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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

In this issue

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

An overview on the scientometric advancement of the eJIFCC

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REPORT

The history of the electronic Journal of the International Federation of Clinical Chemistry and Laboratory Medicine (eJIFCC) dates back over 20 years. Browsing the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) website one can witness that the earliest issues date back to the previous millennium. The eJIFCC publishes Thematic Issues as well as Issues with Free Communications, where the publication types can be Reviews, Original Articles, Case Reports and Letters. While assessing the volume of the published issues, it can be promptly realized that the annual published page count has increased considerably in recent years. All eJIFCC issues from 1999 onward are available online and are indexed, in a searchable, downloadable, and citable from on PubMed as of 2018. Since its conception, the eJIFCC has remained a Platinum Open Access Journal, distributed under the terms of the Creative Commons Attribution Non-Commercial License, that provides a

venue to all individuals who intend to publish in the vast field of laboratory medicine. The number of submissions for Free Communications has significantly increased from year to year, and according to figures from the past four years, and because of a strict peer review process, 71% of the submitted Free Communications were rejected and the reminder, 29%, were accepted, almost always following minor or major revision.

Global interest in the eJIFCC is illustrated by the geographical diversity of the published manuscripts (Table 1). In the past four years, on average 41 papers (range: 36-47) were published annually in a quarterly manner. This represents a significant increase as compared to the earlier years. It is also important that all involved strive to minimize the turnaround time of the accepted papers (i.e., from acceptance to publication). The quality of the published issues and individual papers has improved remarkably over the years thanks to our publisher Insoft Canada Inc.

The query naturally arises whether the increment in the number of publications and printed pages is accompanied with an enhanced recognition of the eJIFCC? In this short report, we delineate the path that the eJIFCC has followed in the past years and seek to characterize the journal by various objective scientometric parameters.

Evaluation by SCImago

The SCImago Journal Rank (SJR) is a widely accepted form of the real influence of a scientific journal. This evaluation accounts for both the number of citations received by a journal and the importance or prestige of the journals where the citations come from. A journal's SJR is a numeric value indicating the average number of weighted citations received during a selected year per document published in that journal during the previous three years. The SJR indicator has been developed to be used in extremely large and heterogeneous journal citation networks. It is a size-independent indicator, and its values rank journals by their "average prestige per article" and can be used for journal comparisons in science evaluation processes. The SJR indicator computation is carried out using an iterative algorithm that distributes prestige values among the journals until a steady-state

Table 1 **Accepted manuscripts in the eJIFCC by geographical distribution in the period 2018-2021**

Europe (76 papers)	Asia (31 papers)	America (20 papers)	Africa (20 papers)	Oceania (1 paper)
18: Italy	12: India	7: Canada, United States	10: Ethiopia	1: New Zealand
16: Spain	6: Nepal	3: Argentina	3: Morocco	
11 : Hungary	5: Turkey	2: Mexico	2: Nigeria, Sudan, South Africa	
6: Greece	3: Pakistan	1: Ecuador	1: Algeria	
5: Belgium, UK	2: Syria			
2: Austria, France Germany, Malta	1: Bangladesh, Japan, Malaysia			
1: Croatia, Czech Republic, Denmark, Lithuania, Portugal, Serbia, Slovenia				

The eJIFCC is open to all who submit valuable materials. As can be seen, accepted papers (reviews, original articles, case reports, letters) published in the period (2018-2021) were submitted from 5 continents and 37 different countries.

solution is reached. The SJR algorithm begins by setting an identical amount of prestige to each journal, then using an iterative procedure, this prestige is redistributed in a process where journals transfer their achieved prestige to each other through citations. The process ends up when the difference between journal prestige values in consecutive iterations do not reach a minimum threshold value anymore. The process is developed in two phases, (i) the computation of Prestige SJR (PSJR) for each journal: a size-dependent measure that reflects the whole journal prestige, and (ii) the normalization of this measure to achieve a size-independent measure of prestige, the SJR indicator. SCImago Journal Rank was developed by Scimago Lab. and it originates from a research group at University of Granada.

Based on the above criteria, journals are categorized into quartiles each representing 25% of the total sum and Q1 being the most highly cited journals. Within this quartile, a D1 group is a subcategory that labels the top 10% of the journals in that category. By using this evaluation, the eJIFCC ranks 24 out of 59 Medical Biochemistry journals and falls into the Q2 (Quartile 2) category along with several other respected laboratory journals.

Evaluation by Impact Factor

The founder of the Institute of Scientific Information, Eugene Garfield, devised the impact factor. For any given year, the ratio between the number of citations received in that year for publications in the given journal that were published in the preceding two years and the total number of citable items published in that journal during the two preceding years is the so-called two-year journal impact factor. Citable items are publications that are classified as article, review, or proceedings paper in the Web of Science database, as such editorials, corrections, notes, retractions, and discussions are excluded. The number of citations is extracted from the Journal Citation Reports (JCR) database, which is published by Clarivate. Furthermore, to accommodate annuals or other publications with irregular frequency, the JCR also includes a five-year impact factor which is a ratio of the citations to a given journal each year and the number of citable items published in the given journal during the preceding five years.

The application for inclusion of eJIFCC to the Web of Science core collection was submitted in March 2019. But following announcement of a new submission system in May 2021, our pending application was earmarked for resubmission, and the eJIFCC is still under evaluation by Clarivate.

Evaluation by Cite Score

The Cite Score (CS) of an academic journal is a measure reflecting the yearly average number of citations to recent articles published in that journal. This journal evaluation metric is relatively new and was launched in December 2016 by Elsevier as an alternative to the generally used JCR impact factors (calculated by Clarivate). Cite Score is based on the citations recorded in the Scopus database rather than in JCR, and those citations are collected for articles published in the preceding four years instead of two or five.

In any given year, the Cite Score of a journal is the number of citations, received in that year and previous 3 years, for documents published in the journal during that period (four years), divided by the total number of published documents (articles, reviews, conference papers, book chapters, and data papers) in the journal during the same four-year period. The calculation date for each given Cite Score as later additions, corrections or deletions to the data will not lead to a score update. Scopus also provides the projected-Cite Scores for the next year, which are updated every month. Before 2020, the score was calculated differently: in a given year, the Cite Score of

a journal was the number of citations, received in that year, of articles published in that journal during the three preceding years, divided by the total number of "citable items" published in that journal during the three preceding years: The Cite Score of the eJIFCC for 2020 was 2.7, and as it looks in early November 2021 the Cite Score tracker for 2021 indicates a value of 4.9. When compared to more established and respected journals, the eJIFCC has now also gained visibility on the Cite Score map (Figure 1).

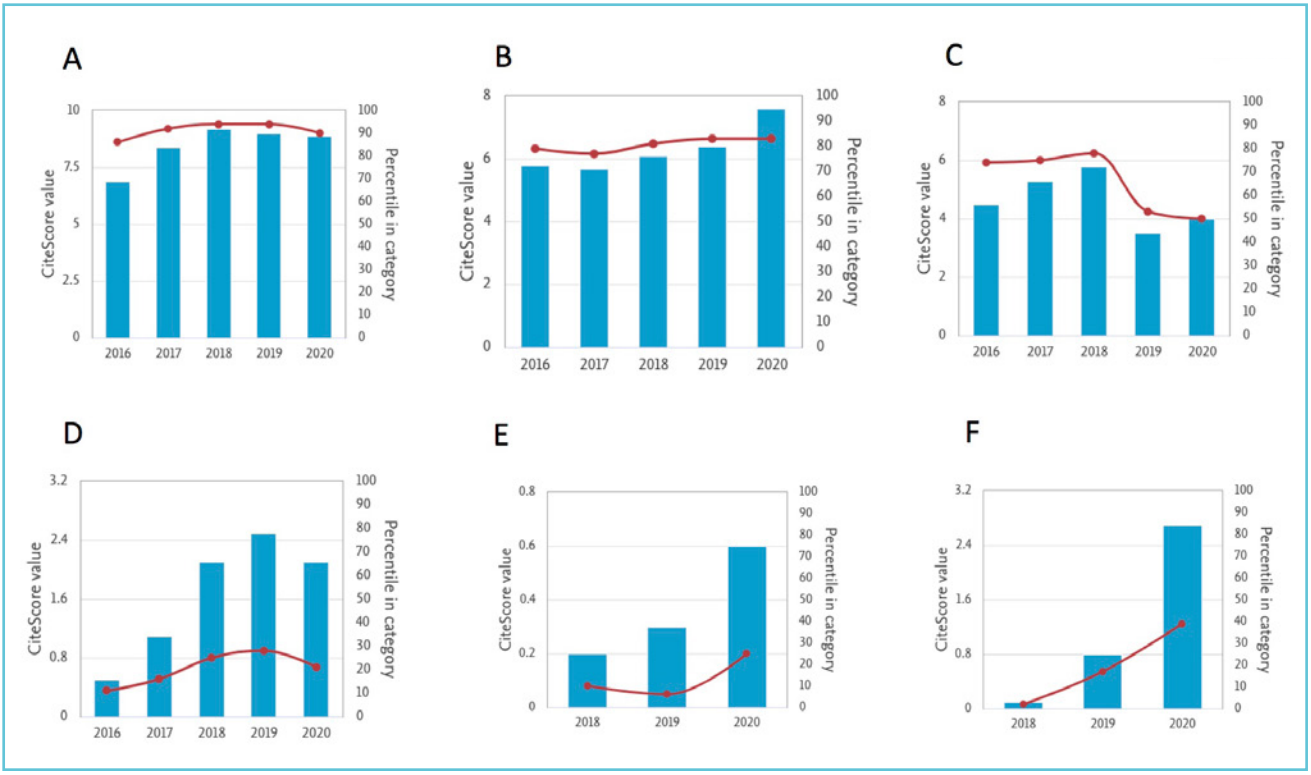
Evaluation by the Source Normalized Impact per Paper

Source Normalized Impact per Paper (SNIP) measures contextual citation impact by weighting citations based on the total number of citations in a subject field. The impact of a single citation is given higher value in subject areas where citations are less likely, and vice versa. Unlike the well-known journal impact factor, SNIP corrects for differences in citation practices between scientific fields, thereby allowing for more accurate between-field comparisons of citation impact. CWTS Journal Indicators also provides stability intervals that indicate the reliability of the SNIP value of a journal. SNIP was created by Professor Henk F. Moed at the Centre for Science and Technology Studies (CWTS), University of Leiden.

The advantage of using SNIP are the followings:

- Measures contextual citation impact by 'normalizing' citation values.

Figure 1 **Cite Score value (blue bars) and Percentile category (red lines) of selected clinical laboratory journals**



Respected clinical laboratory journals have a Cite Score value in the range of 5-10 and a percentile value in the upper quadrant, but with potential fluctuation in some cases (panels A-C). Other less known laboratory journals have lower Cite Score values and some are only present on this metric scale in recent years. Panel F demonstrates the steady increase for both Cite Score and Percentile parameters in case of the eJIFCC.

- Takes a research field's citation frequency into account.
- Considers immediacy - how quickly a paper is likely to have an impact in a given field.
- Accounts for how well the field is covered by the underlying database.
- Calculates without use of a journal's subject classification to avoid delimitation.
- Counters any potential for editorial manipulation.

A SNIP value of 1.0 means that a journal's articles are cited at the average rate for all journals in the same subject area; anything over 1.0 indicates more citations than the average in that field while a SNIP of less than 1.0 is below the average. A SNIP of more than 1.5 generally indicates a very well-cited journal. The 2020 SNIP score of the eJIFCC is 1.177, suggesting that it is higher than the average in the field and is in the range of other well-established laboratory journals.

It is also important how the citations are gained, and which published manuscripts generate the most citations. The 5 top-cited papers published in the eJIFCC based on the Clarivate database are demonstrated in Table 2.

It can be concluded that general laboratory topics as well as specialized areas are both of interest, and it is also interesting that two of the most cited papers were published recently. It is important to note that Issue-2 in 2019, a Thematic Issue guest edited by Béla Nagy jr. on 'non-coding RNAs as potential biomarkers' generated an immense interest. The 9 papers published in that Thematic Issue have an average citation of 25 per paper i.e., roughly 10 times that of an average eJIFCC cited paper. This means that when a hot topic is appropriately addressed it has a high chance of attracting the attention of the laboratory community to our journal.

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Table 2 **The top 5 cited papers published in the eJIFCC based on Web of Science data**

Name of authors	Title	Year	Pages	Times cited
Simundic A.M.	Measures of diagnostic accuracy: basic definitions	2009	203-211	**470**
Bonneau E, Neveu, Konstantin E, Tsongalis GJ, DeGuire V.	How close are miRNAs from clinical practice ? A perspective on the diagnostic and therapeutic market	2019	114-127	**93**
Kelly J, Sadeghien T, Adeli K.	Peer review in scientific publications:benefits, critiques and a survival guide	2014	227-243	**79**
Zakaria R, Allen KJ, Koplin J, Roche P, Greaves R.	Advantages and challenges of dried blood spot analysis by mass spectrometry across the total testing process	2016	288-317	**72**
diResta C, Galbiati S, Carrera P, Ferrari M.	Next-generation sequencing approach for the diagnosis of human diseases: open challanges and new opportunities	2018	4-14	**51**

The top 5 cited papers from the eJIFCC as retrieved from the Clarivate database. These papers represent the versatility of the topics that were published in the journal. The highest total citation is a single author review on the measures of diagnostic accuracy while the highest citation/year paper is a relatively recent review on microRNAs.

Acknowledgement

We are extremely grateful for all the contributing authors, and also for those whose manuscripts could not be accepted, as we learned a great deal from those. In addition, a lot of thanks to our guest editors and reviewers who helped us with maintaining the journal over the past years, and we hope to receive their support in the future as well.

The authors are indebted to Silvia Colli Lanzi, Ildikó Kópis and Valentina László for their excellent secretarial assistance.

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Call for manuscript submissions for a thematic eJIFCC issue on "Laboratory aspects of COVID-19 disease"

Guest editor for the thematic issue: Béla Nagy Jr.

Since the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic in December 2019, the importance of clinical laboratory tests has emerged to manage the hospitalization of patients with different severity of COVID-19 related disorders, to distinguish severe and non-severe clinical conditions and to predict the outcome of the disease. For these purposes, a vast number of clinical studies has recently been conducted to validate the potential role of various laboratory tests. In parallel, the effect of COVID-19 vaccines has also been evaluated. However, due to the rapid accumulation of this enormous amount of patient data, we need to raise the questions where we are now and where we should be heading?

We would like to offer some new insights into the usefulness of routinely available and novel laboratory biomarkers in the still demanding COVID-19 as well as for monitoring of vaccination with an eJIFCC issue dedicated to this disease. We invite you to submit a paper on ***"Laboratory aspects of COVID-19 disease"*** to be published in this thematic issue. Submitted papers will be peer-reviewed according to the regular procedure of the eJIFCC Journal.

Important deadlines

- Deadline for submission of the tentative title (to the Guest Editor): **April 1, 2022**
- Deadline for submission of the manuscript: **May 15, 2022**

Potential types of articles

- Original Article
- Critical Reviews
- Case studies

Manuscripts need to be submitted by e-mail

- to the Editor-in-Chief: ejifcc@ifcc.org
- with a copy to the Guest Editor: nagy.bela@med.unideb.hu

Guest editor

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The detection of hyaline casts in patients without renal dysfunction suggests increased plasma BNP

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Key words:
urinalysis, hyaline casts, BNP, eGFR

ABSTRACT

Background and aim

Casts in urinary sediments are useful in the identification of kidney diseases. Among them, hyaline casts have not previously been considered as pathognomonic. However, hyaline casts can occasionally be found in patients undergoing cardiovascular treatment without renal dysfunction. We evaluated the background of these patients and also investigated their levels of plasma brain natriuretic peptide (BNP).

Materials and methods

Samples from patients who visited the Division of Cardiovascular Disease at Nihon University Hospital (2014-2018) were examined. We set extract conditions from the laboratory information system database, setting the threshold over 60 mL/min/1.73 m^2 for the estimated glomerular filtration rate (eGFR), and proteinuria as absent (-) or trace (±). One hundred

forty-seven of 3137 (4.7%) samples showed hyaline casts (M:F=102:45, mean age 69.5±11.2 years). Samples with hyaline casts were divided into three rank groups. We compared BNP levels among each cast group and age-matched controls using Kruskal-Wallis analysis.

Results

The median BNP levels of the controls and the three casts groups were 23.3 pg/mL in the controls, 31.1 pg/mL in group (1+), 35.5 pg/mL in group (2+), and 45.8 pg/mL in group (≥3+). The median BNP levels differed significantly between two casts groups (group (2+) and group (≥3+)) and the control group ($P<0.05$ and $P<0.01$, respectively).

Conclusion

Hyaline casts could be detected in patients with normal renal function. When hyaline casts are more than 2+, the physician should consider checking plasma BNP levels of the patient.

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INTRODUCTION

Examination of the urinary sediment, especially in conjunction with assessment of proteinuria, is useful in the detection of chronic kidney disease. Casts are components of the urinary sediments, and most of them are useful in the identification of the type of kidney disease, such as erythrocyte casts in glomerulonephritis[1]. Among these casts, hyaline casts have previously not been considered to be pathognomonic, but are commonly detected in all kidney diseases[2]. They may also be seen in normal subjects who perform strenuous exercise and in non-renal disorders such as fever or dehydration[3].

However, hyaline casts occasionally can be found in patients undergoing treatment for cardiovascular disease. We have found a relationship between the number of urinary hyaline casts and the levels of plasma brain natriuretic peptide (BNP) from laboratory data on patients without proteinuria[4]. In that study, the number of hyaline casts and BNP levels were positively correlated, but we used only laboratory data from urine and blood without patient background data. Therefore, it was possible that the data included subjects with a history of kidney disease and heart failure. In the present study, we also reviewed medical histories to assess renal function and patient background. The aim of this study was to investigate whether hyaline casts in urine can be detected in patients with normal renal function, and to examine the relationship between their levels of plasma BNP and the number of hyaline casts.

