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Clinical Practice Article

Epidermal growth factor and remarkable correlations in a case series COVID-19

Factor de crecimiento epidérmico y correlaciones notables en una serie de casos con la COVID-19

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ABSTRACT

Introduction: Molecular links relate epidermal growth factor (EGF) to inflammatory phenomena. In the context of COVID-19, understanding the role of serum concentrations of EGF offered new possibilities for a better understanding of physiopathology and therapeutics.

Objective: To explore the behavior of serum EGF values in patients with COVID-19, as well as to determine possible significant correlations between EGF, and analytical and clinical parameters of interest.

Methods: Cross-sectional observational analytical study, in a series of COVID-19 cases that included 15 patients attended between august-september 2021. Variables included: age, sex, comorbidities, respiratory rate, heart rate, hospital stay, neutrophils, lymphocytes, neutrophil-lymphocyte index. Summary measures: absolute frequency, percentage, and the arithmetic mean were used. The statistical significance of observable differences between groups was explored with the chi-square test or Welch's t test with $\alpha = 0.05$.

Results: Notable correlation observed are $r_{EGF\text{-}age} = -0.6211$ ($p = 0.0206$) overall, with $r_{EGF\text{-}age} = 0.2998$ ($p = 0.4030$) in critically ill patients and $r_{EGF\text{-}age} = -0.9607$ ($p = 0.0000$) in patients with care report. In the case of correlation with neutrophils, this is observed in the case of the subset of critical patients


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($r_{EGF-PNN}$= -0.4471, p= 0.3524); in relation to respiratory rate and heart rate, remarkably strong correlations were observed in critically ill patients (r_{EGF-Rr}= 0.8220, p= 0.1928; r_{EGF-Hr}= -0.9285, p= 0.1207).

Conclusions: There is a plausible relationship between serum EGF values and COVID-19, with notably strong correlations for clinical parameters in the context of serious illness.

Keywords: biomarkers; epidermal growth factor; COVID-19.

RESUMEN

Introducción: Los vínculos moleculares relacionan el factor de crecimiento epidérmico (EGF) con fenómenos inflamatorios. En el contexto de la COVID-19, la comprensión del papel de las concentraciones séricas de EGF, ofreció nuevas posibilidades para comprender mejor la fisiopatología y la terapéutica.

Objetivo: Explorar el comportamiento de los valores séricos de EGF en pacientes con la COVID-19, así como determinar posibles correlaciones significativas con parámetros analíticos y clínicos de interés.

Métodos: Estudio analítico observacional transversal, en una serie de casos de COVID-19, en 15 pacientes ingresados entre agosto-septiembre de 2021. Variables incluidas: edad, sexo, comorbilidades, frecuencia respiratoria, frecuencia cardíaca, estancia hospitalaria, neutrófilos, linfocitos, índice neutrófilo-linfocito. Medidas resumen: frecuencia absoluta, porcentaje y media aritmética. La significación estadística de las diferencias observables entre grupos se exploró con la prueba de *ji* cuadrado o la prueba t de Welch con α= 0,05.

Resultados: Correlaciones destacables observadas son $r_{EGF-edad}$= -0,6211 (p= 0,0206) global, con $r_{EGF-edad}$= 0,2998 (p= 0,4030) en pacientes críticos y $r_{EGF-edad}$= -0,9607 (p= 0,0000) en pacientes con informe de cuidados. En el caso particular de la correlación con los neutrófilos, se observa en el caso del subconjunto de pacientes graves ($r_{EGF-PNN}$ =-0,4471, p= 0,3524); en relación con la frecuencia respiratoria y la frecuencia cardiaca, se observaron correlaciones notablemente fuertes en los pacientes graves (r_{EGF-Rr}= 0,8220, p= 0,1928; r_{EGF-Hr} = -0,9285, p= 0,1207).

Conclusiones: Existe una relación plausible entre los valores séricos de EGF y la COVID-19, con correlaciones notablemente fuertes para los parámetros clínicos en el contexto de la enfermedad grave.


