progastrin, Oral Malignant Disorders, UV Spectrometer, plasma level

RÉSUMÉ

Introduction: La progastrine est surexprimée dans les cellules tumorales colorectales humaines et cible directement la voie oncogénique β -caténine/Tcf4. L'association entre la voie Wnt/ β -caténine et le cancer ne se limite pas au cancer colorectal, car la perturbation de cette voie entraîne une transition épithélio-mésenchymateuse dans la carcinogénèse buccale. Le rôle de la progastrine chez les patients atteints de cancer buccal (CB) étant peu exploré, cette étude a été envisagée pour évaluer le rôle des taux plasmatiques de progastrine chez des sujets à différents stades de carcinome épidermoïde oral.

Méthodes: La présente étude était une étude transversale menée auprès de 60 sujets répartis en deux groupes : le groupe CB (n=30), comprenant des patients atteints d'un carcinome épidermoïde oral confirmé histologiquement, et le groupe C (n=30), constitué de témoins sains. Des échantillons de sang de 10 ml ont été prélevés chez les sujets de l'étude, et le plasma a été analysé pour détecter le peptide de progastrine.

Résultats: Cette étude a démontré que les taux plasmatiques moyens de progastrine (hPG80) étaient significativement plus élevés dans le groupe CB (20,51 pM/L) que chez les témoins (9,9 pM/L). Aucune différence significative n'a été observée dans les niveaux plasmatiques de progastrine entre les CB bien, modérément et peu différenciés, ni avec l'augmentation du stade du CB.

Conclusion: La présente étude a révélé des niveaux accrus de progastrine plasmatique chez les sujets atteints de carcinome épidermoïde oral par rapport aux témoins, soulignant le potentiel d'utilisation des taux plasmatiques de progastrine comme biomarqueur pour la détection précoce des tumeurs malignes buccales.

Mots-clés : Carcinome épidermoïde oral, Progastrine humaine, Affections malignes buccales, Spectromètre UV, Niveau plasmatique

Correspondance

Madan Kumar PD

Ragas Dental College and Hospital, Department of Public Health Dentistry, Chennai, India

Email: madankumar@rdc.ac.in

INTRODUCTION

Oral cancer (OC) is the 6th most common cancer worldwide and the second most common cancer in India [1]. Oral Potentially Malignant Disorders (OPMD) precedes many cases of OC and the prevalence of OPMD is 10.4% in Indian population. Its prevalence is higher in states like Kerala and Gujarat in which 54 per 1 Lakh population are affected [2]. Early detection of cases is key for prevention of malignant transformation and for better survival and treatment. Though visual examination has been the mainstay method for early diagnosis, recent advances in the field of biomarkers have evoked interest for identifying biomarkers for universal screening and early case finding. The National Cancer Institute has defined "biomarker" as a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition of disease [3]. According to the US Food and Drug Administration, an ideal biomarker should be specific, sensitive, predictive, robust, and able to bridge preclinical and clinical trials [4]. Biomarkers play an important role in distinguishing between the presence or absence of disease.

The potential of plasma progastrin, a polypeptide, as a biomarker for a variety of cancers has been studied for more than 30 years. Progastrin, which is produced in gastric antrum G cells, is physiologically processed into gastrin by intracellular proconvertase enzymes and released into the circulation. Consequently, progastrin has been known historically as a precursor of gastrin, without any other physiological functions. GAST, which encodes progastrin, is over-expressed in human colorectal tumor cells and a direct target of the β -catenin/Tcf4 oncogenic pathway, leading to progastrin accumulation. As progastrin is unprocessed or poorly processed into gastrin in colorectal cancer (CRC) cells, it is released from the tumor cells and becomes detectable [5].

The association of the Wnt/ β -catenin pathway and cancer is not merely restricted to CRCs. Progastrin is a ubiquitous biomarker of cancer involving multiple tumor types. Published literature review has highlighted that progastrin is detectable in the blood of at least 11 different types of cancers even at an early stage (breast, uterus, ovarian, prostate, kidney, colorectal, pancreas, esophagus/stomach, liver, skin, melanoma and lung) [5]. However there exist sparse data on the role of progastrin in oral carcinogenesis. Since Wnt/ β -catenin pathway plays a pivotal role in both in oral squamous cell carcinoma and epithelial mesenchymal transition of oral potentially malignant disorders [6], this study was contemplated to assess the role of plasma progastrin levels in subjects with various stages of oral squamous cell carcinoma and compare its levels to a non-cancerous population. The objectives of the study were to assess the level of progastrin in Oral cancer patients and control group, and within the various stages of oral cancer.

