Original Article

Chromogranin A: As A Tumor Marker for Neuroendocrine Tumors Diagnosis, Follow-up & Its Correlation with Response to Somatostatin Analogues

Manwar A. Mohammed AL-Naqqash* MBChB, DMRT, MSc pathology

Abstract:

Background: Chromogranin A is a useful tumor marker for neuroendocrine tumors (NETs) diagnosis & follow-up, Octreotide (somatostatin-long acting repeatable (SAS-LAR)) is an established treatment for NETs. Studies regarding the relation between response to SAS-LAR & the change in Chromogranin A (CgA) plasma level are still lacking.

Objectives: To determine the association between the using of Octreotide (SAS-LAR) and CgA level on time sequence & clinical status.

Fac Med Baghdad 2016; Vol.58, No.4
Received:July.2016
Accepted:Sept..2016

Patients & methods: a prospective observational study included 38 neuroendocrine patients in The Oncology Teaching Hospital/medical city complex/Baghdad, started at September 2013 till May 2016; assessing their circulating chromogranin A (CgA) plasma levels on multiple occasions(0,2 and 4 months) by ELISA technique and its correlation with response to somatostatin analogues (SAS-LAR) in those patients.

Results: the study recruited 38 neuroendocrine patients. 21 (55%) of them were males, 23 (60%) patients were older than 50 years old & 17 (44%) had metastasis to different sites. Somatostatin analogues (octreotide 30mg) was administered to 20 out of 38 (52.6%) studied patients. Serial CgA tests were performed in (17 out of 20) patients used SAS-LAR, with a change in mean value from (225.3 U/L) pre-using the agent to (17.5 U/L) two months after its use & to (8.7 U/L) four months after its use (p=0.009, p=0.002 respectively) while the change in mean of CgA level was from (205.9 U/L) to (200.9 U/L) in 10 patients who did not use Octreotide (p=0.2).Also results showed that no statistically significant difference in mean value of CgA pre & two months after using Octreotide with regard to grade of the tumor.

Conclusions: Plasma CgA is a reliable marker for NETs (regarding diagnosis, prognosis and response to treatment including somatostatin analogues).All patients with NETs should undergo a baseline plasma CgA level at diagnosis. Serial assessment of circulating CgA could be done for NET patients when there is baseline elevation of CgA level in circulation

Keywords: neuroendocrine tumors, chromogranin A, somatostatin analogues.

Introduction:

Neuroendocrine tumors (NETs) is a collective term for a diverse range of neoplasms that arise from cells that originate in the endocrine and nervous systems and share common morphological and immunohistochemical features, including the presence of secretory granules. NETs have generally been considered rare; their incidence has been estimated at 2.5 to 5 per 100,000 people per year and prevalence of 35 per 100,000 people (1). NETs that secrete peptides and neuroamines can cause recognizable clinical syndromes, including carcinoid syndrome. (2) However, due to the indolent nature of NETs, many patients are asymptomatic in the early stages, or present with only vague symptoms such as abdominal pain. (2) As a result, NETs are frequently metastatic at the time of diagnosis: liver metastases are observed in 40 % of patients who present with small intestinal and 60–70 % of patients with pancreatic NETs. (3, 4). Chromogranin A and synaptophysin immunostains identify proteins of neurosecretory granules and are specific immunologic markers for NETs. Serum hormone markers are often elevated in NETs and can be a surrogate marker of symptoms of hormone excess or tumor growth, though none are sensitive enough to be used as a screening test. (5). Chromogranin A (CgA) is found throughout the diffuse neuroendocrine system and is thought to be the best and most sensitive general marker for the diagnosis and follow-up of NETs. Immuno-histochemical detection of CgA represents the milestone in the diagnostic work-up of NETs. Elevated CgA levels have been found in functioning as well as non-functioning tumors, making it a universal marker in NETs (5). CgA is more frequently elevated in G1 and G2 tumors compared to G3 NETs. The highest CgA levels have been found in metastatic midgut NETs, where CgA appears to correlate with tumor burden and biological activity. CgA has recently been described to be predictive of survival