SUBJECTS AND METHODS

Subjects

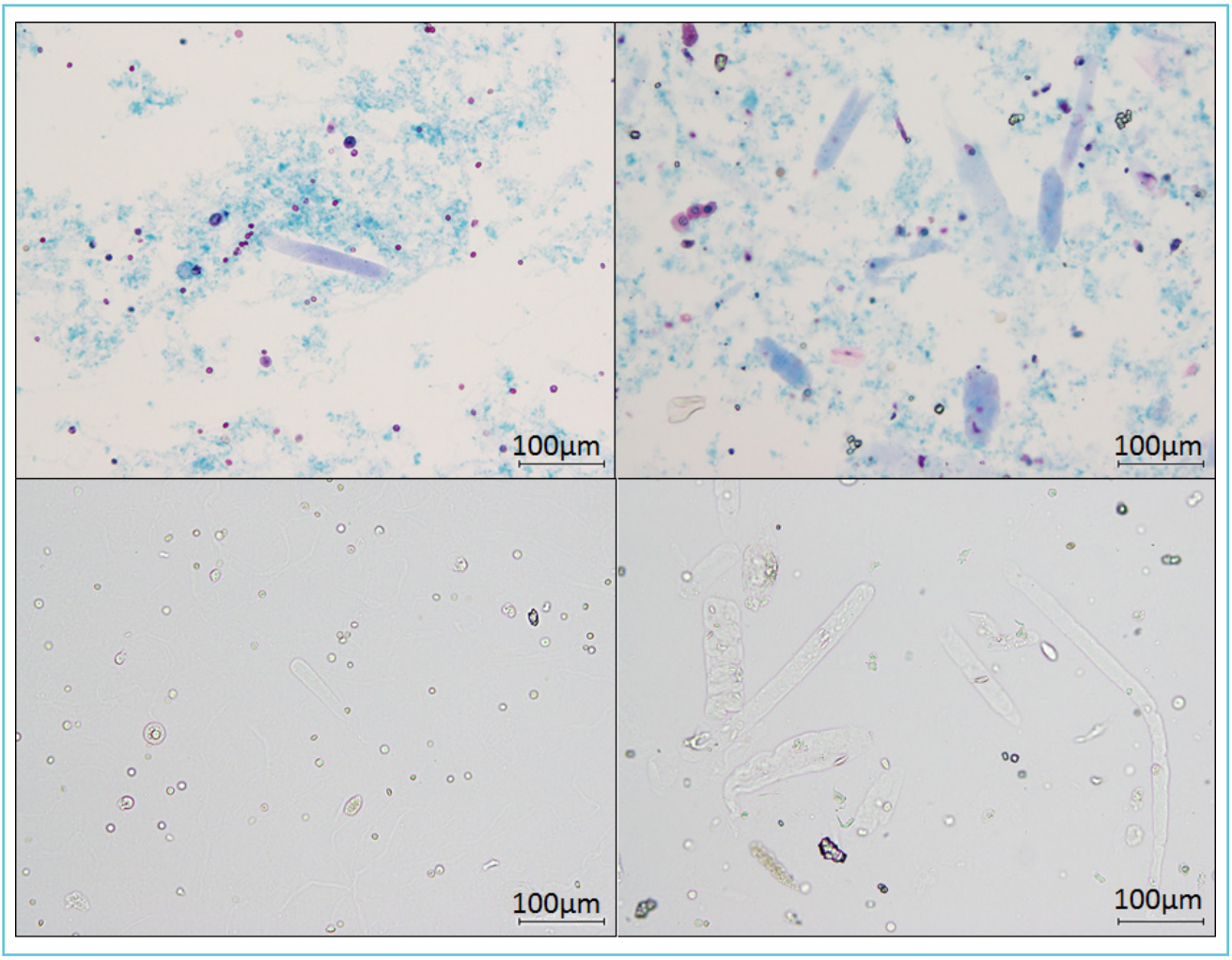
Samples from patients who visited and were tested in the Department of Laboratory Medicine at Nihon University Hospital from October 1, 2014 to December 31, 2018 were examined. We set extract conditions from the laboratory information system database of blood examination and urinalysis as follows. 1) Blood examination and urinalysis including urinary sediment examination were performed on the same day. 2) The threshold was set to over 1.8 mmol/L and under 7.5 mmol/L for blood urea nitrogen (BUN) and over 60 mL/min/1.73 m^2 for the estimated glomerular filtration rate (eGFR), and proteinuria was absent (-) or at a trace level (±). To exclude renal disease and renal dysfunction, the information of all patients' medical records was reviewed for five years. The medical records included demographic data, history of comorbid conditions, and use of cardiac medication. Exclusion criteria were as follows: a known history of renal dysfunction, defined as an eGFR of <60 mL/min/1.73 m^2; proteinuria; detection

of urine casts other than hyaline casts; or a history of acute infectious disease, cardiovascular surgery, myocardial infarction, or cerebral infarction. Because patients with chronic arterial fibrillation and chronic heart failure show increased BNP levels even in a medically stable condition[5,6], they were also excluded from the study.

Measurements

Urine samples were collected and examined using the method proposed by the Japanese Committee for Clinical Laboratory Standards (JCCLS)[7]. Proteinuria was assessed by a dipstick (Uriace, Eiken, Tokyo, Japan). Urine sediment was assessed by two trained laboratory technicians within four hours after collection. In preparing the urine sediment for examination, 10 mL of urine was centrifuged at 500 *g* (1,730 rpm; radius, 15 cm) for 5 min. Urinary casts were counted in whole fields at low power field (100×). Results from microscopic examination are described as approximate counts in the whole field (WF). Examples of hyaline casts under a microscope at low power field are presented in Figure 1.

Figure 1 Examples of hyaline casts under low power field (100×)



The upper two are after Sternheimer staining, and the lower two are without staining. The left panels are examples of group (1+) (1-9/WF) and/or group (2+) (10-29/WF). The right panels are examples of group (≥3+) (≥30/WF).

Serum creatinine (Hitachi Chemical Diagnostics Systems, Tokyo, Japan) and BUN (Serotec, Sapporo, Japan) were measured by enzymatic methods using fully automated analysis (LABOSPECT 008, Hitachi High-Technologies Co., Tokyo, Japan). We used eGFR equations devised by the Japanese Society of Nephrology (JSN) in the main analysis. The equations are: eGFRcreat [mL/min/1.73 m^2] = 194 × (s-Cr /88.4)[µmol/L] (-1.094) × age [years](-0.287) for males; and eGFRcreat [mL/min/1.73 m^2] = 194 ×(s-Cr /88.4) [µmol/L](-1.094) × age [years](-0.287) × 0.739 for females.

Plasma BNP levels (concentrations) were measured by fully automated enzyme immunoassay analysis using AIA-2000ST (TOSOH Bioscience, Tokyo Japan).

Transthoracic echocardiography was performed to assess left ventricular function (Vivid S6, GE Healthcare; Vivid E9, GE Healthcare; EPIQ 7, Philips; CX50, Philips). We measured the ejection fraction (EF) and left atrial diameter (LAD).

Data on medical history, use of antihypertensive medication, blood pressure in the right arm of the seated patient on the same day of blood and urine sample collection, and body weight and height were reviewed in each patient. Body weight and height were measured while the patient was wearing light clothing without shoes. Blood pressure was measured using an electronic sphygmomanometer (H55, TERUMO, Tokyo, Japan). Two consecutive measurements in the right arm of each seated patient were taken, and the second measurement was used for analysis. Hypertension, diabetes mellitus, hyperlipidemia, and angina pectoris were defined according to documentation of the diagnosis or the use of medications.

Statistical analysis

Samples with hyaline casts were divided into three rank groups according to JCCLS. They were defined by the number of the casts (1-9/WF as (1+), 10-29/WF as (2+), and ≥30/WF as (≥3+/WF)).

We compared BNP levels among each hyaline rank group and controls using Kruskal-Wallis analysis. A one-way analysis of variance (ANOVA) for repeated measures was used to compare the intragroup values of age, body mass index (BMI), eGFR, heart rate, and blood pressure. A Bonferroni correction and Tukey's honestly significant difference test were used for *post hoc* analysis. The *t* test was used to assess differences in EF and LAD in echocardiograms between the hyaline cast groups and controls. $P<0.05$ was considered statistically significant. Statistical analysis was performed using commercially available software (IBM SPSS Statistics for Windows, version 22.0.0.0, Armonk, NY). The statistical analysis was conducted at a 95% confidence level.

Ethical principles

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki in line with the Ethical Guidelines for Epidemiological Research by the Japanese government. This study was approved by the Ethics Committee of Nihon University Hospital (No. 20191001).

RESULTS

Initially, 4302 samples (M:F=2925:1377, mean age 65.6±12.2 years, range 18 to 95 years) were extracted from the laboratory information system database. According to the exclusion criteria mentioned above, we excluded 1165 samples out of 4302 (1165/4302, 27%). The remaining 3137 (M:F=2104:1033, mean age 67.9±9.14 years) were examined. One hundred forty-seven (M:F=102:45) out of 3137 (147/3137, 4.7%) samples showed hyaline casts (M:F=102:45, mean age 69.5±11.2 years, range

45 to 92 years). The clinical characteristics of all samples are presented in Table 1.

We used samples from patients without hyaline casts and who had transthoracic echocardiograms within three months as controls. We excluded those under 50 years of age, leaving for the control analysis (N=302, M:F=193:109, mean age 69±9.0 years, range 51 to 88 years) a population with matched age. The characteristics of the control and each hyaline group according JCCLS rank are presented in Table 2. The ratio of hypertension in controls was lower than in the total hyaline cast groups (65% and 80% respectively). The ratios of diabetes mellitus and dyslipidemia in the controls were also lower than in the total hyaline cast groups, but in each group the ratio of diabetes mellitus was around 30%. The ratio of angina pectoris in the controls was higher than in the total hyaline cast groups.

The prescribed antihypertensive drugs are presented in Table 3. Angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) were prescribed in 65% of the control group and in 49% of the cast groups. The most prescribed antihypertensive medications were ACEI/ARB in the control group (65%) and calcium channel blockers in the cast groups (60%). Diuretics were prescribed more frequently in the cast groups (13%) than in controls (2%).

Table 4 shows a comparison among four groups. The median BNP levels of the control and three casts groups were 23.3 pg/mL in the control, 31.1 pg/mL in group (1+), 35.5 pg/mL in group (2+), and 45.8 pg/mL in group (≥3+). These were significantly different in the two cast groups (group (2+) and group (≥3+)) compared to the control group ($P<0.05$ and $P<0.01$, respectively). There was a significant increase in BNP levels as the cast rank increased.

Table 1 Background clinical characteristic of the samples

	All samples (n=3137)
Age (years), mean (SD)	67.9 (9.14)
Sex (M/F)	2104/1033
Weight (kg), mean (SD)	64.23 (11.81)
BMI (kg/m^2), mean (SD)	24.3(3.7)
Medical history diagnosis	**n (%)**
Hypertension	69%
Diabetes mellitus	35%
Dyslipidemia	58%
Angina pectoris	22%

BMI: body mass index.

Table 2 Background clinical characteristic of controls and samples with hyaline casts

	Control (n=302)	Total samples with hyaline casts (n=147)	Hyaline cast rank		
			(1+) (n=70)	(2+) (n=37)	(≥ 3+) (n=40)
Age (years), mean (SD)	69.0 (9.0)	69.5 (11.2)	67.8 (10.5)	69.9 (11)	71.7 (11.8)
Sex (M/F)	193/109	102/45	46/24	23/14	33/7
Medical history diagnosis, n (%)					
Hypertension	197/302 65%	118/147 80%	54/70 77%	28/37 77%	36/40 90%
Diabetes mellitus	83/302 27%	43/147 29%	24/70 34%	10/37 27%	9/40 23%
Dyslipidemia	150/302 50%	94/147 64%	43/70 61%	24/37 65%	29/40 73%
Angina pectoris	59/302 20%	20/147 13%	10/70 14%	8/37 22%	2/40 5%

Table 3 Types and ratios of prescribed antihypertensive agents in control and hyaline cast groups

		Control (n=302)	Total samples with hyaline casts (n=147)	Hyaline cast level		
				(1+) (n=70)	(2+) (n=37)	(≥ 3+) (n=40)
Hypertension		197/302 65%	118/147 80%	54/70 77%	28/37 77%	36/40 90%
Types of antihypertensive medication	ACEI/ARB	132/302 65%	72/147 49%	36/70 51%	15/38 39%	21/40 53%
	Ca-blocker	138/302 44%	88/147 60%	37/70 53%	24/38 63%	27/40 68%
	β-blocker	48/302 46%	65/147 44%	28/70 40%	15/38 39%	22/40 55%
	Diuretic	7/302 2%	19/147 13%	4/70 6%	5/38 13%	10/40 25%

ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers.

Table 4 Comparison between control and hyaline cast ranks

	Control (n=302)	Hyaline cast level		
		(1+) (n=70)	(2+) (n=37)	(≥ 3+) (n=40)
BNP, pg/mL, median	23.3	31.1	35.5*	45.8**
eGFR, mL/min/1.73 m², mean (SD)	73.8 (9.2)	73.9 (8.6)	75.1 (11)	71.3 (8.7)
BMI, kg/m², mean (SD)	24.2 (4.0)	23.7 (3.9)	24.1 (5)	24.1 (4.5)
Heart rate, beats/min mean (SD)	67.5 (11.3)	63.5 (14)	63.5 (16)	64.2 (15.1)
BP, mmHg, systolic, mean (SD)	131.8 (18)	130 (14.8)	126 (13)	129 (14)
diastolic, mean (SD)	78.1* (11.2)	72 (10.3)	70.2 (11)	70.9 (10.2)

*BNP: B-type natriuretic peptide, eGFR: estimated glomerular filtration rate, BMI: body mass index, BP: blood pressure. *P<0.05, **P<0.01.*

No significant differences were observed in age, BMI, and eGFR among the groups. There were no significant differences in heart rate or systolic blood pressure, but diastolic blood pressure was significantly higher in the control group (P<0.05).

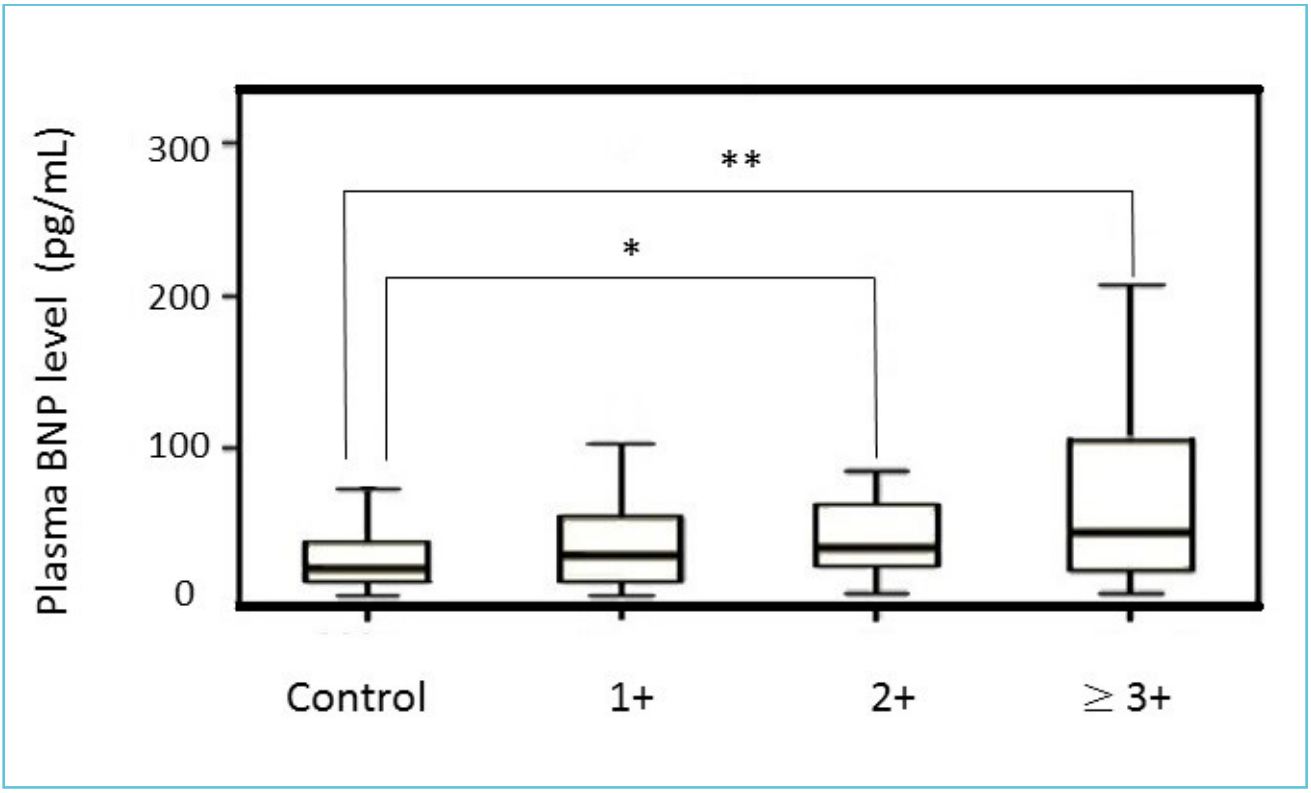
Box plots showing median levels of BNP were compared among the control and three hyaline cast groups (Figure 2). These differed significantly in the two casts groups (group (2+) and group (≥3+)) compared to the control group (*P<0.05 and ** P<0.01, respectively).

Table 5, on the following page, shows a comparison of echocardiography measurements between the control and cast groups. There were no significant differences in EF and LAD from echocardiography measurements between the cast and control groups according to *t* tests.

DISCUSSION

The significance of hyaline casts that appear in patients with normal renal function has rarely been investigated. We previously showed a relationship between the number of urine hyaline casts and plasma BNP levels using only laboratory data[4]. Therefore, patients with a history of kidney disease[8,9] might have been included in that data. Furthermore, diseases that show increased BNP such as congestive heart failure[10], acute infection with fever, and atrial fibrillation[5,6] were not excluded. In the present study, we excluded 27% of samples according to former evaluation criteria. However, the relationship between the number of urine hyaline casts and plasma BNP levels was still significant, showing an increase in BNP levels with an increasing number of hyaline casts. We also found no significant differences in eGFR among groups.

Figure 2 Box plots comparing control and hyaline cast ranks



*Box plots showing median levels of BNP were compared among the control and three hyaline cast groups. Median BNP levels were significantly different between two cast groups (group (2+) and group (≥3+)) and the control group. *P<0.05, **P<0.01.*

Table 5 Comparison of echocardiography measurements between control and cast groups.

	Control (n=302)	Samples with hyaline casts (n=34)
Age (years), mean (SD)	69.0 (9.0)	68.3 (12.2)
Sex (M/F)	193/109	21/13
Echocardiography measurements		
EF (%), mean (SD)	68.8 (7.5)	65.6 (11.1)
LAD (mm), mean (SD)	36.7 (5.8)	37.8 (6.8)

EF: ejection fraction; LAD: left atrial dimension.

These results confirmed that hyaline casts could be detected in urine from patients with normal renal function and that they suggest possibly elevated plasma BNP.

Normally, very few casts are seen in urine examinations without renal dysfunction. Hyaline casts are the most frequently observed, and zero to two casts per low-power field is considered normal. Increased urine concentration, such as from dehydration, and elevated albumin concentration also affect the emergence of hyaline urine casts[11]. In this study, subjects with proteinuria and/or high BUN were excluded. Hyaline casts may also be seen when the decline in renal perfusion leads to sluggish urinary flow[12].

Consistently low urine flow was excluded from the causes of cast formation by our exclusion criteria. Furthermore, there was no significant difference in EF and LAD from echocardiography measurements between the cast and control groups. Imhof et al.[13] reported that loop diuretics, not thiazide diuretics, cause the production of hyaline casts without any proteinuria. In this study, 80% of cast group subjects suffered hypertension, whereas 65% of control group subjects did. Diuretic therapy may contribute to the production of hyaline casts in some patients. However, diuretic use was only at 13% in the cast groups, less than the use of ACEI/ARB and Ca-blocker agents. Therefore, drug use cannot sufficiently explain the emergence of hyaline casts in this study.