METHODS

Study design and Ethical consideration

This cross-sectional study aimed to assess plasma progastrin levels in patients with different stages of oral squamous cell carcinoma (OSCC) and compare them with a healthy control group. The study protocol was approved by the Institutional Review Board, and necessary permissions were obtained from the relevant institutional authorities. The investigation adhered to the principles of Good Clinical Practice (GCP), and all participant data were handled with strict confidentiality. Written informed consent (provided in bilingual format) was obtained from each participant after a thorough review of the study information sheet.

Study setting, location and Phases

The study was conducted in two phases. Phase I involved patient recruitment, screening, and histopathological assessment, while Phase II focused on measuring plasma progastrin levels using UV spectrophotometry.

Phase I was conducted through an outreach program in collaboration with a secondary care hospital, Ranipet district, consisting of Ranipet town and other small towns and villages, is a semi-urban industrial district in Tamil Nadu, India, with a heterogeneous population, similar to other districts in the country. Tobacco and alcohol use in this population are comparable to those in other Indian districts [7].

From an eligible population of 40,852 individuals, a total of 1,389 subjects (1,012 men and 377 women) were screened for oral mucosal lesions, which were subsequently confirmed via histopathological examination. Details of the screening process and case findings have been previously published [8]. Additional subjects were recruited from Vaai Ganam, an oral screening initiative for professional truck drivers in Chennai's suburbs, where 747 drivers were evaluated. The results of this screening have also been published [9]. For all identified lesions, standard punch biopsies were performed, and tissues were histologically examined.

For the present study, 30 subjects with histologically confirmed oral squamous cell carcinoma (OSCC) were selected. Controls (n=30) were recruited from voluntary blood donors at the institution. The study was conducted in full accordance with STROBE guidelines, ensuring methodological transparency and adherence to rigorous reporting standards. The inclusion and exclusion criteria for recruiting subjects for the present study were:

Inclusion Criteria

1. Subjects with histologically confirmed oral squamous cell carcinoma (Group OC)
2. Subjects who give consent to participate.
3. Subjects aged above 18 years.
4. Control subjects included those who were free from any systemic disease

Exclusion criteria

1. Subjects with past history of any major illness such as hepatic disease, tuberculosis, hypertension, gout, renal disease, diabetes or metabolic syndrome, gastritis and those which require any medications.
2. Subjects under drug treatment for any epithelial dysplasia.
3. Subjects undergoing radiotherapy or chemotherapy for cancer or any history of malignancy other than oral cancer.
4. Subjects with history of gastric disease with a potential to influence progastrin levels
5. Subjects, on stool examination who demonstrate presence of occult bleeding or expression of H.Pylori antigen
6. Subjects taking antibiotics or other medications that could influence the clinical characteristics for at least 3 months,
7. Subjects with current history of tobacco use, and pregnancy/lactation.

Sample size calculation

Sample size calculation was done using GPower software [10] for comparing the means of two independent groups. In this case, with an effect size of 0.75, a significance level (α) of 0.05, and a desired statistical power of 0.80, the required sample size was computed using an allocation ratio of 1:1. The resulting sample size for each group was 29, leading to a total of 58 participants. Given practical constraints and to maintain balanced group sizes, the final sample size was rounded to 60, with 30 participants in each group.

For the Phase 2 part of the study, blood samples which were drawn from the study subjects for their systemic health assessment were used for the study. Ten millilitres of venous blood was collected from the antecubital vein with a syringe by a trained phlebotomist and placed in vials of 2 ml each (5 sets). The samples were subjected to centrifuge at 2000 RPM at room temperature for 15 minutes immediately. The supernatant was separated and considered as plasma extract. In most cases Plasma samples were subjected to treatment immediately after withdrawal. However, in case of any delay the samples were stored at 4 degrees and were subjected for analysis within 24 hours.