**Dept. of Surgery, college of medicine, University of Baghdad.*
manwaralnaqqash@gmail.com

and of treatment response in NETs. Its levels may correlate with the tumor burden, tumor progression or regression in response to therapy. CgA has been reported to be useful for the identification of disease progression, and high plasma levels seem to correlate with poor prognosis. A recent prospective Italian study reported that a more than 30%, decrease in CgA levels after acute Octreotide administration allows to discriminate those patients responsive to chronic somatostatin analogues (SSAs) treatment from the ones likely unresponsive.(6).The first-line treatment strategy for NETs is surgery, but this is rarely curative, as most patients present at advanced stages of disease.(3,4). Somatostatin analogues, including Octreotide and Lanreotide, were introduced to control symptoms that result from release of peptides and neuroamines. Octreotide is the most studied SSA. (1). Octreotide acetate LAR is a formulation in which Octreotide acetate is encapsulated in microspheres of a slowly dissolving polymer, providing a predictable pharmacokinetic profile and steady-state kinetics when injected intramuscularly once every 28 days. (7) Usually, the treatment with long-acting preparations of SST analogues consists in an intramuscular injection (i.m.) every 2 or 4 weeks Octreotide-LAR, 10–30 mg. it affects numerous pathways that may confer anti-proliferative effects in NETs through inhibition of tumor angiogenesis and inhibition of secretion of growth factors.(8,9) Direct mechanisms by which Octreotide achieves tumor regression include binding to somatostatin receptors sst_2 and sst_5 , which are found in high density on tumor cells(10), and thereby inhibiting hormone secretion from the tumor. Octreotide may also be used in asymptomatic patients at the time of diagnosis of metastatic disease (11). The pro-apoptotic activity of SST analogues seems to have clinical relevance, as shown by the interesting findings published by Eriksson et al. that reported an increase in apoptosis in bioptic samples of tissues by patients with (gastrointestinal pancreatic (GEP-NETs) after the treatment with SST analogues at high doses. It followed that apoptosis is related to the biochemical response and the disease stabilization (70% of cases) (12, 13). The European Neuroendocrine Tumor Society (ENETS) 2012 guidelines (and National Cancer Comprehensive Network) stated that the use of SSAs, especially Octreotide acetate LAR, is recommended for antiproliferative purposes in functioning and non-functioning midgut tumors. (14, 15)

Aim: To determine the association between the using of Octreotide (SAS-LAR) and CgA level on time sequence & clinical status in NET patients.

Patients and methods:

The design of the study is a prospective observational study. All patients had confirmed histopathological diagnosis. The tumors were classified according to the new Tumor size, lymph Node, Metastasis (TNM) classification for NET tumors, including the grading system whereby tumors with proliferation index Ki67 < 2% belong to the grade 1 (G1) tumor category, whilst tumors with Ki67 between 2 and 20%, and tumors with Ki67 > 20%, belong to the G2 and G3 tumor categories, respectively(16).

This study was conducted in The Oncology Teaching Hospital/medical city complex/Baghdad and recruitment of NET patients' data included all NET patients registered from September 2013 in outpatient clinic. Octreotide 30 was given by deep intramuscular injection to 20 patients every 4 weeks.

The circulating CgA was assessed serially in gastrointestinal hospital laboratory/medical city complex/ Baghdad. Whole blood sample were collected in anticoagulant treated tubes (EDTA), cells are removed from plasma by centrifugation for 10 minutes at 1000-2000 x g. then CgA test were done using ELISA type immunoassay (a kit from Cisbio Bioassays / Parc Marcel Bioteux – BP 84175-30200 Codolet/ France) The kit was designed to detect chromogranin A in serum or plasma, first monoclonal antibody, immobilized on the microplate, captures the CgA proteins contained in the calibrators and samples. After washing, the fixed proteins are then recognized by a second monoclonal antibody conjugated to HRP (Horse –Radish- Peroxidase). After a second intubation, the unfixed reagents are eliminated by washing. Then the colorimetric reaction is started by the addition of an HRP substrate,

TMB (3, 3', 5, 5' Tetrmethyl benzidine). After the reaction is stopped, the optical density (OD) of each well is read at 450nm. The OD values measured are proportional to the CgA protein concentration contained in the calibrators and samples. Three serial measures of CgA were taken for each patient (at baseline before start using SAS LAR, 2 months and 4 months).