BNP is a neurohormone secreted by the cardiac ventricles in response to ventricular volume overload[14]. Both BNP and N-terminal proBNP (which is generated from the same molecule of BNP) are used as reliable diagnostic markers for heart failure and preclude the need for echocardiography in cardiovascular patients[15]. Plasma BNP levels are also related with heart failure severity and are increased in more advanced New York Heart Association (NYHA) functional classes[14]. Furthermore, in asymptomatic cohorts, plasma natriuretic peptide levels predict the risk of cardiovascular mortality, a first cardiovascular event, heart failure, and stroke or transient ischemic attack[16]. NT-proBNP is also an independent predictor of mortality and cardiovascular risk in hypertensive patients[17].

The Japanese Heart Failure Society recommends echocardiography in patients with a BNP level over 100 pg/mL. In cases with many risk factors and with a BNP level of 40–100 pg/mL, chest X-ray, electrocardiogram, and echocardiogram are recommended[18,19]. However, for asymptomatic patients, a physician should check plasma BNP by clinical signs and/or baseline characteristics. There is no definitive recommendation of when physicians should check plasma BNP, and the high cost might make it an impractical screening tool. However, if urinalysis can be used for screening before checking plasma BNP, nearly three-quarters of the cost can be saved. The Japanese Heart Failure Society also states that patients with a BNP level of 40–100 pg/mL may have mild heart failure. In this study, the median BNP level in the (≥3+) group was 45.8 pg/mL. This is within the range that mild heart failure, which is equivalent to NYHA I, may be present.

The mechanism of the interaction between hyaline casts and plasma BNP is unknown. Hyaline casts may appear to be related to blood pressure that is high enough to need an antihypertensive agent including a diuretic, because 80% of patients with casts showed hypertension whereas only 65% of control patients did. However, there were no significant differences in systemic blood pressure, indicating that blood pressure was under control in the cast groups. Temporary high blood pressure therefore does not explain this phenomenon. An arteriosclerosis-related change in some kind of emulgent may affect the emergence of hyaline casts, which could also be related to the increase in plasma BNP.

In conclusion, even though the mechanism is unclear, our findings indicate that physicians should consider measuring plasma BNP levels in subjects presenting hyaline casts in urine, even if they show normal renal function. Especially for samples with ≥2+ number of casts, increased plasma BNP can be expected.

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Ups and downs of COVID-19: can we predict the future? Local analysis with Google Trends for forecasting the burden of COVID-19 in Pakistan

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Google Trends, COVID-19, Pakistan, pandemic, prediction

ABSTRACT

Background

We aim to study the utility of Google Trends search history data for demonstrating if a correlation may exist between web-based information and actual coronavirus disease 2019 (COVID-19) cases, as well as if such data can be used to forecast patterns of disease spikes.

Patients & methods

Weekly data of COVID-19 cases in Pakistan was retrieved from online COVID-19 data banks for a period of 60 weeks. Search history related to COVID-19, coronavirus and the most common symptoms of disease was retrieved from Google Trends during the

same period. Statistical analysis was performed to analyze the correlation between the two data sets. Search terms were adjusted for time-lag over weeks, to find the highest cross-correlation for each of the search terms.

Results

Search terms of 'fever' and 'cough' were the most commonly searched online, followed by coronavirus and COVID. The highest peak correlations with the weekly case series, with a 1-week backlog, was noted for loss of smell and loss of taste. The combined model yielded a modest performance for forecasting positive cases. The linear regression model revealed loss of smell (adjusted R^2 of 0.7) with significant 1-week, 2-week and 3-week lagged time series, as the best predictor of weekly positive case counts.

Conclusions

Our local analysis of Pakistan-based data seemingly confirms that Google trends can be used as an important tool for anticipating and predicting pandemic patterns and pre-hand preparedness in such unprecedented pandemic crisis.

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INTRODUCTION

Pakistan is a low-income country in the subcontinent and also one of the most overpopulated countries in the world, with a high prevalence of communicable diseases. Pakistan ranks 154th out of 189 countries, with Human Development Index value of 0.557 (1). Due to high population densities, lack of skilled medical personnel and resources, low literacy rate and budgetary constraints, among other reasons, Pakistan's healthcare system is especially vulnerable to epidemic infectious disease, and coronavirus disease 2019 (COVID-19) is unfortunately not an exception to this rule.

Pakistan has earlier struggled with controlling other life-threatening infectious diseases such as dengue, hepatitis, acquired immunodeficiency syndrome (AIDS) and so forth, and has lost thousands of lives because of this (2,3,4). This has led researchers to believe that the risk for COVID-19 morbidity and mortality may be higher in Pakistan compared to other worldwide countries (5). The lack of resources, such as test assays, has made it imperative for low-income countries (like Pakistan) to identify reliable alternatives to mass COVID-19 testing, so that the spread of disease could be curbed before becoming unmanageable for society, government and healthcare system.

Since COVID-19 has put more pressure on an already overburdened and underfunded healthcare system, diagnostic testing for severe acute respiratory disease coronavirus 2 (SARS COV-2) infections has been vastly limited. This has potentially led to an underestimation of COVID-19 prevalence in the country (6). However, this problem is not only limited to Pakistan and other developing countries. Most clinical laboratories worldwide, up to 80%, have reported facing difficulties in SARS-COV-2 testing, while more than half reported shortage of supplies needed for routine molecular testing (52%) (7). Hence, a substitute for diagnostics is needed, that could accurately mirror COVID-19 epidemiology in specific geographical areas. In a study conducted by Ginsberg et al, internet search-engine query data was used to predict the course of influenza in the United States (8). This surveillance method provided positive results in non-English speaking countries as well. Studies have correlated the data from Europe, China, Korea and Taiwan with Google Trends for COVID-19 and other epidemic diseases, such as influenza (9, 10). Hence, the application of Google Trends to track disease progression has a far-reaching influence for countries across the globe, regardless of their location or language. It is also

cost-effective, timesaving and does not carry any substantial economical or organizational burdens the healthcare system. Therefore, we ideally conceive that Google Trends may have the potential to assess the prevalence of COVID-19 in Pakistan and anticipate new waves of infection.

A survey conducted in Pakistan showed that nearly half (45%) of patients search about their health-related concerns on the Internet (11). Hence, using epidemiological data from Google trends could significantly represent the Pakistani population. Google search terms regarding key symptoms, such as loss of taste and loss of smell, may help in predicting the epidemiological trajectory of COVID-19 (12,13). Olfactory and gustatory dysfunctions have a strong association with COVID-19 patients, with anosmia showing the highest correlation (14). Tracking these symptoms can be a feasible and viable means for assessing the prevalence of COVID-19 and effectively target the government response.

This study was hence designed to assess the potential utility of Google search trends focused on COVID-19 symptoms (including anosmia and dysgeusia), in projecting the trajectory of the local pandemic outbreak in Pakistan through correlation with ongoing COVID-19 statistics.

MATERIAL AND METHODS

In order to avoid daily variations in the positivity rate, the number of weekly COVID-19 confirmed cases in Pakistan were retrieved from OurWorldInData.org, powered by the Johns Hopkins Coronavirus Resource Center (15). The data was further confirmed from the official website of the Ministry of National Health, Pakistan (16,17). This information was retrieved for a period between March 15, 2020 and June 15, 2021. Data was acquired from Google Trends (Google Inc., Mountain View, CA), using the following search terms, encompassing the most representative symptoms in COVID-19: fever, cough, headache, shortness of breath, taste loss and hearing loss, along with other virus-related keywords such as 'COVID-19', 'coronavirus', 'virus' and 'COVID. A weekly Google Trends score was obtained for each keyword on a scale of 100 points, reflecting the cumulative number of Google searches during the previous week. The maximum attainable score of 100 was defined as the highest search volume during the study period for a particular search.

The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation. This analysis was based on electronic searches in unrestricted, publicly available repositories, such that no informed consent or ethical committee approvals were needed.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Microsoft Excel for Windows (2016) and R Software (version 4.0.2; R Foundation for Statistical Computing). Correlation analysis for individual search terms was used for assessing the time lags which generated the maximum achievable correlations between the weekly positive cases and Google trends timeline. The corresponding P values and 95% confidence intervals (CIs) were also calculated. A P value <0.05 was considered as statistically significant.

To calculate the quantitative effect of Google Trend score increment on subsequent rise in weekly cases, time series linear regression analysis was performed, and the time lag with maximum predictive value was computed. Adjusted R2 values and graphic analysis was undertaken to assess the combined model performance of positivity rate forecasting compared against national surveillance data.

RESULTS

The highest overall trend value for the study duration was achieved for fever (n=3036), followed by cough (n=2120), Coronavirus (n=1669), COVID (n=1417), headache (n=1284), COVID-19 (n=333), virus (n=329), shortness of breath (n=257), loss of smell (n=129) and loss of taste (n=106) respectively. From all searched terms, fever and cough during second week of June and last week of May 2021 attained the highest Google trend value of 100.

Time-series linear regression analysis is provided in Figure 1(a-e) and 2, summarizing the effects of the Google Trends search series when adjusted for the monthly trend of an increase in positive cases.

The linear regression model revealed loss of smell (adjusted R^2 of 0.7) with the significant 1-week, 2-week and 3-week– lagged time series, as the best predictor of weekly positive case counts, as further elaborated in Table 3 and Figure 1(a-e). The combined model yielded an excellent performance for forecasting positive cases with adjusted R^2 value of 0.83 as shown in Figure 2 and Table 2.

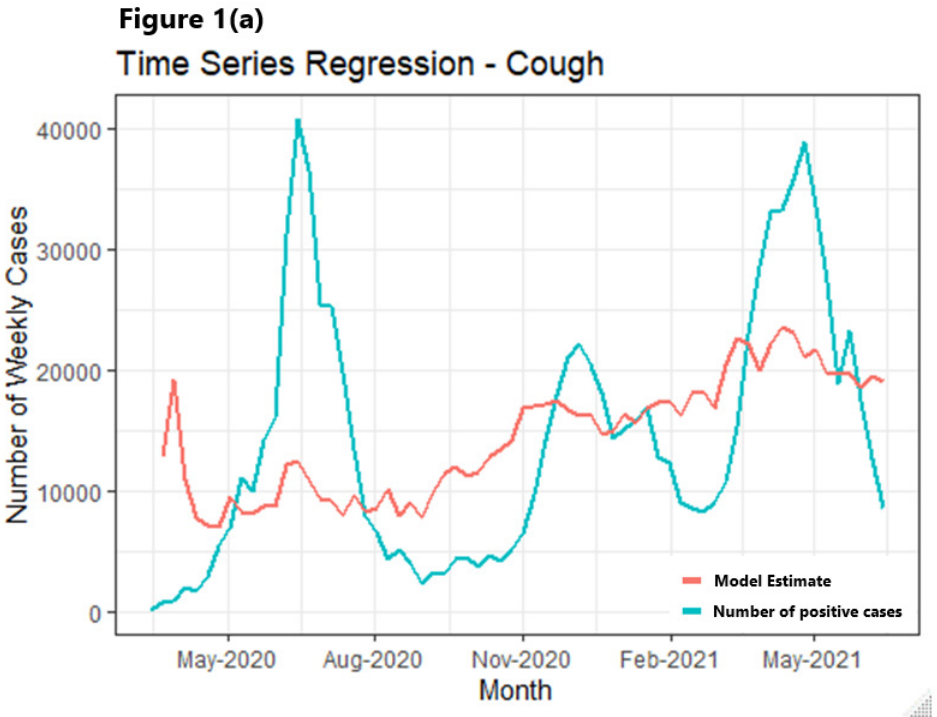
DISCUSSION

The results of our study demonstrate the existence of a statistically significant positive correlation between Google search terms and overall COVID-19 positivity rate in Pakistan. This was especially evident for search terms such as 'fever', 'smell loss', 'taste loss' and 'shortness of breath', with a time lag of 2 weeks, while for 'cough' and

Table 1 Cross-correlation analysis between weekly number of COVID-19 cases in Pakistan with Google Trends scores for suggestive symptoms

Search term	Optimal lag	Correlation	p-value
Fever	-2	0.437	<0.001
Headache	-8	0.349	0.005
Smell loss	-2	0.561	<0.001
Cough	-3	0.260	0.035
Taste loss	-2	0.618	<0.001
Shortness of breath	-2	0.289	0.019
Coronavirus	-3	-0.326	0.008
COVID	-1	0.501	<0.001
COVID-19	-7	-0.353	0.004
Virus	-4	0.322	0.009

Figure 1 (a-e) **Time-series linear regression analysis for weekly number of COVID-19 in Pakistan with Google Trends scores for suggestive symptoms**



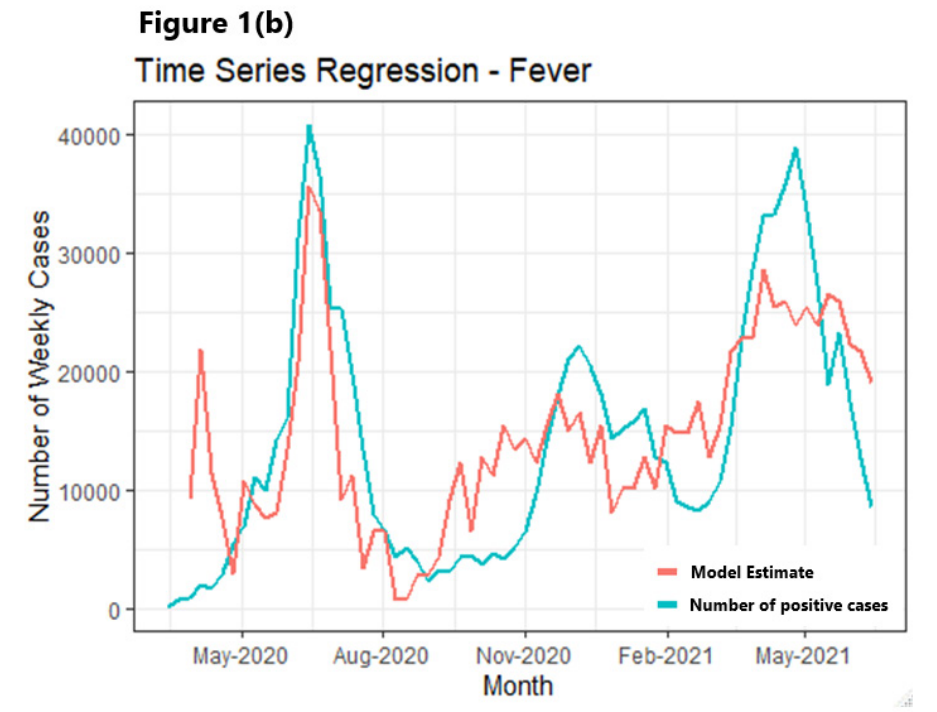


Figure 1(c)

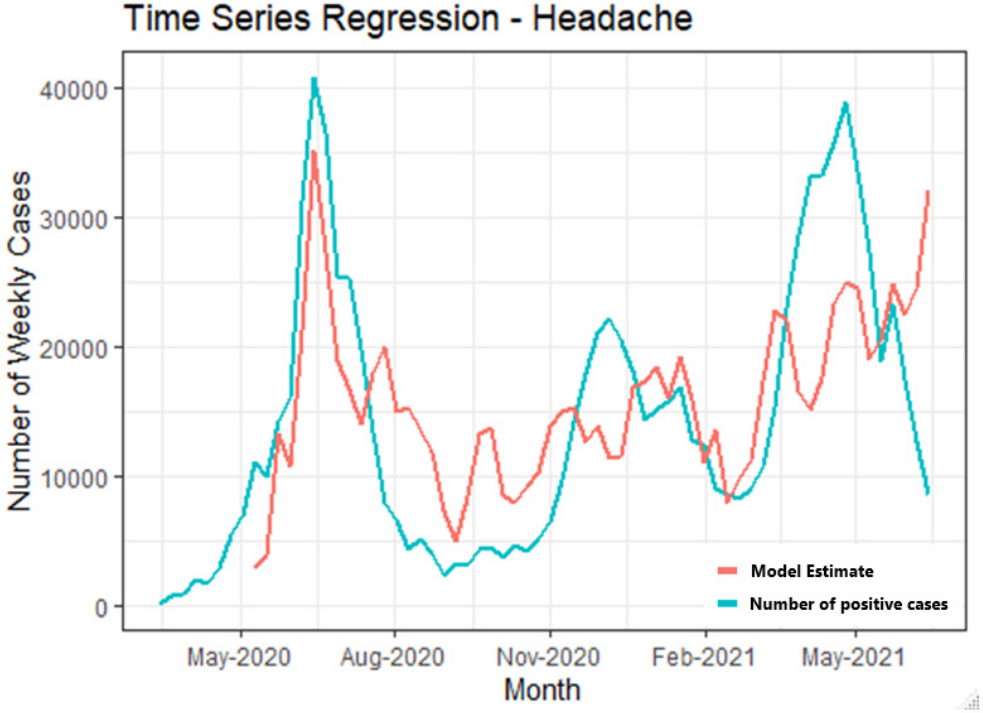


Figure 1(d)

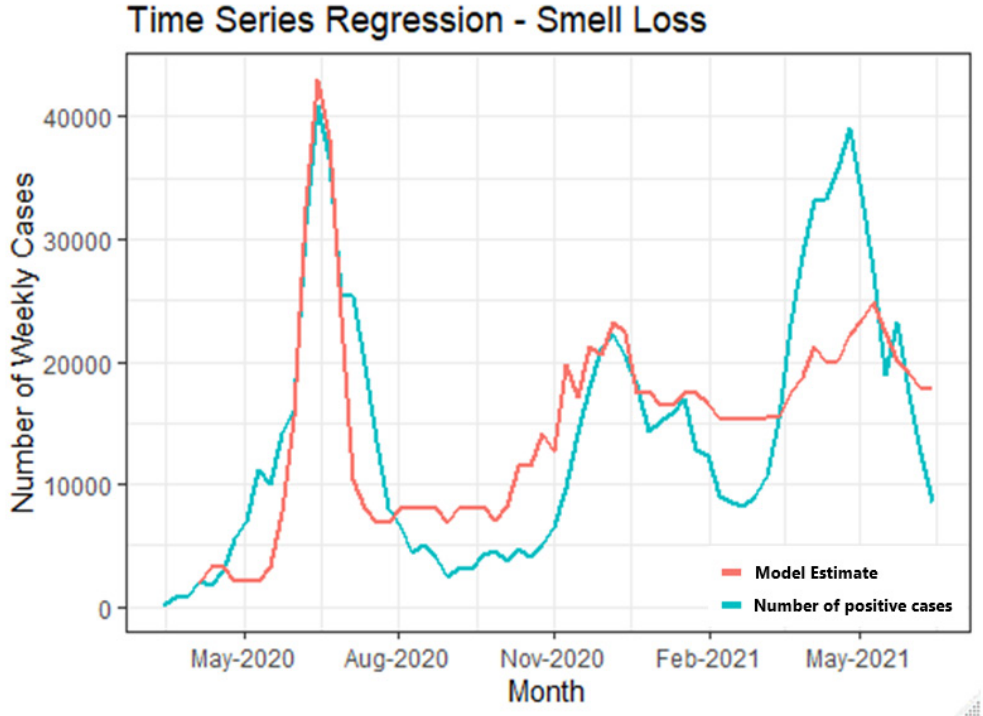


Figure 1(e)