Treatment of Samples

Two ml of plasma was taken in a 15 ml graduated centrifuge tube. 4ml of 0.05% trifluoroacetic acid (TFA) along with 2ml of plasma sample was mixed with 60 μ l of 10% TFA. First centrifugation was carried out for 15 minutes at 2000 rpm. 2ml of the supernatant was added with 10 ml of 0.05% TFA and 2nd centrifugation was done for about 15 minutes at 2000rpm. Again, 2ml supernatant was mixed with 3ml of 80% acetonitrile and 3rd centrifugation was carried out for 15 minutes at 2000rpm. The final collected 2ml supernatant was subjected to UV-1800 UV spectrophotometer at 214nm.

UV Spectrographic Analysis

For all analysis, a standard solution and a control solution was prepared. For preparation of standard - 400microg/L samples, 800microg/L samples and 1200 microg/L samples were prepared and analysed under UV spectrometer. The standards were demonstrated at 214 nm wavelength and the Optical density (OD) values were 0.887, 1.880 and 2.414 respectively. For all the test solutions, OD values were recorded at 214 nm and the progastrin levels were calculated using the following formula:

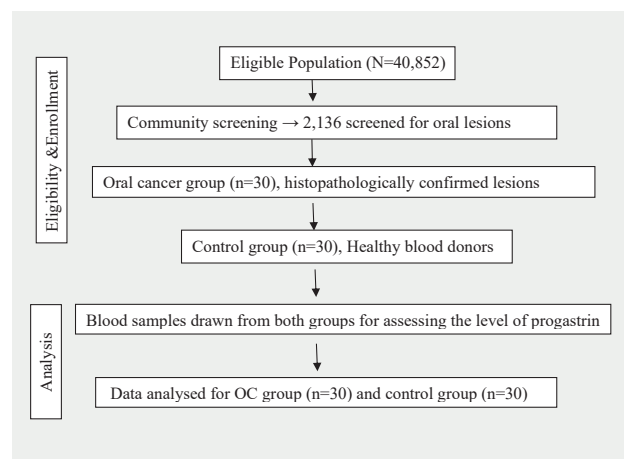
Observed OD x Standard nm/Standard OD x volume of test sample

All samples were prepared in triplet and the mean value was calculated. Sample collection and testing of the samples were done at the Department of Biochemistry, Sathyabama University and Lifecell Diagnostics, Chennai.

Data analysis

The quantitative data were entered into a Microsoft Excel spreadsheet and analysed using IBM SPSS software (Version 20). Descriptive statistics were used to summarize the baseline characteristics of the study population. Since the data followed a non-normal distribution, non-parametric inferential tests were employed for further analysis. The Kruskal-Wallis test was conducted to determine whether significant differences existed in the mean plasma progastrin levels among study subjects based on clinical and histological staging. For all tests, the alpha error was set at 5%, with a p-value of less than 0.05 considered statistically significant.

RESULTS



A total of 60 patients were evaluated, with 30 individuals assigned to each group. Plasma progastrin levels were significantly higher ($p < 0.001$) in the oral cancer (OC) group (20.47 ± 0.14 pM/L) compared to the control group (9.92 ± 1.03 pM/L). Table 1 summarizes the mean and standard deviation of participants age and plasma progastrin levels for both groups.

Table 1. mean age of participants and Plasma Progastrin levels of group OC and control group

	Group OC	Control Group
Age (Mean $\pm$ SD)	59.13 $\pm$ 11.45	50.067 $\pm$ 11.16
Level of HpG80 detected in pmol/L of plasma	20.47 $\pm$ 0.14	9.92 $\pm$ 1.037