Serial CgA testing were performed on 27 NET patients (17 patients were on Octreotide 30mg every 4 weeks, 10 patients were on follow up only).

Statistical analysis: The Statistical Package for Social Science (SPSS) version 20 was used for data entry and analysis. Graphs and tables (number and percentage) were used to describe the data and suitable non -parametric statistical tests which make no assumptions about the probability distributions of the variables were used accordingly. P value < 0.05 was considered significant.

Results:

Thirty eight NET patient were recruited in this study. 21(55%) of them were males, 23 (60%) were older than 50 years of age & 17 (44%) had metastasis to different sites.

Table.1-Mean value of CgA level pre- and two months after using the somatostatin analogue in NET patients.

	SAS-SAR	N	Mean	Std. Deviation	p-value
CgA level /pre-using SAS-LAR	Used	17	225.3	291.775	0.2
	Not used	10	205.9	352.819	
CgA / two months after using SAS-LAR	Used	17	17.5	11.975	0.03
	Not used	10	200.9	329.920	

Table. 2- NET patients with their CgA levels (U/L) measured at baseline, 2 months and 4 months after treatment with SAS-LAR (n=17)

NET patients on SAS-LAR	CgA U/L (baseline level)	CgA U/L 2 months after using SAS-LAR	CgA U/L 4 months after using SAS-LAR
1	600	6	4
2	70	20	8
3	14	8	6
4	137	45	3
5	950	40	20
6	280	12	8
7	110	16	3
8	400	20	6
9	185	35	30
10	55	16	10
11	800	8	6
12	64	14	10
13	40	10	8
14	60	20	10
15	20	10	6
16	40	12	8
17	6	6	2
Mean (U/L)	225.3	17.5	8.7

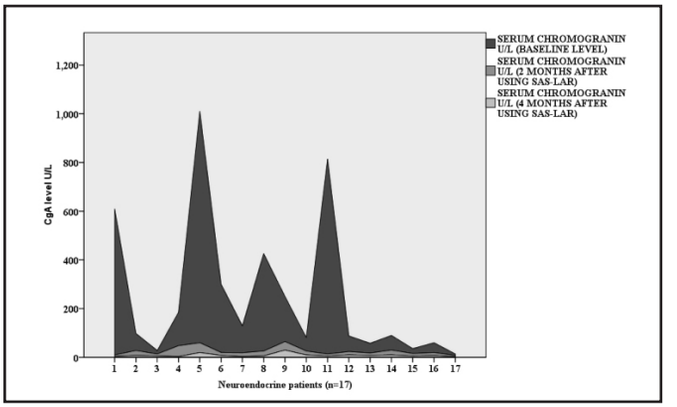

Figure 1: histogram showing changes in CgA levels in NET patients (at baseline, 2 months , 4 months) after using SAS-LAR

Table.3- mean value of CgA level on time sequence (0, 2 and 4 months) after using the somatostatin analogue in NET patients

SAS-SAR- used	N	Mean	Std. Deviation	Paired sample t test
CgA level /pre-using SAS-LAR	17	225.3	291.775	P= 0.009 (after 2 months)
CgA / two months after using SASLAR	17	17.5	11.785	
CgA/ four months' after using SAS-LAR	17	8.7	12.652	P=0 .002 (after 4 months)

Table.4- mean value of CgA level after using the Octreotide according to metastasis status in NET patients

	Metastasis status	N	Mean	Std. Deviation	p-value
CgA / two months after using SAS-LAR	Yes	7	22.12	15.858	0.1
	No	10	12.90	5.225	

Table.5- mean value of CgA level after using the Octreotide according to grade of disease in NET patients.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		P-value
					Lower Bound	Upper Bound	
G1	2	13.7	8.500	2.250	0.22	27.28	0.5
G2	7	12.7	5.187	1.960	7.92	17.51	
G3	6	25.6	16.120	6.581	8.75	22.58	