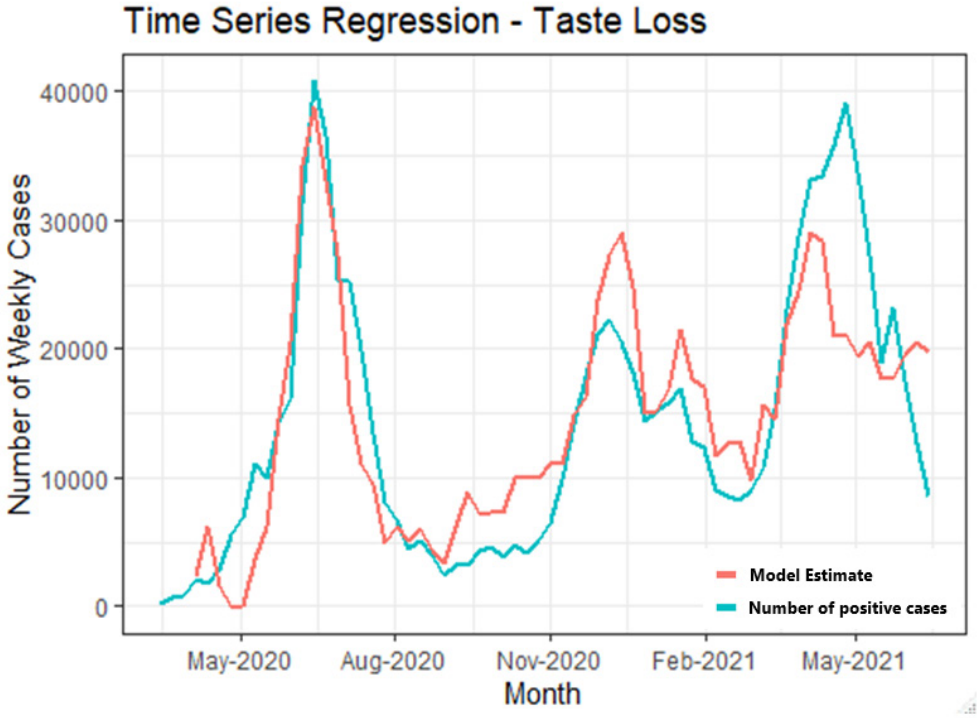


Table 2 Adjusted R^2 based on time-series linear regression analysis for the combined model for weekly number of COVID-19 in Pakistan with Google Trends scores

	Estimate	Std. Error	p-value
Month	798.4	204.1	<0.001
Fever (Lagged -2)	219.1	66.5	0.002
Taste Loss (Lagged -1)	1478.9	480.2	0.003
Taste Loss (Lagged -3)	1717.6	507.2	0.001
Covid (Lagged -1)	237.8	50.0	<0.001
Covid-19 (Lagged -7)	-1725.8	420.5	<0.001
Adjusted R^2 0.83			

Figure 2 Time-series linear regression analysis for the combined model for weekly number of covid-19 in Pakistan with Google Trends

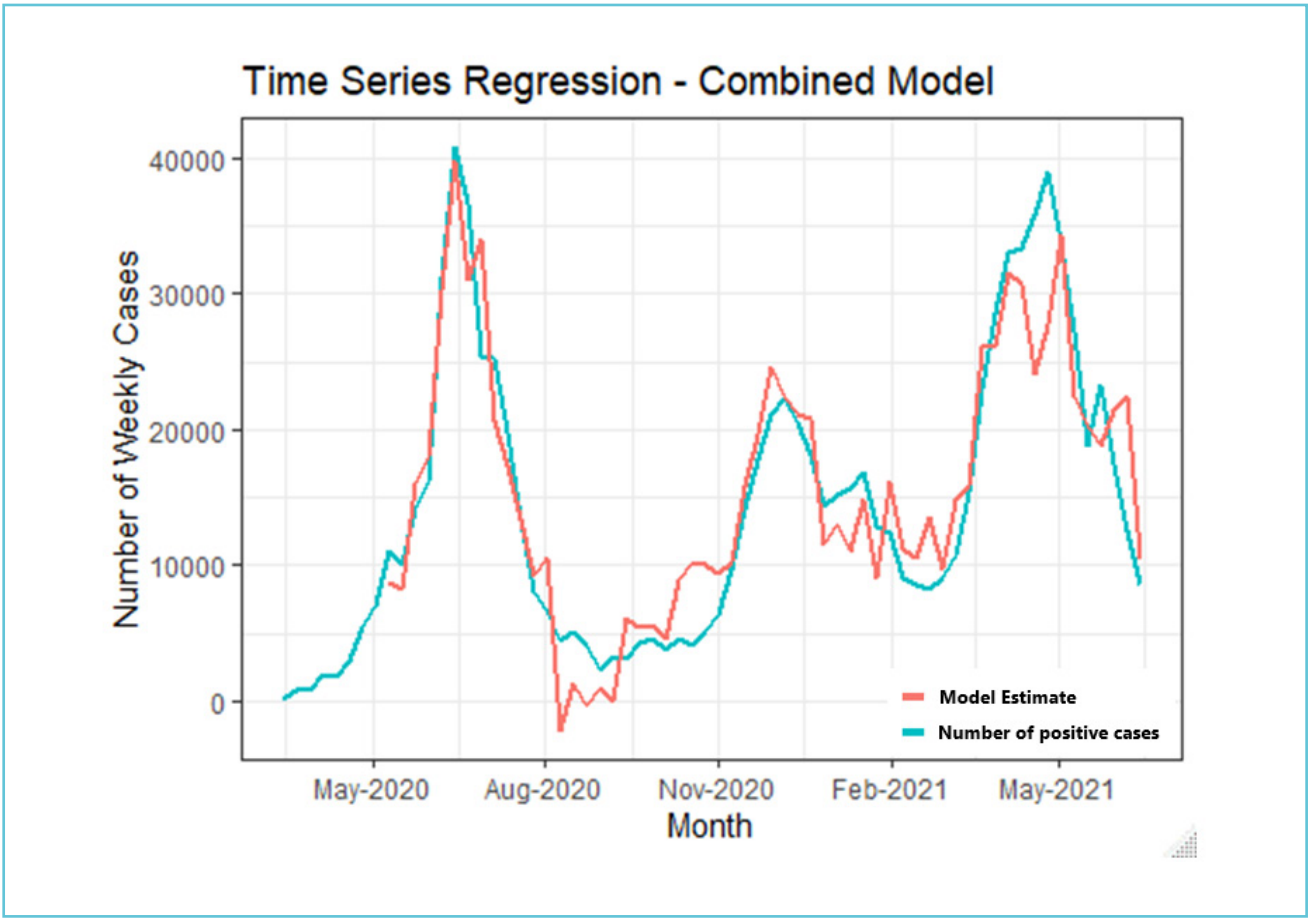


Table 3 Adjusted R^2 based on time-series linear regression analysis for weekly number of covid-19 in Pakistan with Google Trends scores for suggestive symptoms

Symptom	Adjusted R^2
Fever	0.36
Cough	0.44
Headache	0.60
Loss of smell	0.70
Loss of taste	0.70

'coronavirus' the time lag was 3 weeks. This model can hence successfully predict an increase in COVID-19 cases 2-3 weeks ahead of official diagnosis, thus allowing government and healthcare system to adapt and be prepared for the oncoming burden.

Google Trends is a useful tool for forecasting both healthcare and non-healthcare related epidemiological trends. The freely available data can help health authorities to anticipate increases in demands of testing capacity, as well as treatment facility, including availability of hospital beds, oxygen supply, access to ventilators and availability of adequate number of physicians and ancillary staff.

Although this study is a first-of-its-kind based in Pakistan, other countries have successfully used Google Trends to predict changes in the ongoing COVID-19 pandemic outbreak. Cherry et al. studied Google Trends data for 137 regions from 5 different countries, reporting that pathognomonic symptoms such as anosmia and dysgeusia can accurately predict the future incidence patterns of COVID-19 (12). Henry et al. reported similar results in Poland (18). In another study, Lippi et al. also describe significant associations of fever, fatigue and dyspnea with the COVID-19 outbreaks in Italy (19). The same group reported that the correlation between Google searches and COVID-19 cases became stronger with a lag of 2 weeks, as compared to the same week (13). The results of these studies are hence in keeping with the findings of our Pakistan-based analysis. The use of Google Trends in health policy making and management of pandemic could be especially useful for low-middle income countries (LMIC) like Pakistan, where resources are limited and strict and timely management of these resources can help curb the increasing pandemic.

The model we developed could be hence used in other LMIC, to direct resources where most required. Although our study utilized data from the whole country, region specific data can also be used to focus resources to regions which require them the most in near future.

The limitations of our study include the limited use of internet decrease literacy rate in developing countries. Also, the internet use behavior can be influenced by media communications, and possibly serve as a cofounder. Increased knowledge about self-reported symptoms can also decrease the use of internet for searching COVID-related information.

CONCLUSION

Google Trends is an effective tool for forecasting trends of the ongoing COVID-19 pandemic outbreak. We found a high correlation between Google searches for COVID-19 symptoms and diagnoses of SARS-CoV-2 infection, which can be used to direct resources where required or needed. Such data can help government authorities and health policy-making agencies to make well-informed decisions related to imposition of lockdown and provision of resources. Utilizing such data can help developing countries like Pakistan streamline their efforts against the pandemic and possibly prepare of outbreaks before the actually happening to minimize morbidity and mortality as well financial losses that may pursue.

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Data availability statement

The data that support the findings of this study are openly available by accessing https://trends.google.com/trends/?geo=PK and the links in references [15, 16].

Ethics statements

This analysis was based on electronic searches in unrestricted, publicly available repositories,

so that no informed consent or ethical committee approvals were needed.

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Author contribution

SA performed the literature search, data analysis and write-up of the work in the first draft. MAA was involved in the write up, literature search and data collection. MSO did the data analysis and prepared graphs and tables. AS assisted in the writing of the first draft. ZAA and IS were involved in the critical revision of the article for the intellectual content. LJ conceived the idea, coordinated the writing of the paper and reviewed the final draft. GL provided supervision of the project, contributed to discussion of the results along with review and amelioration of the draft. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Profiling of lactate dehydrogenase isoenzymes in COVID-19 disease

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ABSTRACT

Introduction

Serum total lactate dehydrogenase (LDH) activity was elevated and showed a positive correlation with disease severity and outcome in severe COVID-19 disease. However, it is still unknown whether the relative abundance or calculated activity of any LDH isoenzyme is predominately increased in COVID-19 subjects.

Methods

Twenty-two consecutive patients suffered from moderate or severe COVID-19 pneumonia were recruited into this study who showed enhanced total LDH activity. The ratio of LDH isoenzyme activities was further investigated using gel electrophoresis (Hydragel®, Sebia) with densitometric evaluation. Calculated activity values of these isoenzymes were correlated with routine laboratory parameters, the degree of lung

parenchymal affection based on chest CT and clinical outcome.

Results

Total LDH activity was raised in the range of 272-2141 U/L and significantly correlated with calculated LDH-3 and LDH-4 activities (r=0.765, P=0.0001; and r=0.783, P=0.0001, respectively). In contrast, the relative abundance of neither LDH isoenzyme was exclusively abnormal in COVID-19 patients. Calculated activity of LDH-3 and LDH-4 demonstrated a modest but statistically significant association with serum ferritin (r=0.437, P=0.042; r=0.505, P=0.016, respectively). When the relationship between the severity of pulmonary affection by SARS-CoV-2 infection and relative abundance of LDH isoenzymes was studied, a larger ratio of mid-zone fractions was observed in the presence of ≥ 50% lung parenchymal involvement. Finally, regardless of LDH isoenzyme pattern, abnormal relative ratio of LDH-4 and higher calculated LDH-3 and LDH-4 activity values were detected in subjects with unfavorable outcome.

Conclusion

No characteristic profile of LDH isoenzymes can be detected in COVID-19 pneumonia, however, elevated activities of LDH-3 and LDH-4 are associated with worse clinical outcomes.

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INTRODUCTION

Since the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic in December 2019, the importance of clinical laboratory tests has emerged to manage the hospitalization of patients with different severity of COVID-19 related disorders, to distinguish severe and non-severe clinical conditions and to predict the outcome of the disease. For these purposes, an enormous amount of clinical data has recently accumulated to evaluate and validate the potential role of routinely available as well as novel laboratory biomarkers (1). There are several parameters which have been identified as independent risk factors to assess disease severity, such as C-reactive protein (CRP) (2,3), interleukin-6 (2,3), circulating ACE2 activity (4,5), D-dimer (3,6), total lactate dehydrogenase (LDH) (7-10) and cardiac markers, i.e. high-sensitive cardiac troponin I (cTnI) with myoglobin, CK-MB activity and NT-pro-BNP (11). In parallel, CRP (12) and total LDH (10,12) were useful to recognize early lung injury and failure, whilst total LDH (7,8,11), CRP (13), D-dimer (6,14) and soluble ACE2 activity (5) were able to predict unfavorable outcome of COVID-19. Furthermore, the combination of increased total LDH with other blood-based biomarkers or clinical parameters could aid the clinical estimation of COVID-19 severity and mortality (15,16).

Regular analysis of total LDH activity has got in focus in this disease, however, only limited amount of data is available on the profile of LDH isoenzymes that was analyzed in plasma samples of some COVID-19 subjects (17). Hence, our aim was here to further investigate the relative abundance and calculated activity of LDH isoenzymes in serum by gel electrophoresis in hospitalized COVID-19 subjects in connection with the disease severity and worse clinical outcome.

METHODS

Patients

In this study, 22 consecutive patients (13 males and 9 females) at the age of between (min-max) 27-81 years of age were recruited from March 1 to 14, 2021 at the Clinical Center and Gyula Kenézy Campus, University of Debrecen, Debrecen, Hungary (Table 1). These subjects suffered from severe (n=14) or moderate (n=8)

pneumonia at sampling time point and were confirmed to be positive for COVID-19 disease by reverse transcription polymerase chain reaction (RT-qPCR) test of a nasopharyngeal swab. All these patients underwent chest CT scan to evaluate the extent of pulmonary lesions, such as ground-glass opacities and consolidation using a visual scoring system. Also, enrolled subjects suffered from various diseases, such as hypertension, cardiomyopathy, diabetes mellitus, renal disorders, cataract or angina based on their pre-COVID-19 history (Table 1). Severely ill patients were transferred to the Intensive Care Unit (ICU), while those with moderate symptoms were treated at the Department of Infectious Diseases, Gyula Kenézy Campus, University of Debrecen, Debrecen, Hungary. Despite ICU treatment all severe subjects died of COVID-19 within 28 days of the initiation of the disease, while patients in moderate clinical status were effectively treated and survived (Table 1).

Laboratory analyses

Total LDH activity and serum creatinine were determined by kinetic colorimetric assays on a Cobas® 8000 analyzer (Roche Diagnostics, Mannheim, Germany). In parallel, white blood cell (WBC) counts were determined by an Advia 2120 Hematology System analyzer (Bayer Diagnostics, Tarrytown, NJ, USA). The concentrations of C-reactive protein (CRP), ferritin, and cTnT were determined by electro-chemiluminescent immunoassay (Cobas® e 411 analyzer, Roche Diagnostics), while D-dimer was analyzed by immunoturbidimetry (BCS® XP, Siemens, Munich, Germany). The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation was used to estimate the glomerular filtrate rate (GFR).

The five isoenzymes of LDH were separated by electrophoresis using LDH Hydragel® 7 kit (Sebia, Norcross, GA, USA) on alkaline buffered (pH 8.4) agarose gel. The separated isoenzymes were visualized using a specific chromogenic substrate, and the amount of formazan precipitate was proportional to the LDH enzymatic activity. A semi-automated HYDRASYS® electrophoresis instrument (Sebia) was applied to obtain gels ready for interpretation. The dried gels were processed for densitometry to achieve an accurate relative quantification of individual zones. Abnormal ratio of LDH isoenzymes was evaluated based on the manufacturer's instructions of LDH Hydragel® 7 kit.

Statistical analyses

Kolmogorov–Smirnov test was used for evaluation of the normality of data. To compare the data of two groups, we applied Mann–Whitney U test. Correlations between total LDH and LDH isoenzyme activities as well as the link between LDH-3 or LDH-4 activity and other laboratory parameters were determined using Spearman's test. Statistical significance was defined when P value was < 0.05. Statistical analyses were performed using GraphPad Prism software (version 6.01, La Jolla, CA, USA).

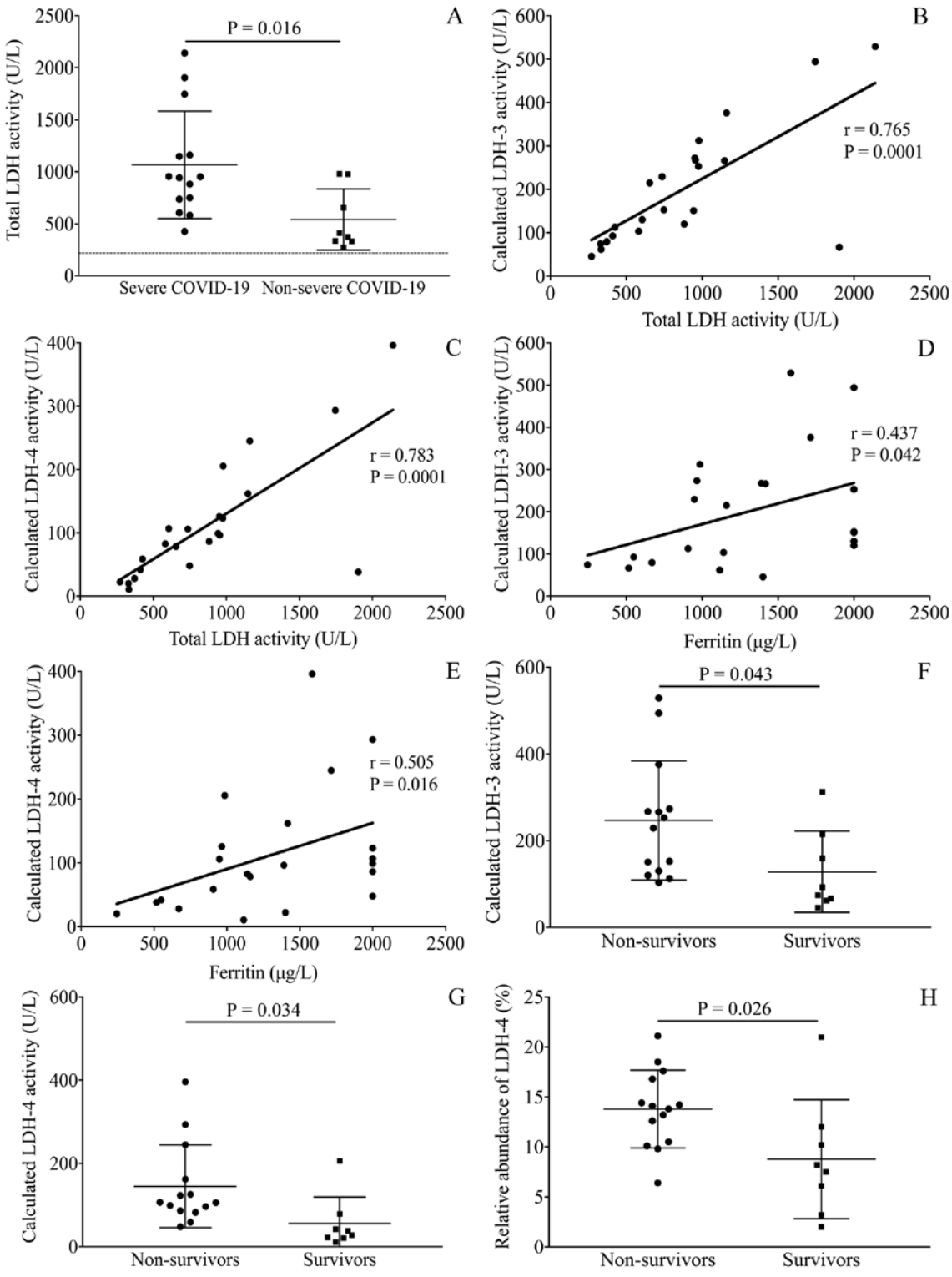
RESULTS

Based on routine laboratory tests, inflammatory clinical conditions were indicated by elevated WBC count, serum CRP and ferritin levels. Importantly, based on its upper reference limit (URL) *i.e.* 220 U/L, total LDH activity in sera was higher than normal in all recruited COVID-19 patients within the range of 272-2141 U/L (Table 1, Figure 1A). Moreover, total LDH activity was significantly higher in severe compared to non-severe COVID-19 patients (median [IQR] 947.5 [704.3-1307.0] vs 391.5 [331.8-895.8] U/L, P = 0.016) (Figure 1A). Although these subjects suffered from various comorbidities in the pre-COVID-19 era, these conditions did not substantially modulate LDH activities during COVID-19 disease (Table 1).