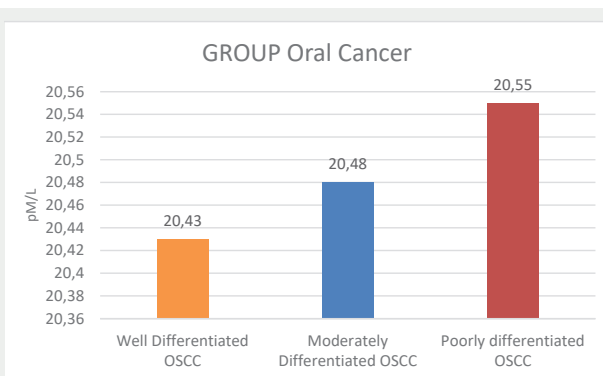
Further analysis of the OC group revealed no statistically significant differences ($p = 0.542$) in progastrin levels across clinical stages (Stage I: 20.46 $\pm$ 0.14 pM/L; Stage II: 20.46 $\pm$ 0.14 pM/L; Stage III: 20.48 $\pm$ 0.13 pM/L; Stage IV: 20.66 $\pm$ 0 pM/L) (Table:2).

Table 2. Mean Plasma Progastrin levels of group A study participants based on clinical stage of Oral Cancer

STAGES	MEAN PLASMA PROGASTRIN VALUE pM/L	P value*
Stage I	20.46 $\pm$ 0.14	0.542
Stage II	20.46 $\pm$ 0.14	
Stage III	20.48 $\pm$ 0.13	
Stage IV	20.66 $\pm$ 0	

*Kruskal-Wallis Test, P-value < 0.05 statistically significant

Similarly, histopathological differentiation of oral squamous cell carcinoma (OSCC) showed no significant association with progastrin levels ($p = 0.06$; Figure 1).

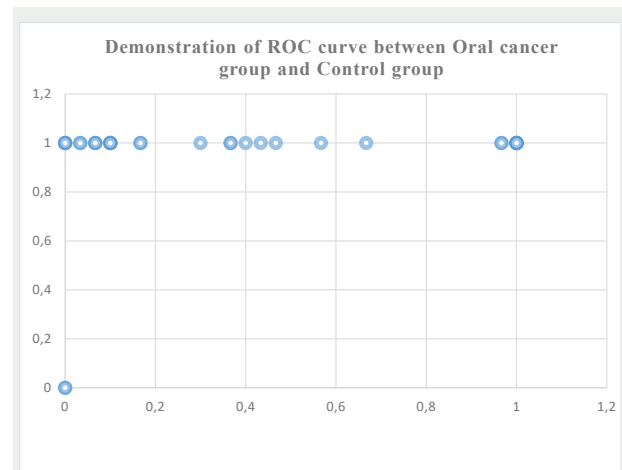
**Figure 1.** Mean Plasma Progastrin levels of group A study participants based on histopathological differentiation

Receiver operating characteristic (ROC) analysis demonstrated outstanding diagnostic performance for plasma progastrin in distinguishing OC cases from controls, with an area under the curve (AUC) of 98.67%. The test exhibited high sensitivity (95%) and specificity (81.3%), supporting its potential as a robust biomarker for oral cancer detection (Figure 2).

DISCUSSION

The present study was an initial attempt to assess the role of human plasma progastrin (hPG80) in patients with oral squamous cell carcinoma compared to the control. As results shows that mean plasma progastrin level in oral cancer group (20.47 $\pm$ 0.14 pM/L) is two times higher compared to the control group (9.92 $\pm$ 1.03 pM/L). The

levels of hPG80 increased with increased clinical staging and histo-differentiation (more for poorly differentiated when compared to moderate and well differentiation) of OSCC subjects. Although these differences were not statistically significant, this linear difference could throw light on the tumour promoting potential of hPG80 in oral carcinogenesis. Numerous studies and systematic review have demonstrated the role of plasma progastrin as a potential biomarker for various malignancies. You B et al have demonstrated that human plasma progastrin have been overexpressed in more than 11 different types of epithelial and non-epithelial cancers [5].