Total =15 (2 patients were not included in this table due to unknown tumor grade)

Discussion:

CgA is a member of the chromogranin family; its transcription and peptide processing are well characterized, but its precise function remains unknown. Levels are detectable in the circulation but vary substantially (~25%) depending on which assay is used. Serum and plasma measurements are concordant. CgA is elevated in ~90% of gut NETs and correlates with tumor burden and recurrence. CgA is currently the best available biomarker for the diagnosis of NETs. It is critical to establish diagnosis and has some utility in predicting disease recurrence, outcome, and efficacy of therapy. Measurement of plasma CgA is mandatory for the effective diagnosis and management of NET disease. (17) SST analogues remain the main symptomatic therapeutic modality for the management of NETs. Generally, their effects are limited to symptom control and stabilization of the disease progress. While decrease in tumor size rarely occurs, the recent PROMID study using Octreotide LAR demonstrates a clear effect on time to tumor progression compared with placebo and tumor disease stabilization. The decrease in biochemical tumor markers is evident in about 50% (16) Somatostatin analogues (Octreotide 30), was administered to 20(52.6%) patients and there was an established role in the symptom and tumor control in NET patients as were stated according to published guidelines. (18) Serial CgA tests were performed in (17/20) patients used SAS-LAR, with a change in mean value from (225.3 U/L) pre-using the agent to (17.5 U/L) two months after use & to (8.7 U/L) four months after use (p=0.009, p=0.002 respectively) while the change in mean of CgA level was from (205.9 U/L) to (200.9 U/L) in 10 patients who did not use Octreotide 30mg (p=0.2). These findings are comparable to data from Italian study in which there was a successful objective response occurred in 21/31 patients (68%). Successful symptomatic response occurred in 13/18 patients (72%)& biochemical response in 25/31 (81%). (19) but the percentage of reduction is different from that published in previous studies (16) in which > 50% reduction in tumor marker was observed in less than 40%. This difference is more likely to be related to the fact that not all patients received SAS-LAR underwent serial CgA (due to limited availability of the test). However, more studies are still required.

Size of the primary tumor, and Ki67 did not influence the response rate to SSA therapy (20). These findings were consistent with our results that showed no statistically significant difference in mean value of CgA pre & two months after using Octreotide between patients with (G1, G2 or G3) & between patients with presence or absence of metastasis(p=0.5 ,0.1) respectively.

Conclusions:

Plasma CgA is a reliable marker for NETs, reflecting the clinical evolution of the disease, diagnosis, prognosis, follow-up, and assessment of response to therapy and all patients with NETs should undergo a baseline plasma CgA level at diagnosis. Serial assessment of circulating CgA could be done for NET patients when there is baseline elevation of CgA level in circulation and the Response to Octreotide LAR therapy correlates with reduction in circulating CgA level and it can be used to assess response to treatment of NETs.

References

1-Frederico Costa, Brenda Gumz , Octreotide – A Review of its Use in Treating Neuroendocrine Tumours , European Oncology & Hematology,[internet] 2013;9(2):105–9 DOI: available from: http://doi.org/10.17925/EOH.2013.09.2.105

2-Modlin IM, Oberg K, Chung DC, et al., Gastroenteropancreatic neuroendocrine tumors, Lancet Oncol, 2008;9:61–72.

3-Yao JC, Hassan M, Phan A, et al., One hundred years after 'carcinoid': epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States, J Clin Oncol, 2008;26:3063–72.

4-Lal A, Chen H, Treatment of advanced carcinoid tumors, Curr Opin Oncol, 2006;18:9–15.