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Palabras clave: biomarcadores; factor de crecimiento epidérmico; COVID-19.

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INTRODUCTION

COVID-19 still sets the international scientific scene today. Despite the coordinated results of immunization campaigns, social control measures and the typical biology of viral diseases, the study of their pathophysiological mechanisms is still active. The risk of resurgence is not a chimera.

Among the molecular effects of SARS-CoV-2 replication, in the context of inflammation as the first immune response, overexpression of a signal transducer and activator of transcription 3 (STAT3), and reduced cytoplasmic levels of signal transducers and activator of transcription 1 (STAT1),[1,2] enhanced by activation of EGF-dependent receptors (EGFR) both overexpressed and positively regulated in the inflammatory framework[3,4] apparently play a crucial role in the development of severe forms of the associated lung disease.

EGFR has as its main ligand a small molecule, of only 53 amino acids, called epidermal growth factor (EGF).[5] In models of kidney damage, in the context of COVID-19, high levels of serum IL-6 have been observed in contrast to low levels of EGF in urine.[6,7,8] However, no scientific publications have been found that specifically analyze the role that EGF could presumably play in the progression of the disease. Despite the obvious molecular networks linking EGF to multiple inflammatory processes, data from the clinical context are scarce. Contributing to elucidate the possible correlation between EGF and COVID-19 will provide new possibilities for a better understanding of the inflammatory phenomenon in the context of pathogen-triggered immune response.

The purpose of current research is to explore the behavior of serum EGF values in the context of patients diagnosed with COVID-19, on the analysis of field data generated during the propagation of beta and


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alpha viral strains in eastern Cuba, aspects that have not been reported, on the basis of serum studies, in previous studies.

METHODS

Study design

Observational cross-sectional study, in a series of cases (15 patients) with COVID-19, treated at "Saturnino Lora" Hospital and admitted between August-September 2021. The diagnostic criteria used were those declared by Ministry of Public Health (MINSAP)[9] based on World Health Organization (WHO) recommendations. The diagnoses were made by experienced, certified professionals.

Clinical and analytical data

Clinical and analytical data were collected by the professional teams during patient care in the corresponding medical institutions. The sample consisted of 15 patients.

The information used was collected from medical records and includes age, sex, comorbidities, respiratory rate, heart rate, hospital stay, neutrophils, lymphocytes, neutrophil-lymphocyte index (NLI).

It was verified that the acquisition of the analytical parameters was carried out in strict compliance with the standardized procedures of the clinical laboratory of the institutions and in accordance with current Center for State Control of Medicines, Equipment and Medical Devices (CECMED) regulations.[10]

Serum EGF levels

Serum EGF detection was performed using the commercial kit UMELISA-EGF from the Cuban Immunoassay Centre. In all cases, 5 mL of blood was collected by a puncture of the cephalic vein in the flexure of the arm using disposable syringes of 10 mL capacity, with 21 G hypodermic needles, deposited in a dry test tube, obtaining the serum by the coagulum retraction technique for 4 hours and centrifugation (according to the manufacturer's recommendations at 1500 rpm for 10 minutes at 24 ^{0}C). The serum obtained was dispensed by Eppendorf micropipettes into 1.5 mL Eppendorf vials, after which they were stored frozenly at -20 ^{0}C, until processing at the SUMA laboratory, certified by CECMED, at the Juan Bruno Zayas Hospital. Results were expressed in picograms per milliliter (pg/mL).