**Figure 2.** ROC characteristics of Plasma Progastrin levels between Oral Cancer group and Control group

Findings from the scoping review done by Madan K et al has shown concrete evidence on the association between plasma progastrin levels in colo-rectal cancers and Lung carcinoma [11]. The study by wojcik and kulpa showed the relation between progastrin serum biomarker and diagnosis of pulmonary cancer and the utility of progastrin as a diagnostic marker [12]. Recent studies have highlighted the role of hPG80 affecting tumour progression by disrupting cell junctions, cell proliferations, inhibiting apoptosis, regulating cancer stem cells and angiogenesis. Of particular interest is the role of hPG80 in colorectal cancer, where it is believed to harbour tumour promoting functions with impact on cancer stem cell self-renewal and antibodies targeting hPG80 leads to increased apoptosis, reduced proliferation and migration and invasion of colorectal cancer [13]. In addition, the relationship between hPG80 and cancer progression is also explained by its role in activating WNT –m oncogenic pathways. Since the latter is an important pathway in the pathogenesis of oral cancers, it is hypothesised that hPG80 plays a vital role in oral carcinogenesis [14, 15]. In the study conducted by You B et al the diagnostic accuracy of lung cancer patients was high with ROC curve AUC of 0.75, compared to present study, diagnostic performance with an area under the curve (AUC) of 98.67%[5].

The present study showed increase levels of plasma hPG80 among the subjects with OSCC when compared to the control. A study conducted by Amjadi O et al showed no significant difference in hPG80 levels in patients with gastric cancers when compared based on clinicopathological

findings related to survival like stage, grade, and distant metastasis, metastasis to lymph nodes and perineural and lymphatic involvement [13, 16]. Chauhan A et al, in their study showed hPG80 values similar to the results of the present study. This study used a commercial ELISA kit for assessment while the present study used a cost-effective UV spectrometer for assessment with comparable results [17]. This hPG80 is a novel blood-based multi-cancer biomarker with superior clinical utility. The assay-based tests enable cost-effective implementation in standard laboratories, overcoming limitations of existing tests [18]. Retrospective studies confirm its diagnostic potential across multiple cancer types [5, 19]. Hence, Progastrin serves as a clinically relevant biomarker detectable in blood during both cancer progression (primary and metastatic tumors) and precancerous stages (e.g., adenomatous polyps), enabling effective early detection and patient monitoring [20].

Strength and Limitations

This study ensured all participants were free from H Pylori which could have affected the study results. The present study also used a UV spectrometer analysis method which tested the samples in triplets. This method is an acceptable method with good sensitivity and specificity and can be used in a resource limited setting for universal screening of samples.

The results of the present study have to be interpreted with the following limitations. By nature, this study was of a cross-sectional design and hence issues related to temporality cannot be addressed. Though efforts were taken to rule out any other types of malignancies or systemic disease among the cases and control, errors related to selection bias of the study and control subjects could confound the results. Further, being an initial attempt, a convenience type of sample was chosen for the study for ease of implementation. A randomly selected study population who could be followed up prospectively may provide better evidence for this association.

CONCLUSION

The present study showed increased levels of hPG80 in subject with OSCC when compared to the control. The present study highlights the potential use of hPG80 levels as a biomarker for oral malignant and potentially malignant disorders. Since early diagnosis is key for survival and treatment prognosis, hPG80 offers to be ubiquitous biomarker for a variety of cancers including oral malignancies. As hPG80 increases with increasing staging and histo differentiation of oral cancers future research should focus on the role of Progastrin measured in the peripheral blood of patients on treatments, and also whether medications targeting hPG80 could improve treatment outcomes.