Table 1 Main demographical, clinical and laboratory parameters of 22 consecutive COVID-19

ID	Age (y)	Sex (F/M)	WBC (G/L)	CRP (mg/L)	Ferritin (ug/L)	Total LDH (U/L)	Abnormal relative abundance of LDH isoenzymes (%)	cTnT (ng/L)	GFR-EPI (mL/min/ 1.73 m2)	D-dimer (mg FEU/L)	COVID-19 severity	History, pre-COVID-19 comorbidities	28-day outcome
1	77	M	17.6	39.9	1401	272	-	17.1	24	1.0	moderate	HT, kidney stones	survivor
2	81	F	8.5	11.1	670	372	42.8 (LDH-2)	41.7	10	3.7	moderate	HT, drug induced nephropathy	survivor
3	63	F	24.2	67.3	949	737	31.1 (LDH-3)/ 14.4 (LDH-4)	13.1	81	1.5	severe	HT, stable angina	non-survivor
4	29	M	14.2	423.3	1391	955	42.1 (LDH-2)/ 28.0 (LDH-3)	10	71	0.7	severe	iron deficiency, epilepsy	non-survivor
5	32	M	3.1	16.8	245	331	42.6 (LDH-2)	16.8	90	0.5	moderate	inherited cardiomyopathy	survivor
6	64	M	7.2	37.5	1115	334	34.1 (LDH-1)/ 43.4 (LDH-2)	33.7	90	1.4	moderate	HT, cholecystectomy, cataract	survivor
7	73	M	13.7	21.1	549	411	-	16.5	90	0.5	moderate	HT, kidney stones, arthritis	survivor
8	71	F	9.4	92.2	907	426	26.5 (LDH-3)/ 13.8 (LDH-4)	13.1	80	0.9	severe	HT, polyarthritis	non-survivor
9	52	F	14.5	59.9	1715	1161	32.4 (LDH-3)/ 21.1 (LDH-4)	n.m.	90	0.9	severe	HT, instable angina	non-survivor
10	73	F	33.8	50.9	2000	749	43.7 (LDH-2)	12.9	76	3.7	severe	HT, cataract	non-survivor
11	27	F	8.2	94.3	985.3	979	31.9 (LDH-3)/ 21.0 (LDH-4)	10	81	1.3	moderate	cholangitis	survivor
12	68	M	7.6	112.5	2000	882	51.6 (LDH-5)	70.4	14	1.5	severe	HT, poststreptococcal GN	non-survivor
13	60	F	11.5	20.1	965	952	28.7 (LDH-3)/ 13.2 (LDH-4)	17.2	90	91.8	severe	HT, spinal osteoarthritis	non-survivor
14	65	M	17.3	12.8	1585	2141	18.5 (LDH-4)/ 18.3 (LDH-5)	n.m.	10	6.2	severe	HT, renal dysfunction	non-survivor
15	53	M	23.6	10.7	1418	1148	14.1 (LDH-4)/ 22.0 (LDH-5)	n.m.	90	2.1	severe	HT, cardiomyopathy	non-survivor
16	71	F	13.2	132.1	2000	943	31.2 (LDH-5)	93.7	17	2.2	severe	HT, nephrosis syndrome	non-survivor
17	49	M	17.2	40.6	2000	976	26.1 (LDH-3)/ 12.6 (LDH-4)	n.m.	60	2.3	severe	chronic alcohol consumption	non-survivor
18	65	M	11.8	9.9	516	1904	67.4 (LDH-1)	36.2	74	0.5	moderate	HT, iron deficiency	survivor
19	80	F	17.5	292	2000	606	17.6 (LDH-4)/ 29.9 (LDH-5)	n.m.	77	3.8	severe	HT, stroke, diabetes mellitus	non-survivor
20	46	M	43.8	156	1141	582	14.2 (LDH-4)/ 29.5 (LDH-5)	20	73	3.1	severe	aortic insufficiency	non-survivor
21	36	M	5.1	60.6	1160	655	32.8 (LDH-3)	10	90	0.8	moderate	cholecystectomy	survivor
22	51	M	12.5	13.3	2000	1746	28.3 (LDH-3)/ 16.8 (LDH-4)	53.1	90	22.9	severe	HT, diabetes mellitus	non-survivor

Figure 1 **Analysis of the associations between serum LDH activities and different clinical parameters**

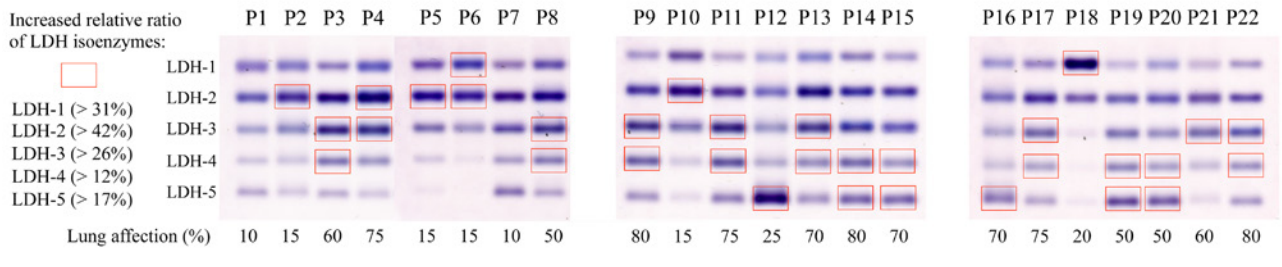


To further investigate the background of high total LDH activity, gel electrophoresis was performed to determine the relative abundance and to quantify the calculated activity of LDH isoenzymes. According to the subsequent densitometry analysis, LDH isoenzymes with increased activity had no universal pattern in COVID-19. Out of 22 subjects, nine patients showed a larger ratio of mid-zone fractions, *i.e.* increased LDH3 with or without LDH-4 or LDH-2, while single elevated LDH-2 activity was seen in case of three patients. Also, there were six individuals with increased LDH-5 activity with or without LDH-4 and two patients had higher LDH-1 level. In contrast, two persons did not show an altered ratio of LDH isoenzymes despite high total LDH activity. Overall, there was no typical profile of LDH isoenzymes in COVID-19 pneumonia (Figure 2).

In contrast, when we statistically correlated the measured activity of total LDH with the calculated activity of each individual isoenzyme, a significant relationship was observed between total LDH with LDH-3 activity (r=0.765, P=0.0001) (Figure 1B) and LDH-4 activity (r=0.783, P=0.0001) (Figure 1C), while no association was found with other isoenzymes (data not shown). The direct link between calculated LDH-3 and LDH-4 activity and other laboratory parameters typically altered in COVID-19 disease was also studied, and a modest but statistically significant association was demonstrated in case of both isoenzymes only with serum ferritin (r=0.437, P=0.042; r=0.505, P=0.016, respectively) (Figures 1D and E).

Lung parenchymal involvement expressed in (%) caused by SARS-CoV-2 infection was variable among recruited patients with different disease severity based on chest CT examination (Figure 2). The extent of pulmonary lesions was significantly larger in non-survivors with severe symptoms compared to survivors having only moderate alterations (70 [50-76] vs 15 [50-76] %, P = 0.003) (data not shown). When the relationship between CT findings and abnormal relative percentage of LDH isoenzymes was studied, a larger ratio of mid-zone fractions was observed in patients suffered from ≥ 50% pulmonary parenchymal involvement. On the other hand, elevated relative abundance of LDH-2 alone was present at moderate (< 20%) parenchymal extension (Figure 2). Pneumonia and COVID-19 related severe liver failure together resulted in augmented LDH-5 ratio (n=6), while intravascular hemolysis in two critically ill patients showed high LDH-1 level (n=2). Based on these data, the severity of pulmonary affection was strongly related to abnormal relative abundance of LDH isoenzymes which belong to the mid-zone fractions, however, the manifestation of other comorbidities causing the release of other LDH isoenzyme(s) modified the overall results of LDH isoenzyme ratio.

Figure 2 **Evaluation of relative ratio of serum LDH isoenzymes by gel electrophoresis**



Finally, in regard to the clinical outcome, significantly larger activity values of LDH-3 (241.0 [127.7-299.0] vs 83.7 [63.0-200.9] U/L, P = 0.043) and LDH-4 (106.4 [85.5-182.7] vs 33.0 [20.9-69.4] U/L, P = 0.034) were seen in non-survivors vs survivors (Figures 1F and G). Furthermore, the relative abundance of LDH-4 (P = 0.026) but not LDH-3 (P = 0.368) was higher in patients with poor outcome than recovered subjects (Figure 1H).

DISCUSSION

In recent clinical studies, increased total LDH activity in sera has been investigated as a biomarker to estimate disease severity (7-10), to indicate early pulmonary damage (10,12) and to predict unfavorable outcome of COVID-19 (7,8,13). Since total LDH has been considered as a reliable biomarker for variable inflammatory conditions and related pulmonary damage for a long time, such as in sepsis, cardiovascular disorders or cancers (18), that is why the routine measurement of LDH needs to be verified in COVID-19 as well (8). However, it has not been revealed whether elevated total LDH level in COVID-19 was generally due to a release of one particular LDH isoenzyme. For this purpose, serum samples of 22 hospitalized patients with severe or non-severe COVID-19 disease showing abnormal total LDH activity were analyzed in this study to quantify the relative abundance and activity of LDH isoenzymes by gel electrophoresis. Apart from the analysis of LDH isoenzyme pattern, calculated activity values were correlated with other laboratory parameters and clinical data.

Total LDH activities were found abnormal in all cases ranging from slightly elevated level up to 9 times URL value and were significantly higher in severe compared to non-severe COVID-19 patients, which were in accordance with the latest clinical data (8,15). Although there was a positive correlation between calculated activity of LDH-3 or LDH-4 and measured activity of total LDH, neither of them was found to exclusively contribute to high total LDH activity in this cohort based on gel electrophoresis. Hence, there was no typical profile of LDH isoenzymes in COVID-19 pneumonia. Serrano-Lorenzo *et al.* have recently investigated the different LDH isoenzymes in plasma samples and showed no correlation between the activity of total LDH and its isoenzymes (17). In addition, these authors did not find any association of relative LDH activities with various routine hematological and chemical laboratory parameters in contrast to our results where LDH-3 and LDH-4 activities were related to serum ferritin levels. Due to the limited number of our patients, we could not determine the odds ratio of total LDH activity for the recognition of severe COVID-19 cases, but others have determined that LDH had a powerful predictive value for disease severity (9,10). Similarly, Poggiali *at el.* found that total LDH showed a substantial ROC-AUC value (0.76, $P < 0.0001$) at the cut-off value of 450 U/L to distinguish severe and moderate respiratory distress states in COVID-19 (12).

Based on chest CT examination, various degree of lung parenchymal involvement was detected among recruited patients with different disease severity. The level of pulmonary impairments was significantly larger in non-survivors with severe symptoms compared to survivors having only moderate alterations. These results are in line with clinical data of Canovi *et al.* suggesting a direct role of detectable lung lesions provoked by SARS-CoV-2 infection with induced inflammatory response and reduced oxygen saturation (19). In addition, there was a strong negative correlation between total LDH and PaO_2/FiO_2 values in a large group of COVID-19 subjects (12). In our study, when the relationship between CT findings and abnormal relative ratio of LDH isoenzymes was studied, a larger ratio

of mid-zone fractions was observed in patients who suffered from ≥ 50% pulmonary parenchymal involvement. On the other hand, elevated LDH-2 activity was present at moderate (< 20%) parenchymal extension. Accordingly, the severity of pulmonary affection was strongly related to abnormal activities of LDH isoenzymes which belong to the mid-zone fractions. However, pneumonia and COVID-19 related severe liver failure together resulted in augmented LDH-5 activity, while intravascular hemolysis caused high LDH-1 level. Although Serrano-Lorenzo and his colleagues have analyzed in-gel LDH isoenzymes in COVID-19 subjects, altered relative activities of these isoenzymes were not studied in detail in the aspect of other comorbidities and clinical outcome (17). In this study, based on their pre-COVID-19 history, subjects suffered from various diseases, such as hypertension, cardiomyopathy, diabetes mellitus, renal disorders, cataract or angina, but these conditions did not substantially modulate LDH activities in COVID-19 disease. Finally, considering the clinical outcome of these patients, significantly larger LDH-3 and LDH-4 activity values were seen in non-survivors vs survivors. Furthermore, the relative abundance of LDH-4 but not LDH-3 was higher in patients with poor outcome than recovered subjects. Recently, elevated total LDH activity has been described to provide a prognostic value for survival having a 16-fold increase in odds of mortality (7) and showing an increased risk for death at cut-off value of 395 U/L (13).

In conclusion, no characteristic profile of LDH isoenzymes can be detected in COVID-19 pneumonia, however, elevated calculated LDH-3 and LDH-4 activities are associated with unfavorable clinical outcomes. Based on these data, there must be a direct link between increased LDH activity and SARS-CoV-2 induced lung injury, but a more widespread tissue damage can simply overwhelm the relative activities of LDH isoenzymes. Hence, further clinical studies are required with a larger number of patients to validate the usefulness of in-gel activities of LDH isoenzymes in COVID-19.

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Conflict of interest

There are no competing interests to declare among the authors of this work.

Ethical approval

The study was approved by the Scientific and Research Ethics Committee of the University of Debrecen and the Ministry of Human Capacities under the registration number of 32568-3/2020/EÜIG.

Figure legends

Table 1. Main demographical, clinical and laboratory parameters of 22 consecutive COVID-19 patients. To investigate the background of increased total LDH activity in sera of subjects suffered from severe (n=14) or moderate (n=8) COVID-19 condition, gel electrophoresis was performed for the quantitation of relative abundance and activity of LDH isoenzymes. Enrolled patients showed elevated WBC count with high CRP and ferritin levels. cTnT was not determined

in all cases. Renal function and prothrombotic conditions were determined by GFR-EPI and D-dimer level, respectively. Based on the data collection by the clinicians, pre-COVID-19 comorbidities, COVID-19 disease severity and clinical outcome were also available. Abbreviations: WBC: white blood cells, CRP: C-reactive protein, cTnT: cardiac troponin T, GFR-EPI: glomerular filtrate rate calculated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, HT: hypertension, poststreptococcal GN: poststreptococcal glomerulonephritis. n.m. means 'not measured'.

Figure 1. Analysis of the associations between serum LDH activities and different clinical parameters. (A) Comparison of total LDH activity in severe (n=14) and non-severe COVID-19 patients (n=8). (B, C) Correlation between total LDH and calculated LDH-3 and LDH-4 isoenzyme activities in the entire patient cohort. (D, E) Relationship between calculated LDH-3 and LDH-4 activity and serum ferritin concentration in recruited subjects. (F, G) Analysis of calculated activity of LDH-3 and LDH-4 between non-survivors (n=14) and survivors (n=8). (H) Association between the relative abundance of LDH-4 and 28-day outcome of COVID-19 patients. To compare the data of two groups, Mann–Whitney U test was used, while correlations were determined using Spearman's test. Dotted line in part A depicts the URL value of measured total LDH activity (i.e. 220 U/L).

Figure 2. Evaluation of relative ratio of serum LDH isoenzymes by gel electrophoresis. The five isoenzymes of LDH were separated by electrophoresis using LDH Hydragel® 7 kit on alkaline buffered (pH 8.4) agarose gel. The separated isoenzymes were visualized using a specific chromogenic substrate, and its amount was proportional to the LDH enzymatic activity according to densitometry. Abnormal relative ratio of LDH isoenzymes indicated by red quadrants was evaluated based on the manufacturer's instructions of the kit. P1-P22 indicate the ID number of enrolled patients. Below the gel images, the degree of lung parenchymal involvement (%) based on chest CT examination is depicted in the presence of various LDH isoenzyme patterns.

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Is it true hypoparathyroidism? A root cause analysis of unusually low Intact Parathyroid Hormone (iPTH) at a clinical laboratory

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Parathyroid hormone, error, audit, pre-analytical phase

ABSTRACT

Introduction

Intact Parathyroid Hormone (iPTH) has a short half-life i.e. two to four minutes therefore the sampling regimen has to pass through a stringent pre-analytical process control. The aim of this study was to identify the causes of apparently falsely low iPTH encountered while signing out Laboratory reports by the Clinical Chemistry professionals.

Material and methods

This report was conducted at the section of Clinical Chemistry, The Aga Khan University Hospital (AKUH) Karachi Pakistan from July to December 2017. Audit tool utilized was Plan-Do-Check-Act Cycle. After correlating with available clinical details and lab parameters, all low iPTH values (<16 pg/ml) were investigated by phone interview. A fresh sample was requested for non-correlating cases.

Appropriate interventions were undertaken and a re-assessment was done from January to March 2018.

Results

During the audit, 2559 iPTH samples were analyzed. 110 (4.3%) were identified as apparently falsely low. After recollection, the above 110 samples were immediately centrifuged, and cold chain maintained until re-analysis. 60 (2.4%) resulted with normal or elevated levels. The causes identified were poor compliance of staff with pre-analytical steps including delayed sample separation and unfavorable temperature chain maintenance. Interventions included online meetings with the staff country-wide and circulation of flyers detailing the pre-analytical steps via emails and hard copies. Re-audit showed reduction in number of apparently falsely low results to 30 out of a total of 1448 samples and 14 (0.96%) were investigated to be falsely low.