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Statistical analysis

A digital database was used to record the data, created using the technical facilities of the Excel software of the Microsoft Office 2010 platform (Microsoft, USA), on a Hewlett-Packard laptop computer. Data processing was carried out on the same technological platform. In the statistical analysis, measures of central tendency (arithmetic mean), dispersion (standard deviation and confidence interval) and Pearson's correlation coefficient (r) were used as summary parameters. Q-Q and Jarque-Bera (JB) normality tests were performed; in addition, the statistical significance of observable differences between groups was explored with the chi-square test (categorical variables) or Welch's t-test (continuous variables) with $\alpha = 0.05$, the effect size was estimated using Hedge's g-formula (g).[11]

Ethical Statement

The study was controlled by the general principles established in the documents adopted by the international community in relation to biomedical research on human subjects, set out in the Declaration of Helsinki (update of the World Medical Assembly held in Brazil, 2013),[12] with the state regulations in force according to the requirements of the national regulatory authority (Regulation M 83-15 of the CECMED, approved by Resolution 165/2000 of MINSAP),[13] as well as in the Guide to Good Clinical Practice of the International Conference on Harmonization (ICH E6).[14] The research was approved by the Research Ethics Committee at “Saturnino Lora” Hospital, and the corresponding certification by the Regional Ethics Committee for the southeastern region of Cuba (provided as metadata Complementary File). Prior to the inclusion of each subject in the study, Informed Consent was requested and obtained.

RESULTS

Average age of 63.5 ± 9.1 years (JB=1.31, p= 0.5179), 60% male, with a prevalence of comorbidities of 86.6%, 60% with more than one chronic non-communicable disease; in the particular case of hypertension blood (HB) the prevalence was 66.6%, followed by ischemic heart disease with 40% and diabetes mellitus (DM) with 26.6%. Particular data on the behavior of these parameters in relation to the severity of COVID-19 are shown in table 1.


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Table 1- General characteristics

Parameter	Critical report n= 6	Care report n= 9
Age	63.8 ±17.05 years	63.3 ±10.99 years
Sex		
Men [% (n)]	83 (5)	44 (4)
Women [% (n)]	17 (1)	55 (5)
Comorbidities		
HB [% (n)]	100 (6)	44 (4)
DM [% (n)]	33 (2)	33 (3)
Ischemic heart disease [% (n)]	50 (3)	22 (2)
Other [% (n)]	33 (2)	55 (5)

HB: hypertension blood, DM: diabetes mellitus.

Overall hospital stay was 9.8 ± 2.03 days (JB= 33.7, p= 0.000), with a mean respiratory rate of 20.26 ± 1.24 breaths per minute (JB= 3.61, p= 0.1637), and a mean heart rate of 84.86 ± 6.07 beats per minute (JB= 0.8, p= 0.6670). Regarding analytical parameters the mean EGF was 168.76 ± 35.64 pg/mL (JB= 3.39, p= 0.1831), with peripheral blood monocyte values of 589.33 ± 157.02 cells x 10^9 /L (JB= 0.61, p= 0.7352), with neutrophils at 5973 ± 1641 cells x10^9 /L (JB= 2.32, p= 0.3119) and lymphocytes at 1694 ± 325 cells x10^9 /L (JB= 2.12, p= 0.3454), with an average neutrophil/lymphocyte ratio of 3.65 ± 0.89 units (JB= 2.59, p= 0.2727). Particular data regarding the particular behavior of these parameters in relation to the severity of COVID-19 are shown in table 2.


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Table 2 - Clinical and analytical parameters

Parameter	Critical report	Care report	*p-value*
Stay	10 ± 1.67 days	9.66 ± 3.29 days	0.8631
Rr	22 ± 2.42'	19 ± 0.68'	0.0659
Hr	90 ± 6.41'	81 ± 8.62'	0.1206
EGF	180.20 ± 71.95 pg/mL	161.13 ± 38.45 pg/mL	0.6590
PMN	8655 ± 2056 x10 /L^9	4184 ± 1500 x10 /L^9	0.0063
Lymphocytes	1802 ± 516 x10 /L^9	1622 ± 438 x10 /L^9	0.6138
INL	5.03 ± 1.23	2.74 ± 0.83	0.0147

Rr: respiratory rate; Hr: heart rate; EGF: epidermal growth factor;
PMN: Neutrophils; INL: Neutrophils/ Lymphocytes index.