REFERENCES

1. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. *Sens Int*. 2020;1:100046.
2. Pahwa V, Nair S, Shetty RS, Kamath A. Prevalence of oral premalignant lesions and its risk factors among the adult population in Udupi Taluk of coastal Karnataka, India. *Asian Pac J Cancer Prev*. 2018;19(8):2165–70.
3. Henry NL, Hayes DF. Cancer biomarkers. *Mol Oncol*. 2012;6(2):140–6.
4. Murdoch-Kinch CA. Oral medicine: advances in diagnostic procedures. *J Calif Dent Assoc*. 1999;27(10):773–82.
5. You B, Mercier F, Assenat E, Langlois-Jacques C, Glehen O, Soulé J, et al. The oncogenic and druggable hPG80 (Progastrin) is overexpressed in multiple cancers and detected in the blood of patients. *EBioMedicine*. 2020;51:102574.
6. Silveira DA, Gupta S, Sinigaglia M, Mombach JC. The Wnt pathway can stabilize hybrid phenotypes in the epithelial-mesenchymal transition: a logical modeling approach. *Comput Biol Chem*. 2022;99:107714.
7. Prasanna Samuel, Belavendra Antonisamy, Palani Raghupathy, Joseph Richard, Caroline HD Fall, Socio-economic status and cardiovascular risk factors in rural and urban areas of Vellore, Tamilnadu, South India, *International Journal of Epidemiology*, Volume 41, Issue 5, October 2012, Pages 1315–1327, <https://doi.org/10.1093/ije/dys001>
8. Madankumar PD, Iyer K, Soni S, Nagarajan L, Kumar K, Solomon S, et al. A simple screening program for oral cancer in a defined geographic area in southern India: A community-based cross-sectional study. *Cancer Res Stat Treat*. 2022;5(2):226–31.
9. Kumar PD, Loganathan K, Kannan R, Roa UD, Bhagiyalakshmi AN. “Vaai Ganam”-a screening program for early detection of oral potentially malignant disorders and oral cancer among truck drivers in Chennai—a cross-sectional survey. *Int J Occup Saf Health*. 2024;14(1):117–23.
10. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–91.
11. Madan Kumar PD, Ranganathan K, Narayanan MBA, Kuzhalmozhi M. Role of Plasma Progastrin Phenotypes as a Potential Biomarker for Screening and Diagnosis of Cancer - A Scoping Review. *Asian Oncol Res J*. 2022;5(1):53-60. Available from: <https://journalaorj.com/index.php/AORJ/article/view/51>.
12. Wojcik E, Kulpa JK. Pro-gastrin-releasing peptide (ProGRP) as a biomarker in small-cell lung cancer diagnosis, monitoring and evaluation of treatment response. *Lung Cancer Targets and Ther*. 2017;8:231. doi: 10.2147/LCTT.S149516.
13. Amjadi O, Alizadeh-Navaei R, Rezapour M, Omrani-Nava V, Moosazadeh M, Azadeh H, et al. Association between Serum Progastrin Biomarker Level and Gastric Cancer. *Asian Pac J Cancer Prev*. 2022;23(10):3595–600.
14. Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC. Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. *J Clin Invest*. 2000;106:533–9.
15. Giraud, J., Foroutan, M., Boubaker-Vitre, J., Grillet, F., Homayed, Z., Jadhav, U., Crespy, P., Breuker, C., Bourgaux, J. F., Hazerbroucq, J., Pignodel, C., Brulin, B., Shivdasani, R. A., Jay, P., Hollande, F., & Pannequin, J. (2021). Progastrin production transitions from Bmi1+/Prox1+ to Lgr5high cells during early intestinal tumorigenesis. *Translational Oncology*, 14(2), 101001. <https://doi.org/10.1016/j.TRANON.2020.101001>
16. Li L, Yin X, Meng H, et al. Increased progastrin-releasing peptide expression is associated with progression in gastric cancer patients. *Yonsei Med J*. 2020;61:15–9. doi: 10.3349/ymj.2020.61.1.15.
17. Chauhan A, Prieur A, Kolesar J, Arnold S, Payen L, Mahi Y, et al. hPG80 (circulating progastrin), a novel blood-based biomarker for detection of poorly differentiated neuroendocrine carcinoma and well differentiated neuroendocrine tumors. *Cancers*. 2022;14(4):863.
18. Cappellini M, Flaceliere M, Saywell V, et al. A novel method to

detect hPG(80) (human circulating progastrin) in the blood. Anal Methods. 2021;13:4468–77.

19. Prieur A, Harper A, Khan M, et al. Plasma hPG(80) (Circulating Progastrin) as a novel prognostic biomarker for early-stage breast cancer in a breast cancer cohort. BMC Cancer. 2023;23:305.
20. Fioretzaki R, Sarantis P, Charalampakis N, Christofidis K, Mylonakis A, Kostas E, Karamouzis MV, Sakellariou S, Schizas D. Progastrin: An Overview of Its Crucial Role in the Tumorigenesis of Gastrointestinal Cancers. Biomedicines. 2024 Apr 17;12(4):885. doi: 10.3390/biomedicines12040885