Conclusion

Stringent pre-analytical process control is vital for quality reporting and patient safety.

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INTRODUCTION

Parathyroid hormone (PTH) is a biologically active 84 amino acid peptide hormone, which serves various vital physiological roles in the human circulation but more extensively appraised in literature as a regulator of bone metabolism. Specifically, it functions to enhance renal reabsorption of calcium, bone resorption and activation of vitamin D, whereas has inhibitory effect on renal phosphate reabsorption, bone turnover and mineralization (1).

A number of circulating molecular forms of PTH have been identified namely the fragment containing carboxyl (C) - or amino (N)-terminal portions of the molecule that results from either intra-glandular or peripheral degradation of the hormone, but their biological significance is ill-defined (2). Starting from the second half of the 20th century the first generation of radioimmunoassays were pioneers to measure PTH activity but suffered from certain limitations including lack of diagnostic accuracy. Second generation immunometric assays (IMAs) are graded as specific for the whole PTH molecule (3).

From a global perspective, PTH measurements are now routinely carried out on the second-generation assays for the diagnosis and management of hypo-parathyroidism and hyperparathyroidism (4, 5). The widely used IMA two-site sandwich assays, characteristically termed as “intact PTH” (iPTH) assays, recognize the intact as well as large C-terminal fragments as they employ a capture antibody against the C-terminal part of the PTH molecule and a radioiodinated detection antibody directed towards the N-terminal portion of PTH (6). Improved clinical specificity of third generation assays has not yet been widely established and they are not in widespread use (7).

Addressing the pre-analytics, iPTH is a relatively unstable hormone possessing a plasma half-life of two to four minutes (8). Stability differs depending on sample type, whether fresh frozen or lyophilized etc. It is therefore imperative for clinical laboratories to specify the preferred specimen type and to provide clear advice about storage if specimens are not assayed immediately (9). A study has reported bovine thrombin in rapid serum tubes to decrease PTH compared to serum separator tubes by 14.1% after 4 hours at room temperature (10). Furthermore, there is inconsistent evidence in literature regarding the potential effects of serum separator tubes on iPTH measurement. Other potential influences beyond the control of laboratory are prior

food intake, vegetarian diet, strenuous exercise, gender, race and menopausal status (1).

While signing out laboratory reports the Clinical Chemistry Consultants were encountering an unusually high number of iPTH results that were below the reference interval and not correlating with other laboratory parameters and clinical details. The aim of this audit was to identify the causes of apparently falsely low iPTH and rectify the root cause.

MATERIAL AND METHODS

This study was conducted within the section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital (AKUH) Karachi Pakistan from July to December 2017. The clinical laboratory of AKUH is accredited by the College of American Pathologists and has more than 10 stat laboratories and a network of 264 phlebotomy stations and collection points across Pakistan. This study was approved by the departmental quality management committee (DQMC) of Pathology & Laboratory Medicine, AKUH. Samples for iPTH analysis from across the country are transported following the appropriate protocol i.e. 3-5 cc of venous blood sample in ethylenediaminetetraacetic acid (EDTA K_2) tube is collected and subjected to immediate centrifugation at 3000 *g* for 15 minutes, frozen and sent in dry ice to main campus in Karachi. Main campus is the hub of AKU clinical laboratories for analysis at the clinical chemistry section.

The iPTH was analyzed on Siemens IMMULITE 2000 a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay (Siemens diagnostics USA). In our laboratory all internal quality control, validation and proficiency testing are accomplished according to Clinical and Laboratory Standards Institution (CLSI) guidelines (11). Two levels of commercially available controls were run with each batch.

Audit tool utilized was Plan-Do-Check-Act Cycle. In the planning phase the audit team comprising of three Consultant Chemical Pathologists and a laboratory technologist prospectively reviewed and discussed all cases with low iPTH (<16 pg/ml). In the next phase after correlating with available clinical details and laboratory parameters including 25-OH-Vitamin D and serum calcium, clinical history was obtained by phone call for all low iPTHs (<16 pg/ml). In order to appropriately correlate the clinical history with the iPTH results the sectional post graduate residents were trained using direct lecture methodology and an hour teaching session by a Consultant Chemical Pathologist was taken. This session was centered across a refresher of the pre-analytical and analytical steps for serum iPTH and clinical indications of iPTH alongside causes of low iPTH were also reviewed. They were further directed to request a fresh sample for confirmation of the results for non-correlating cases based on the clinical history and other laboratory parameters after independent consultation by at least two Consultant Chemical Pathologists. The findings were presented in the DQMC meeting as a potential patient safety issue and meetings minutes were recorded. Grounded on the findings analyzed appropriate interventions were undertaken and implemented for the entire network of the clinical laboratory and re-audit was done from January to March 2018 to check the compliance and the outcome. Data was analyzed using Microsoft Excel version 2013.

RESULTS

Audit findings

During the audit period, 2559 iPTH samples were analyzed, 2242 were out-patients and 317 were in-patients. Inpatients' samples were collected at the main AKUH and outpatients' samples were received from phlebotomy centers

in Karachi and also from phlebotomy centers across Pakistan for the months of July to December 2017.

The audit tool utilized i.e. the Plan-Do-Check-Act Cycle is summarized in Figure 1. After correlating with available clinical details and laboratory parameters including 25-OH-Vitamin D, serum calcium and, based on telephonic history, 110 (4.3%) were identified as apparently falsely low by the two independent Pathologists.

On re-analysis of a fresh sample under instructions of immediate centrifugation and cold chain maintenance, 60 (2.4%) were found to have normal or elevated levels with respect to reference intervals used.

Gap analysis

A few Phlebotomy centers staff were not aware regarding the sample stability of plasma iPTH, furthermore delayed centrifugation was also evident in most cases. Specimens collected at main campus laboratory were not being delivered to the laboratory labelled as STAT leading to delayed centrifugation and analysis. Samples drawn and collected outside the laboratory collection unit i.e. at different patient care facilities and home were being received at the collection units with unfavorable temperature control and without appropriate transportation protocol. The root causes are depicted with the Ishikawa diagram in Figure 2.

Corrective actions

An online meeting regarding iPTH sampling collection and transportation issues was held by the audits team and laboratory manager with regional managers/coordinators in which refresher was given on sample collection of iPTH; all people were asked to reinforce it in their respective areas. In this meeting detailed pre-analytical steps were outlined, and number of discordant results were shared with the respective regions. The regional managers/coordinators were directed to take a verbal pre-test of the phlebotomist at their respective regions regarding the pre-analytical steps of iPTH and provide feedback and educate. The flyer with pictorial description of pre-analytical steps of iPTH for easy comprehension was circulated to all phlebotomy centers by laboratory manager via email and hard copy as shown in Figure 3. The flyer was designed in line with ACB recommendations for iPTH which emphasize that as PTH is labile; serum or plasma should be separated as soon as possible, preferably using a refrigerated centrifuge (12).

Furthermore, it encompasses the step-by-step process from collection of 3-5 cc blood sample in EDTA k_2 (purple top) tube, followed by cold chain maintenance as specimen must be kept cold (2–8°C) through the collection & separation process. Separated Plasma specimen should be stored at -20°C and should be transported to the laboratory for analysis in frozen condition with dry ice.

Two follow-up meetings were repeated in the subsequent months and compliance data was shared and feedback was given. The laboratory manager issued a memo that all inpatient iPTH samples should be transported directly to the Clinical Chemistry section for immediate processing and analysis rather than being transported to the main laboratory and further routed to the section.

Phlebotomy staff at main laboratory were reinforced to send all iPTH samples collected from outpatients at main laboratory to the section of Clinical Chemistry in ice immediately labelled as STAT. Clinical Chemistry processing bench staff were reinforced to processes all iPTH specimens immediately as STAT specimen on receipt in the section for immediate analysis.

Figure 1 Plan - Do - Check - Act - Cycle

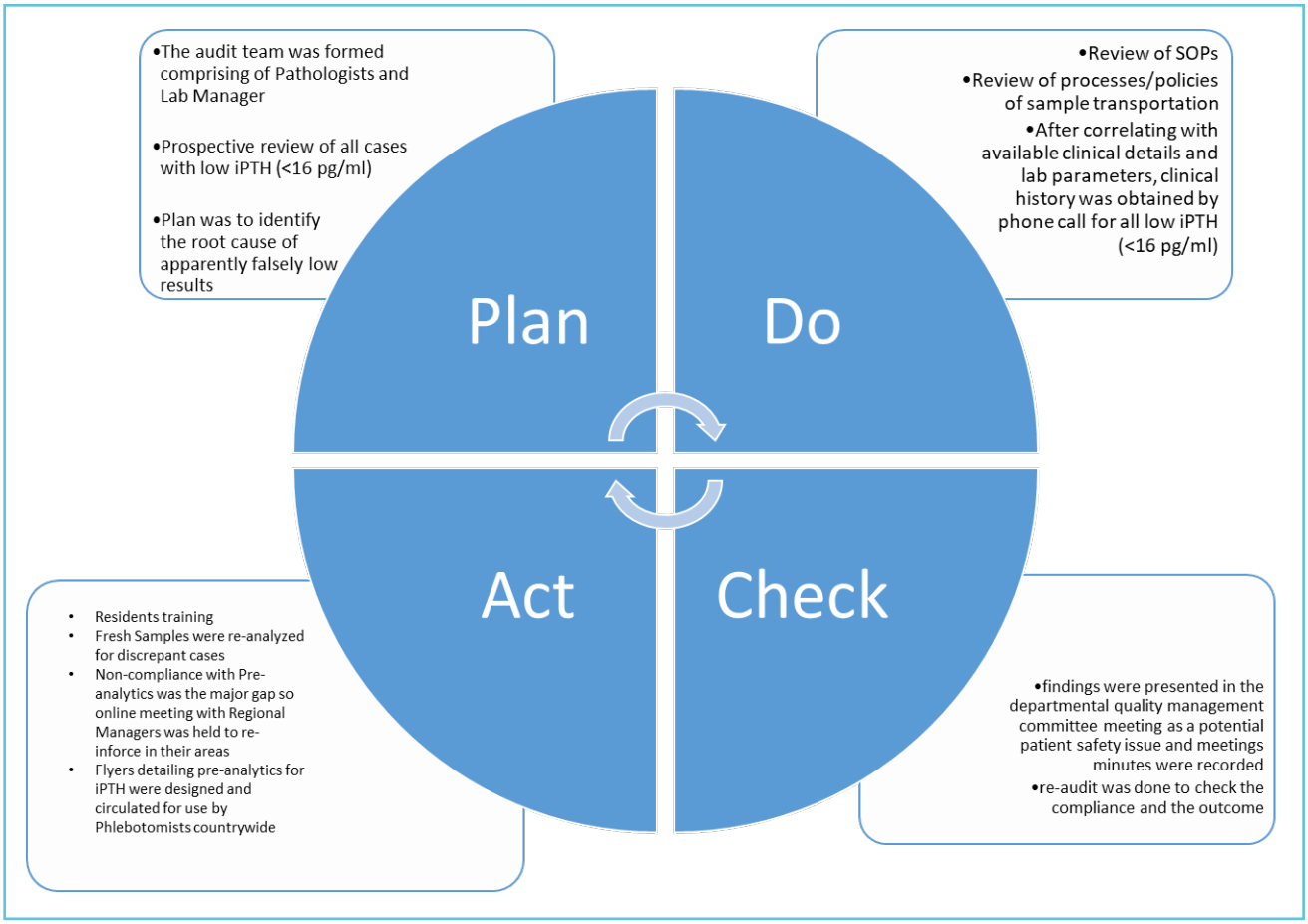


Figure 2 Ishikawa diagram depicting the major causes and consequences

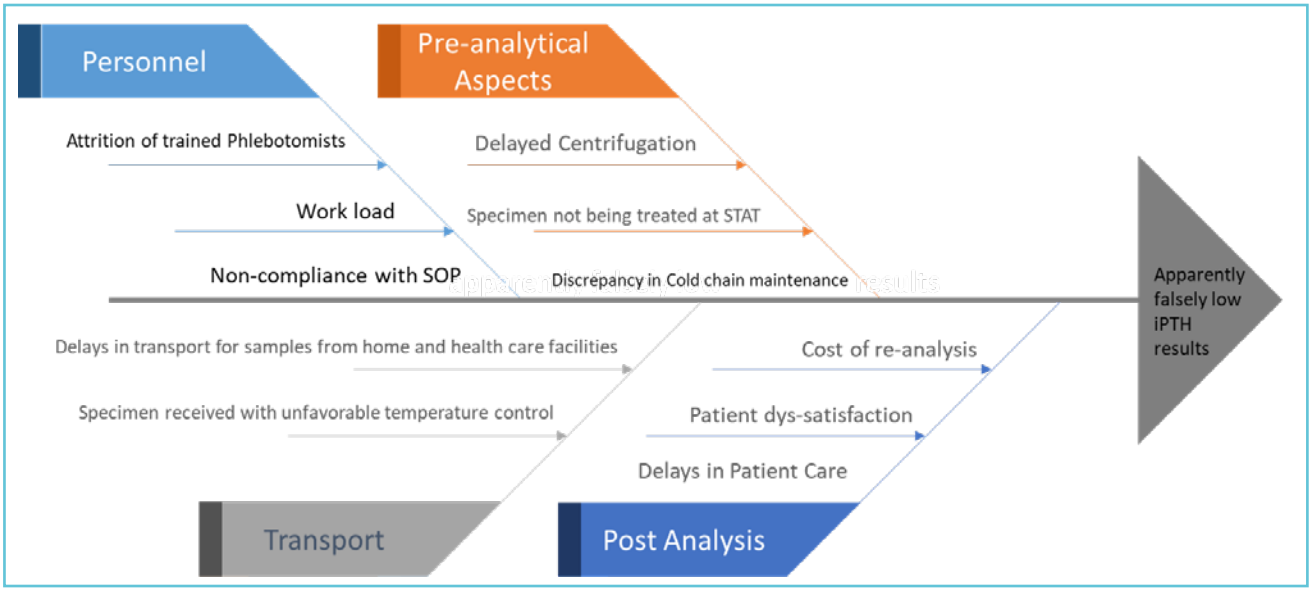
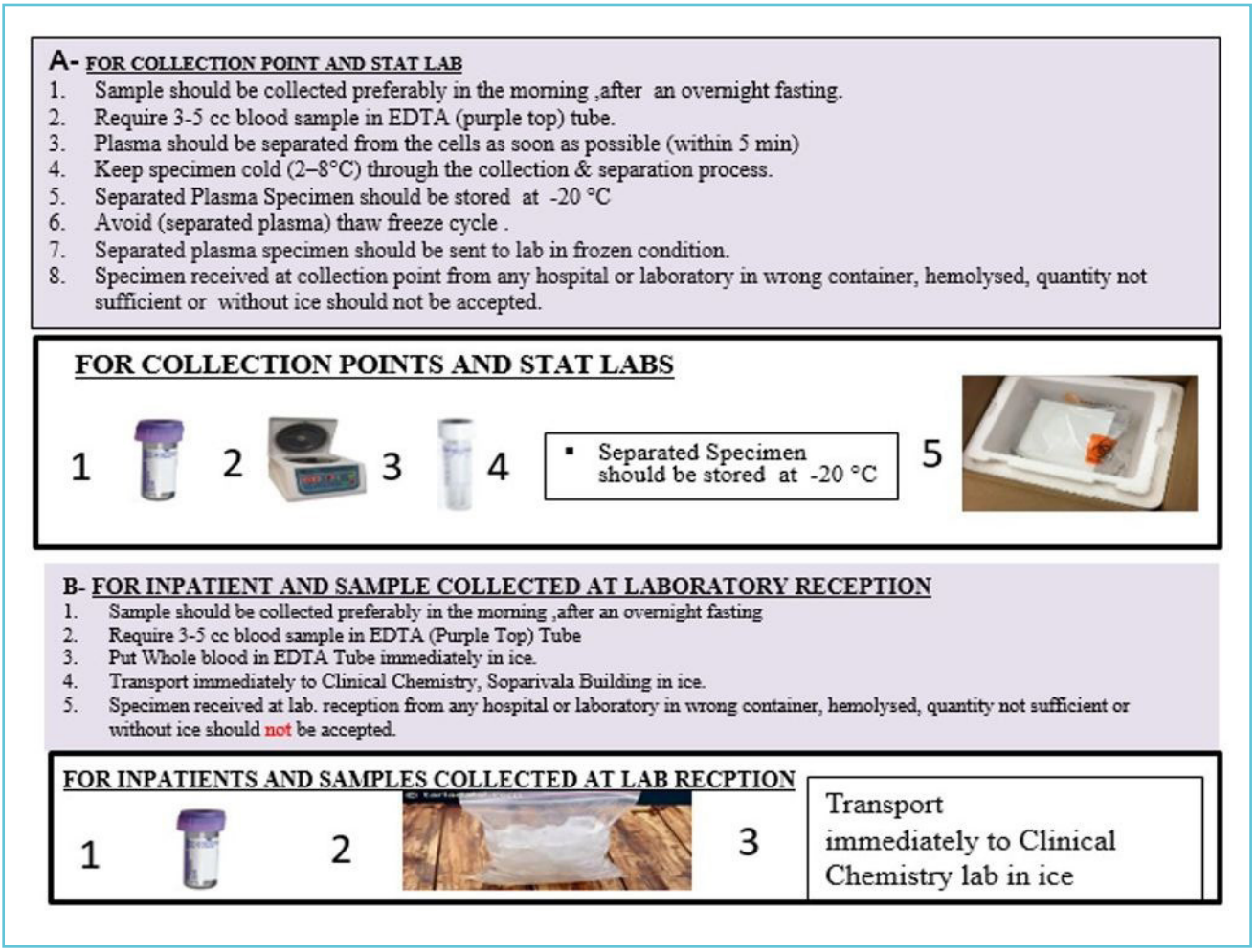


Figure 3 iPTH (Intact Parathyroid Hormone) specimen collection guide – flyer circulated for staff countrywide



Re-audit

A re-audit was done in April, 2018 and data collected from January to March 2018 was critically reviewed and analyzed by the audit team.

During the re-audit period 1448 samples were analyzed for iPTH. After the implementation of corrective actions identified by the audit team a reduction in number of apparently falsely low results to 30 (2.1%) was recorded.

Furthermore, the rate of change in report was reduced by more than 50% from a total of 60 (2.4 %) in the audit phase to 14 (0.96%) in the re-audit phase. A significant difference was observed in the median iPTH before and after the corrective action from the different blood collection sites, being 58.4 (IQR: 25.02-265) and 66.2 (IQR: 30.6-221) respectively.

DISCUSSION

The backbone of a high-volume clinical laboratory functioning is laid on the pillars of total quality management in the laboratory process involving the pre-analytic, analytic, and post-analytic phases. It ensures the integrity of all the stages involved in producing laboratory results, initiating from test order entry to the final interpretation of reports issued, with attention

to reduce or eliminate the errors that may arise during the various steps (13). Establishment of ideal sample collection and processing protocols and compliance with the sample transport guidelines are a pre-requisite for the standard operating protocols (SOPs) for a clinical laboratory (14).