5- DeVITA, Hellman & Rosenbergs. Cancer Principles & Practice of Oncology. 10th edition. Wolters Kluwe, 2015

6-Roberta Elisa Rossi, Jorge Garcia-Hernandez, Tim Meyer, Christina Thirlwell, Jennifer Watkins, Nicholas Guy Martin, Martyn Evan Caplin, and Christos Toumpanakis. Chromogranin A as a predictor of radiological disease progression in neuroendocrine tumors. Ann Transl Med [internet] 2015 Jun; 3(9): 118.doi: 10.3978/j.issn.2305-5839.2015.04.23. Available from:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4481369/

7-Astruc B, Marbach P, Bouterfa H, et al., Long-acting octreotide and prolonged-release lanreotide formulations have different pharmacokinetic profiles, J Clin Pharmacol, 2005; 45:836–44.

8-Florio T, Molecular mechanisms of the antiproliferative activity of somatostatin receptors (SSTRs) in neuroendocrine

tumors, Front Biosci, 2008; 13:822–40.

9-Susini C, Buscail L, Rationale for the use of somatostatin analogs as antitumor agents, Ann Oncol, 2006;17:1733–42.

10-Chadha MK, Lombardo J, Mashtare T, et al., High-dose octreotide acetate for management of gastroenteropancreatic neuroendocrine tumors, Anticancer Res, 2009;29:4127–30.

11-Oberg K, Kvols L, Caplin M, et al., Consensus report on the use of somatostatin analogs for the management of neuroendocrine tumors of the gastroenteropancreatic system, Ann Oncol, 2004;15:966–73.

12-. Eriksson B, Renstrup J, Imam H, Oberg K. High-dose treatment with lanreotide of patients with advanced neuroendocrine gastrointestinal tumors: clinical and biological effects. Ann Oncol (1997) 8:1041–4. doi:10.1023/A:1008205415035

13-Faiss S, Rath U, Mansmann U, Caird D, Clemens N, Riecken EO, et al. Ultra-high-dose lanreotide treatment in patients with metastatic neuroendocrine gastroenteropancreatic tumors. Digestion (1999) 60:469–76. doi:10.1159/000007693

14-Pavel M, Baudin E, Couvelard A, et al., ENETS Consensus Guidelines for the management of patients with liver and other distant metastases from neuroendocrine neoplasms of foregut, midgut, hindgut, and unknown primary, Neuroendocrinology, 2012;95:157–76.

15-NCCN, National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology™ Neuroendocrine Tumors. National Comprehensive Cancer Network Web site. Available at: http://www.nccn.org/professionals/physician_gls/ pdf/neuroendocrine.pdf. (Accessed 20 November 2013).

16- C. TOUMPANAKIS, J. GARLAND, L. MARELLI, R. SRIRAJASKANTHAN, J. SOH, P. DAVIES, J. BUSCOMBE andM. E. CAPLIN. Long-term results of patients with malignant carcinoid syndrome receiving octreotide LAR. Alimentary Pharmacology & Therapeutics
Volume 30, Issue 7, pages 733–740, October 2009 [internet] version of Record online: 2 JUL 2009
DOI: 10.1111/j.1365-2036.2009.04083.x. available from: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2036.2009.04083.x/full.

17-Irvin M. Modlin , Bjorn I. Gustafsson et al. , Chromogranin A—Biological Function and Clinical Utility in Neuro Endocrine Tumor Disease, Annals of Surgical Oncology[internet] September 2010, Volume 17, Issue 9, pp 2427-2443, available from : http://link.springer.com/article/10.1245/s10434-010-1006-3

18- NCCN guidelines 2016.Neuroendocrine Tumors (version 1.2015):MS-2

19- Faggiano A, Ferolla P, Grimaldi F, Campana D, Manzoni M, Davi MV, et al. Natural history of gastro-entero-pancreatic and thoracic neuroendocrine tumors. Data from a large prospective and retrospective Italian epidemiological study: the NET management study. J Endocrinol Invest. 2012;35:817–23. [PubMed]. Available from:
http://www.ncbi.nlm.nih.gov/pubmed/22080849.

20- Prommegger R, Bale R, Ensinger C, Sauper T, Profanter C, Knoflach M, et al. Gastric carcinoid type I tumour: new diagnostic and therapeutic method. Eur J Gastroenterol Hepatol (2003) 15:705–7.doi:10.1097/00042737-200306000-00020.