In the exploration of notable correlations in relation to epidermal growth factor was observed $r_{EGF\text{-}age}$= -0.6211 (p= 0.0206) in general, with $r_{EGF\text{-}age}$= 0.2998 (p= 0.4030) in critical patients and $r_{EGF\text{-}age}$= -0.9607 (p= 0.0000) in patients with care report. In the particular case of correlation with neutrophils, there is no apparent linear relationship ($r_{EGF\text{-}PNN}$= -0.04, p= 0.4479) in general, but there is in the case of the subset of critical patients ($r_{EGF\text{-}PNN}$= -0.4471, p= 0.3524). Likewise, in relation to the respiratory rate, a notably strong correlation was observed in the case of critical patients ($r_{EGF\text{-}Rr}$= 0.8220, p= 0.1928), as well as in relation to the heart rate. ($r_{EGF\text{-}Hr}$= -0.9285, p= 0.1207).

In the exploration of the correlations according to INL we observed, in relation to EGF with $r_{INL\text{-}EGF}$= -0.0539 (p= 0.4374) in general and in critical patients $r_{INL\text{-}EGF}$= 0.4010 (p= 0.3686). Regarding age, the correlation was notably strong in critical patients ($r_{INL\text{-}Edad}$= 0.9941, p= 0.0344). Likewise, in relation to Rr, a notably strong correlation was observed in the case of critical patients ($r_{INL\text{-}FR}$= 0.8512, p= 0.1758), but not in relation to Hr ($r_{INL\text{-}FC}$= -0.0334, p= 0.4893).

DISCUSSION

While standard or normality levels for serum EGF concentrations are not determined, approximations to their values in the context of COVID-19 and their severity are not available. The data presented here correspond to the period of dissemination in eastern Cuba of the alpha and beta variants of SARS-CoV-2.


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Beyond its binding to the receptor, elucidating the effects of EGF is of greater complexity; the well-documented pro-inflammatory effect of EGFR,[15,16,17] in contrast to the anti-inflammatory effect reported for EGF[18] leads to a re-understanding of the associated molecular phenomena in a logic that goes beyond the traditional role of EGF as an inducer of EGFR heterodimerization.

Although there is a slight trend towards differences in mean EGF values between groups, these are not significant. Also interesting is the fact that in the clinical order no differences attributable to the severity of COVID-19 pneumonia are highlighted, indicating that in the case of critical patients the symptoms that conditioned the report of severe pneumonia were attributable to a decompensated metabolic syndrome, and not to an aggravated underlying respiratory disease. This context could better explain the absence of significant differences in EGF between groups.

Although the evidence suggests a role for the molecule in the clinical setting, the data is inconclusive. There is an apparent inverse proportional relationship with respect to age, which is blurred in the case of critically ill patients, with greater variability of EGF values, compared to care report patients.

When comparing the EGF values with those reported by *Idania G* et al,[19] notable differences are evident, with markedly lower values in COVID-19 patients compared to healthy subjects (168.76 ± 35.64 pg/mL vs. 468.70 ± 73.03 pg/mL); other studies[20,21] where serum EGF values have been reported with technological platforms similar to those employed in this study show similar trends.

This study explores in a pioneering way the behavior of serum EGF concentrations in the pandemic context dominated by SARS-CoV-2 alpha and beta sepsis. Its design is an obvious limitation, as well as the repertoire of analytical parameters to explore correlations, despite which the observed results serve as a basis for the design of further studies in comorbidities that share important links with COVID-19 due to their pathophysiological similarities.

Conclusively, based on important differences between EGF values in COVID-19 and those reported in healthy patients; evidence of an inverse linear relationship between EGF and age in non-critically ill COVID-19 patients; as well as an apparent relationship with neutrophils in the context of severe disease. It is tenable to propose the existence of a plausible relationship between serum epidermal growth factor values and COVID.19, with notably strong correlations for clinical parameters in the context of severe disease.


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Conflict of interest

The author declares that both himself and the institution to which he is affiliated with have not received payment from third parties for any aspect of the work presented; however, he points out the existence of a scientific collaboration with the Centre for Molecular Immunology.

Author contributions

Sole author.