Pre-analytical errors have been reported in literature as a leading proportion of errors in laboratory processes and cause disruption in the assurance of patient safety (15). Moreover, failures in following the best practices of quality management can inflict potential harm to patient safety and dys-satisfaction of requesting physicians (16). The International Federation of Clinical Chemistry (IFCC) Working Group on laboratory errors and patient safety has defined a number of quality indicators for the pre-analytical stage in order to ensure continual quality improvement (17).

This study was planned in keeping with the view that the clinical audits are an essential element of a laboratory's quality management system and should include scheduled and timely inspections (18). Our audit showed a rate of 2.4% pre-analytical errors as depicted in Figure 2, pertaining to varied phlebotomy practices with non-adherence to pre-requisites, transportation errors particularly for specimens brought to the phlebotomy centers from homes and other patient care facilities and samples received from in-patient areas without cold chain maintenance.

The knowledge gaps of phlebotomists were linked with the high attrition rate, as with a widespread network of phlebotomy centers where the staff turnover is a potential confounder.

When planning corrective actions to control error rates, a 'system'-based approach has been shown to be effective rather than an 'individual' approach (19). This study was undertaken accordingly, with emphasis on the check of total laboratory processes, and after collecting data on the flaws that in the pre-analytical processes were identified as opportunities for improvement.

Previous research has shown that online learning and use of effective teaching modalities, like flyers with pictorial depiction of the process for awareness and reinforcement of key steps, prove effective and improves productivity (20, 21). Using the same strategy the timely online meetings with the staff by the audit team and circulation of flyers proved to be effective as unveiled by the reduction in error rates, able to improve patient safety.

Furthermore, repeat test requests lead to patient dissatisfaction and increase laboratory expenditure with negative effects on patient outcomes particularly linked to delays in reporting (22). The corrective actions can lead to substantial improvement in the rate of errors and patient safety can be improved alongside cost cutting of repeat analysis for the clinical laboratory.

Although the re-audit showed noteworthy improvement, still the highest benchmark was not achieved. The audit team recommended that as quality improvement should be a continuous process, timely checks should be done and staff education and reinforcement of appropriate pre-analytics should be regularly scheduled to achieve the highest targets of quality. This strategy based on regular online meetings at virtually no cost and circulation of flyers served as a possible resource pack for continued education in order to attain highest levels of quality and compliance.

The results were shared in the departmental quality management committee meeting and it was decided to include monitoring of falsely low iPTH in the key performance indicators in order to ensure compliance and achieve a near zero target of falsely low results.

CONCLUSION

Since iPTH has a short life, stringent pre-analytical process control is vital for appropriate analysis and result reporting. For improving the pre-analytical quality control regularly scheduled clinical audits, online feedbacks and meetings with staff at outreach phlebotomy centers and refresher based educational activities proved to be beneficial and led to attainment of optimal outcomes alongside a momentous and sustained reduction in laboratory errors. The clinical audit driven interventions catalyzed a commitment to improve pre-analytical error control across the umbrella of a high-volume clinical laboratory, ultimately leading to improved patient safety, end user satisfaction and cost-effectiveness for all stakeholders.

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Disclosure

This study was presented as a poster presentation at the 5th EFLM Conference on Pre-analytical Phase, held in Zagreb, Croatia, 22-23 March 2019.

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The detection of postprandial hypoglycemia with 5-hour oral glucose tolerance test

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five-hour oral glucose tolerance test (5-OGTT), hypoglycemia, insulin resistance, insulin sensitivity

ABSTRACT

Introduction

Postprandial hypoglycemia (PH) is a poorly understood phenomenon. Five-hour oral glucose tolerance test (5-OGTT) is often a useful laboratory investigation to understand and establish a diagnosis of PH. The aim of this study is to present the patterns observed during 5-OGTT performed in cases with PH in a tertiary hospital in Nepal.

Methods

5-OGTTs were performed on 52 patients who complained symptomatic postprandial neuroglycopenic symptoms, at the Nepal Medicity hospital during the period of 2 years from 2017 to 2019. The anthropometry, medical history, serum glucose; insulin and cortisol were obtained. The homeostatic model assessment score for insulin resistance (HOMA-IR) based

on fasting glucose and insulin levels were calculated. Data was analyzed using SPSS (Version 20.0).

Results

21 (40.4%) patients out of 52 developed hypoglycemia [blood glucose < 55mg/dl (3.1mmol/L)], among them nine patients developed hypoglycemia at 3 hours, 11 at 4 hours and one at 5 hours post glucose load. The fasting insulin level in patients who developed hypoglycemia was 12.1 ± 5.8 µU/ml compared to the insulin level analyzed at the point of hypoglycemic episode which was 6.4 ± 1.8 µU/ml, P<0.005.

Conclusion

The level of insulin is disproportionately high in the setting of hypoglycemia where it was expected to be nearly absent. The disturbance in physiological mechanism between insulin sensitivity and insulin secretion may be the possible cause of PH.

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INTRODUCTION

The presence of neuroglycopenic symptoms in patients without diabetes is strongly suggestive of a hypoglycemic disorder. There are two types of non-diabetic hypoglycemia where the first one is postprandial hypoglycemia (PH) and the second is fasting hypoglycemia. Reactive or postprandial hypoglycemia causes blood glucose to decrease two to five hours after a diet with high carbohydrate content. Early and late postprandial hypoglycemia occurs in 2-3 hours and 3-5 hours after a meal respectively. (1) Demonstration of hypoglycemia while symptomatic and alleviation of symptoms following normalization of glucose levels is the gold standard test for diagnosing PH. We have previously reported that the PH is the commonest cause of non-diabetic hypoglycemia in a cohort of patients assessed in a tertiary hospital in Nepal. (2) It is our practice in a metabolic clinic to carry out five-hour oral glucose tolerance test (5-OGTT), with insulin measurement at the onset of the symptoms, to diagnose and observe the pattern of PH.

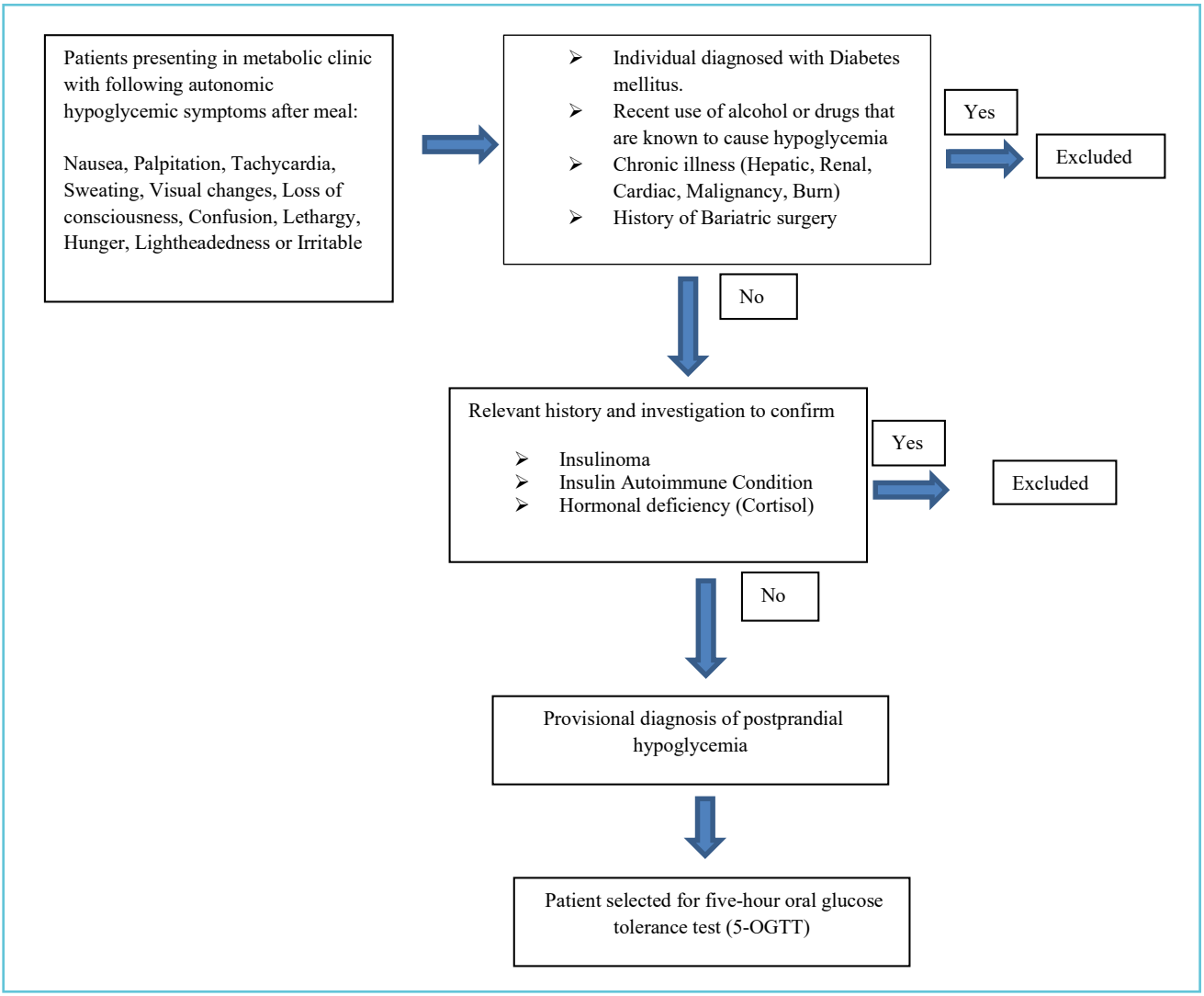
However in clinical practice, the use of 5-OGTT is discredited for the diagnosis of PH as it may show false-positive results. (3) Nevertheless, the 5-OGTT demonstrates the insulin sensitivity of a patient and guides individual therapeutic approach. The aim of this study is to present the patterns observed during 5-OGTT performed in cases with PH in Nepal and discuss the pathophysiological basis behind that pattern. This test is done only in few centers in Nepal.

METHODS

5 hr-OGTT were performed in 52 patients presenting with autonomic hypoglycemic symptoms after meal, at the Nepal Mediciti hospital during the period of 2 years from 2017 to 2019. The sampling scheme is shown in figure 1.

The patients diagnosed with or history of diabetes mellitus, cortisol hormone deficiency, bariatric surgery, drug induced hypoglycemia, Insulinoma and insulin autoimmune syndrome were excluded based on clinical history and relevant investigation. Apart from the antidiabetic medications, the drugs that are known to cause hypoglycemia are quinolones, pentamidine, non selective beta blocker, ACE inhibitors, salicylates, hydroxychloroquine and artemisinins.(4) The adrenal causes of hypoglycemia were ruled out by performing serum cortisol assay in all cases. Insulin glucose ratio (Turners amended ratio) was calculated in each case using [Fasting insulin (µU/ml) × 100 / blood glucose (mg/dl)-30)] formula. (5) The cases with Turners ratio less than 50 were only included in the study to exclude the possible cases of Insulinoma.

Figure 1 Sampling scheme



Biochemical measurements

The clinically diagnosed cases of PH were subjected to 5-OGTT. Patients were advised for 3 days of normal diet and physical activity followed by 10 hours fasting before the investigation. Serum sample for glucose and insulin was collected before the oral intake of the glucose solution (referred as baseline value). 75 grams of anhydrous glucose in 200 ml of water was provided for oral administration. Serum sample were collected and analyzed for glucose every hour after glucose solution intake and insulin was also analyzed from the same sample if there was any event of hypoglycemia. The cut off glucose value to label hypoglycemia was below 55 mg/dl (3.1mmol/L). (6) The homeostatic model assessment score for insulin resistance (HOMA-IR) based on fasting glucose and insulin levels was calculated for cases who developed hypoglycemia.

Serum glucose and insulin were measured using Vitros "ECiQ immunodiagnostic system" Enhanced Chemiluminescence Immunoassay (ECI) after daily maintenance and running internal control samples, which were found to be within the normal range.

Statistical analysis

All data sets were tested for normality using the Kolmogorov-Smirnov test and are expressed as mean ± standard deviation (SD) if normally distributed, or and as median if not normally distributed. The comparison was evaluated using t-test and the Wilcoxon rank test, as appropriate.

RESULTS

The mean age of 52 patients (16 men and 32 women) was 33.8 ± 11.5 years. 21 patients out of 52 developed hypoglycemia at certain hour during 5-OGTT test. Out of 21 patients, nine patients developed hypoglycemia at 3 hours, 11 at 4 hours and one at 5 hours post glucose load.

The mean ± standard deviation of fasting insulin in patients (n=21) who developed hypoglycemia at a certain hour during 5-OGTT was 12.1 ± 5.8 µU/ml compared to the insulin level analyzed during hypoglycemic episode which was 6.4 ± 1.87 µU/ml and this was significant (P <0.005).

When the insulin concentrations during hypoglycemic episode at 3 hours and 4 hours were compared, it was also significantly lower than at the baseline (5.9 µU/ml and 6.5 µU/ml respectively) as shown in Figure 2.

The homeostatic model assessment score for insulin resistance (HOMA-IR) based on fasting glucose and insulin concentrations was calculated for the cases who developed hypoglycemia at three hours and four hours and the average value was 1.9 and 2.9, respectively.

The average glucose concentration in groups that triggered insulin measurement due to symptomatic hypoglycemia was compared with groups that did not trigger insulin measurement. The average blood glucose within two hours post glucose load was 128.3 mg/dL (7.1 mmol/L) versus 118.6 mg/dL (6.6 mmol/L) in a group that later developed and did not developed hypoglycemia respectively (P <0.005). (Figure 3)

Figure 2 **Average insulin and fasting HOMA-IR in patients developing hypoglycemia at 3 hour (3-hour OGTT) and 4 hour (4 hour OGTT) post glucose load**

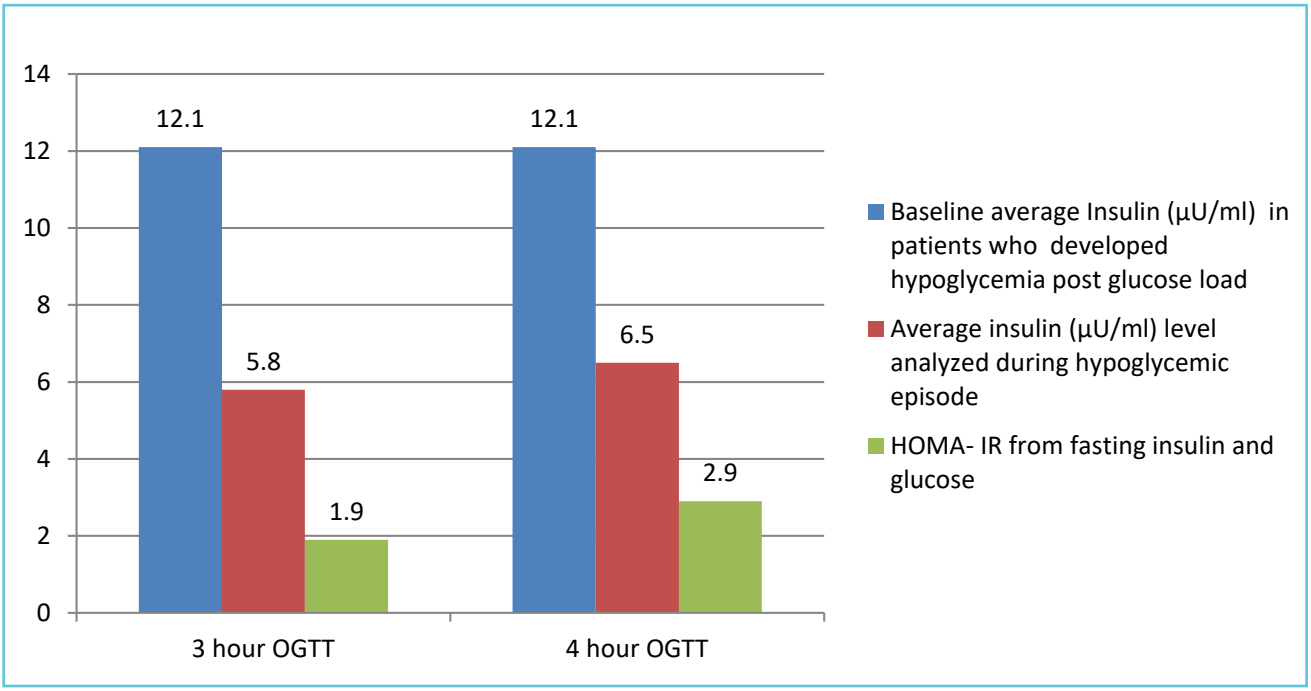
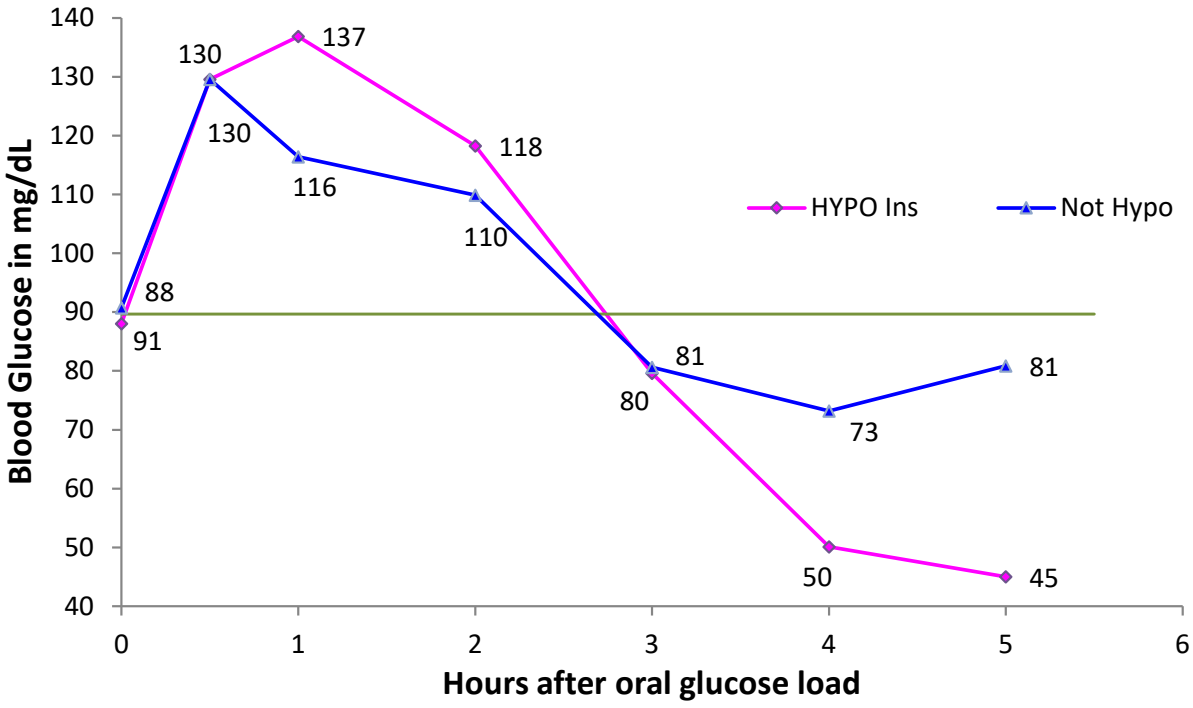


Figure 3 Average glucose concentration in groups triggering (HYPO Ins) and not triggering (Not Hypo) insulin measurement



DISCUSSION

The results of the 5-OGTT carried out at our metabolic clinic have shown various biochemical patterns. The relationship between blood glucose and factors influencing it such as insulin sensitivity, insulin resistance, and counter regulatory hormones has role in this differing pattern. It was proposed back in 1996 by Leonetti F et al that PH may arise from an increased insulin response, which might be related either to insulin resistance or to increased glucagon like peptide-1 (GLP-1), renal glycosuria, defects in glucagon response and high insulin sensitivity. (7)

We have observed symptomatic hypoglycemia both at early (within three hours after the glucose load) and late phase of the 5-OGTT (after three hours). In our study, nine cases (42.9%) developed reactive hypoglycemia at three hours post glucose load. The mean value of HOMA-IR for these nine cases was 1.9 suggestive of mild insulin resistance. Similarly, it is evident from our study that the cases who developed PH at four hours post glucose load, but not at three hours post glucose load, had higher HOMA-IR (2.9 versus 1.9). Similar finding has been reported earlier. (8, 9) The mild form of insulin resistance, observed in cases with PH at 3 hours post glucose load, does not explain the hypoglycemic episode. Tamburrano *et al* reported that this type of idiopathic PH is due to the increased insulin sensitivity. (10)

In our study, baseline insulin level in patients who developed hypoglycemia was significantly higher compared to insulin level during of hypoglycemic episode. It is interesting to note that the insulin level during hypoglycemic episode at 3 hours and 4 hours was not clinically significant

though it was statistically (5.9 µU/ml versus 6.5 µU/ml). The level of insulin is disproportionately high in the setting of hypoglycemia where it was expected to be nearly absent. The insulin half-life is reported to be only 4-5 minutes. This phenomenon could be linked to the abnormal delayed insulin secretion. The delayed insertion of insulin mediated glucose transporter (GLUT-4) has been suggested as a possible cause for this phenomenon. (11, 12) The delayed insertion of GLUT-4 results in very less amount of glucose to be handled by it, as the glucose entry through insulin independent transporters (GLUT 1-3) increases. The continued hyperinsulinemia recruits greater number of GLUT-4 inappropriately at a late stage where hyperglycemia is already approaching normoglycemia and causes rapid entry of glucose into cells resulting in hypoglycemia.

Abnormalities in circulating insulin do not explain all cases of reactive hypoglycemia, since there have been reports of many patients with normal insulin response. (13) Defects in counter regulatory response of glucagon (14), exaggerated response of GLP-1 (15), renal glycosuria (16), defect in hepatic glucose-6-phosphate enzyme system (17) and accelerated stomach emptying (18) are among the commonest causes described in literature. However, in our study, we did not measure the glucagon and GLP-1 level.

A complete work-up including measurement of glucose, insulin, C-peptide, proinsulin, oral hypoglycemic agent screen, and insulin antibodies should be performed at the onset of any hypoglycemic event. Insulin and C-peptide are co-products of proinsulin, and therefore released in the bloodstream at equimolar amounts, with insulin metabolized in the liver, and C-peptide excreted by the kidneys. When the C-peptide concentration is low or undetectable but the insulin concentration is high, then inappropriate insulin administration is a possible cause of hypoglycemia. In our study we measured the insulin level but not the C-peptide, to study the patterns of PH, since the cases with inappropriate insulin administration were already ruled out.

In our study, the pattern of the individual patients reveals a considerable "between individual" variation, which is not surprising but requires some more observations for lasting conclusions. While this disease is often ignored by many physicians and the 5-hour oral glucose tolerance test is often disregarded, it may add therapeutic benefit to the individual patient.

CONCLUSION

Postprandial hypoglycemia represents disturbances of balance between glucose utilization and glucose supply. The disturbed homeostatic loop between insulin sensitivity and insulin secretion may be the characteristic aspect of PH. The pathophysiology varies between individual, and 5-OGTT may be useful to understand this.

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Conflict of interest:

The authors declare that there is no conflict of interest in the publication of this manuscript.

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Impact of a novel strategy for critical values communication for the management of patients treated with clozapine

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ABSTRACT

Introduction

Clozapine is an antipsychotic drug used to treat resistant schizophrenia and other disorders. Based on the actual Spanish legislation, patients treated with clozapine must undergo periodical haematological examinations and treatment should be reviewed when the haemogram shows either a leukocyte count of ≤ 3500/mm^3 or neutrophil count < 2000/mm^3. An automatic notification system has been developed to optimize patient management and it's utility was assessed following the implementation of the new system.

Material and methods

When clozapine (CLO) laboratory test request was made, a reflex complete blood count test was also done. An automatic e-mail was sent by the laboratory information system to the physician when a CLO was ordered and low leukocyte or neutrophil counts were detected, or when a patient with an ordered CLO test did not attend the laboratory for blood drawing.

Results

For patients with haemogram alterations, the time to take clinical action was significantly decreased from 23 to 7 days (p = 0.02). Moreover, the adherence to Spanish Agency of Drugs and Sanitary Devices recommendations significantly increased from 45% to 76% (p = 0.02). For not attending patients, the days out of control decreased from 29 to 12 days, although it was not statistically significant (p = 0.06).

Conclusions

This strategy has allowed the compliance of legal requirements, the improvement of patient safety, and the optimisation of clinical and laboratory procedures.

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INTRODUCTION

Clozapine (CLO) is an antipsychotic drug used in resistant schizophrenia, schizophrenia with extrapyramidal symptoms not responding to other drugs, or psychotic symptoms in Parkinson's disease (1-7).

CLO was discovered in 1958, but in 1975 its commercialisation and prescription were detained since some clinical cases of agranulocytosis, patients presenting neutrophil count under 0.5/mm^3, were detected (9-10).

Basing on subsequent studies including haematological examinations, the Food and Drug Administration (FDA) approved its use for resistant schizophrenia in 1990. In Spain, CLO was re-introduced for clinical practice in 1993 under severe regulatory control conditions. All patients treated with CLO must undergo haematological examinations (haemogram cell counts), weekly during the first 18 weeks of treatment and monthly during the lifelong treatment. The AEMPS (Spanish acronym of Spanish Agency of Drugs and Sanitary Devices) (11) defined these conditions, that remain valid at the time of submitting this manuscript.

The AEMPS defines that, if the haemogram shows a leukocyte count less than 3500/mm^3 or neutrophil under 2000/mm^3, CLO treatment must be reviewed, modifying drug dose or analytic haemogram control frequency (Table 1).

In Spanish population, agranulocytosis is a rare event, but the incidence of neutropenia and leukopenia is estimated to be 3% and 1.3%, respectively (12).

To avoid unnecessary visits, patients receiving CLO and attending our hospital are first directed to the laboratory for blood drawing for the haemogram analysis and then immediately to the Mental Health Department for a visit with the clinician or a specialised psychiatric nurse.

Upon following the above practice, at the moment of the clinical visit the haemogram results (leukocyte/mm^3 and neutrophil/mm^3) are not yet available and can't be reviewed by psychiatric professionals, delaying therapeutic measures for patients presenting with a leukocyte count of less than 3500 /mm^3 or neutrophil count of under 2000/mm^3.

Given the implication to patient safety, developing a good laboratory result communication strategy is essential for clinical practice. A critical result involving therapeutic modifications must be immediately informed to the physicians to ensure the best patient management, avoiding unnecessary treatments or examinations.

Some international organisations have published guidelines and recommendations to ensure the appropriate information communication between the laboratory and clinical departments, like the Joint Commission on Accreditation of Health Care Organization (JCAHO) (13) for laboratory accreditation in the USA, or the Clinical and Laboratory Standards Institute (CLSI) (14) in Europe.

Employing automated rules ensures the detection of 100% of the values requiring immediate communication, minimize human errors and significantly reduce the time to communicate with the physicians, which in turn can prioritize patient visits and results in an overall circuit optimization.

In Spain, some studies have demonstrated that communicated values via e-mail only comprise 1% of all the communications (15), and was a novelty for our hospital.

In May 2018, our clinical laboratory implemented a new laboratory information system (LIS), which made it possible to create informatics algorithms to send an automatic e-mail when certain predefined criteria were met.

This strategy was first applied to patients treated with CLO for automatic notification of altered haematological values, and patients not attending the laboratory. The purpose of this study was to evaluate the impact of a new automatic notification system employing informatics algorithms and e-mails in the clinical management of patients treated with CLO.

MATERIALS AND METHODS

The new LIS employed by the laboratory was Smartlis (Lab Technologies S.A.) v46®, which allows implementation of algorithms and information fluxes with the Mental Health Department to develop a novel strategy of communication.

A new laboratory test named "Clozapine patient control" (CLZ) was created. For patients treated with CLO, when CLZ was ordered by psychiatric physicians or nurses, a reflex haemogram test was also done, this could be differentiated from the other haemograms.

Two different algorithms for automatic advice rules were created.

1. Automatic advice rule: leukopenia/neutropenia detected in a patient treated with CLO

The rule was configured to send an automatic e-mail when the following conditions were met: CLZ test ordered, and leukocyte count $\leq$ 3500/mm^3 or neutrophil count $\leq$ 2000/mm^3.

To assure a quick response e-mails are sent to the physician or nurse ordering the test and to three psychiatric physicians, three laboratory specialists, and one laboratory secretary. To confirm that information was received by the Mental Health Department, it was established that the first psychiatric physician or nurse reading the e-mail had to respond to the Laboratory and take charge of the case. If after 24 hours there was no answer, laboratory staff should contact the Mental Health Department by phone.

The project began in March 2019 and the e-mail text was: "A leukopenia/neutropenia has been detected in the patient Hxxxx". To ensure data protection only patient clinical history number was included and the e-mail addresses employed were the corporative ones.

Three months later, the text was modified to include AEMPS recommendations on treatment and analysis frequency modifications, when a leukopenia/neutropenia was detected (Table 1).

Table 1 AEMPS recommendations

Haematological count		Required action
Leukocyte (mm^3)	Neutrophil (mm^3)	
≥ 3.500	≥ 2.000	Continue same clozapine treatment
Between ≥3.000 and ≤3.500	Between ≥1.500 and ≤2.000	Continue same clozapine treatment but haematological analysis every 2 weeks, until stabilisation or increased cellular count
< 3.000	< 1.500	Stop clozapine treatment, and haematological analysis every day until resolution. Monitor possible infections. No re-introduction of the drug

1.1 Evaluation of rule utility

To investigate the utility of this rule the following parameters were registered for each patient treated with CLO when leukopenia/neutropenia was detected:

a. AEMPS recommendations followed by the physician/nurse: Yes/No

b. Number of days to take a clinical decision

All these parameters were obtained by reviewing patient histories and e-mail accounts.

Data obtained from March to December 2018 (n=11) were compared to those from March to December 2019 (n=17), after implementing the automatic notification.

The difference between AEMPS recommendations fulfilment was compared employing a X^2 test, and the time to take a clinical decision with a U Mann-Whitney test.

Also, during March to December 2019, we calculated the percentage of advices made via mail from the total of laboratory advices to notify critical results (mail and telephone advices).

1.2. Evaluation of proper CLZ requisition

We also evaluated if CLZ test was properly ordered, reviewing all the haemograms ordered by the Mental Health Department from July to October 2019 (n=1811). When leukopenia/neutropenia was detected, we checked if the corresponding patient was treated with CLO.

2. Automatic advice rule: patient treated with CLO not attending the laboratory for blood analysis

Since many patients treated with CLO were absent at the scheduled follow-up visit (neither laboratory analysis nor psychiatric visit), they remained uncontrolled until the next visit. To reduce the time between follow-up visits, the new advice rule sent an e-mail to the coordinating nurse of the Mental Health Department when a patient did not attend the laboratory for the analysis, and a CLZ test was ordered. Thus,

psychiatric professionals could contact the patient and a new analysis could be rescheduled.

The text of the e-mail was: "Patient Hxxxx who had scheduled an analysis for CLZ on xx-xx-xx, did not come to the laboratory". To evaluate the time without follow-up for these patients, the employed parameter was the number of days without follow-up: data of the follow-up analysis - data on the patient who did not go for laboratory analysis.

In order to assess whether time without follow-up was reduced since this automatic rule was implemented, patients not going to the laboratory for blood-drawing between December 2018 (n=16) and December 2019 (n=35) were evaluated, and the differences in the number of days without follow-up were compared using a U Mann Whitney test. The MedCalc® v 7.2.1.0. statistical program was used for the statistical comparisons.

RESULTS

1. Automatic advice rule: leukopenia/neutropenia detected in a patient treated with CLO

From March to December 2019, 1591 CLZ tests were ordered, and 17 met criteria for e-mail sending (1.06%). From the 17 communications, only two corresponded to inpatients, and the other 15 corresponded to outpatients.

These 17 advices (e-mails) corresponded to eight patients: one patient presented five mail advices during the study period, one patient three advices, one patient two advices, and the last five patients presented one advice each during the study period.

Three patients were men and five women and the average age was 32.5 years.

1.1. Evaluation of rule utility

Confirmity to the AEMPS recommendations increased significantly from 45% (5/11) for patients in 2018, to 76% (13/17) for patients in 2019 (p = 0.02).

Table 2 shows the implemented clinical actions for those patients.

The number of days elapsed until the clinical action was implemented significantly decreased from 23 days in the year 2018 to 7 days in 2019, after the application of the advice (p = 0.02).

From March-December 2019, 1.22% (17/1389) from all advices made in our laboratory employed an automatic e-mail.

Table 2 Implemented actions in patients with advice during 2019 and 2018

Action	Number of patients	
	2019	2018
New haematological analysis control until leukocyte/ neutrophil count recovery	9	1
Stop clozapine	1	0
New haematological analysis + stop other drugs	2	0
No modifications	5	10

1.2. Evaluation of proper CLZ requisition

From July to October 2019, 1811 haemograms were ordered by Mental Health Department.

We detected 3 leukopenia/neutropenia in patients treated with CLO that were not advised by e-mail because a haemogram instead of a CLZ test was erroneously ordered by the psychiatric physician/nurse. Two corresponded to a patient with a previous notice and the haematological alteration was already known. However, the last one corresponded to a patient without previous haematological disorders, thus the e-mail advising the alteration and the potential danger was not sent.

2. Automatic rule: patient treated with CLO not attending the laboratory for blood-drawing

During December 2019, 215 CLZ tests were requested. Thirty-five patients (16.3%) did not present for blood drawing. All e-mails were sent to alarm them, and 27 were rescheduled within 1 month (77%). In December 2018, 149 patients had an analysis scheduled, 16 did not attend (10.7%), and nine of them presented for analysis within 1 month (56.25%).

The days without follow-up decreased from 29 days in December 2018 to 12 days in December 2019 after implementing the warning rule, although statistical significance was not reached (p = 0.06).

DISCUSSION

A quality reporting system is defined as the delivery of correct results to the appropriate clinicians in a time frame that ensures patient safety without overburdening both the clinicians and laboratory team (16).

Communication of critical values and clinically significant results is a Joint Commission patient safety goal (17). Failure in communication can significantly delay patient management (18), and it continues to be one of the most common contributing factors to the development of adverse events (19). Data available in the literature shows an error rate of 3.5% for all telephone calls made from laboratories (20). For this reason, automated informatics systems facilitating the generation of alerts for information transmission ensure patient safety. However, these strategies are not yet widely employed. In a 2008 survey of critical value reporting, the College of American Pathologist found that only 8.6% of 623 institutions communicate critical values using wireless technologies. (21) In Spain e-mail notifications only comprise 1% of all the communications (15).

These data are comparable to the results of our study, in which 1,22% of the communications is made via e-mail after the implementation of the first advice rule.

In our laboratory, the implementation of a novel LIS enabled the development of an automatic communication system employing informatics algorithms and e-mail to send an alert when some established criteria were met.

For patients presenting haematological alterations, the implementation of the first rule has advanced the application of the required clinical action. Also the compliance of the AEMPS recommendations has significantly increased, although it was not applied for 24% of the cases (n=4). In two of the four cases, patients were controlled in another hospital and for the other two cases the clinical specialist evaluated the patient and decided to continue with the treatment and analysis frequency. This automatic strategy also ensured the detection of 100% of haemogram alterations. Some clinical situations, such as bacterial infections, may cause leukocyte count elevations. In these cases, decreases in leukocyte count caused by CLO treatment may be masked. Therefore, these

situations must be taken into account in this type of patients.

As we have reported, most of the advices (85%) were sent to outpatients, who are the most benefited from employing this communication strategy.

It is important to remark that demanding the correct CLZ test is essential for the correct operation of the circuit, to avoid the loss of leukopenia/neutropenia cases detected. In our opinion, for the future, automatic systems which could link patient treatment information with analytical tests ordering could be essential to avoid errors attributed to manual test ordering.

For patients that usually renege on their visits, the second reminder rule reduced the time these patients spent without follow-up. Statistically significant differences were not achieved, maybe due to the high standard deviation observed in both groups and the low number of not attending patients in December 2018. Future studies with higher number of patients would be probably required to find significant differences.

Moreover, the two rules allowed to identify a group of patients defined as "well-controlled patients": patients without haematological alterations and generally adhering to the visits/analysis. For these patients, the frequency of their visits to the Mental Health Department had been spread and the quality of the clinical visit improved. For instance, during the clinical visits physicians may spend more time giving recommendations for a healthy life and emotional aspects, instead of spending time reviewing laboratory data.

Globally, all patients have benefited from the application of both rules.

For the Mental Health Department, this strategy is supposed to ensure the fulfilment of actual legislation and the improvement of patient management. The demonstrated benefits of this collaboration should motivate the adoption of similar strategies in many other clinical ambits of our hospital. Many patients treated with other drugs could benefit from this type of warning rules, for example those treated with azathioprine which also cause agranulocytosis.

Some experiences have been published in relation to automated communication, using e-mail or SMS, vs. oral communication. For example, in Spain, an SMS system was implemented to notify microbiological results with many useful benefits, such as the reduction in the time of notification, and elimination of risk errors when there is no repetition of the information received by the recipient to the laboratory staff (22). Another Italian study was performed employing two instant notification systems: the short message service (SMS) and an alert message on the desktop computer (23). Computerised communication demonstrated a reduction in notification time and yielded additional benefits: it eliminated the risk of errors occurring by phone notification and erroneous patient identification and test and value reporting, which occurs if the read-back step is not used.

All studies support the importance of the read-back step, our study established that if no answer were received 24 h after sending the advice e-mail, laboratory staff would contact the Mental Health Department to ensure communication.

Many studies have demonstrated that automatic alerting systems reduce the time until the provision of appropriate treatment in patients with critical laboratory results, showing a reduction of time until the resolution of patient abnormality by 29% (24,25). In our study the reduction of time until a clinical decision was applied was significantly reduced by 67% (22.36 to 7.29) days, showing significant reductions than published data.

CONCLUSION

This strategy has allowed the compliance of legal requirements, the improvement of patient safety, and the optimisation of clinical and laboratory procedures.

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Asymptomatic hypernatremia in an infant with midline defects

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ABSTRACT

Holoprosencephaly is a developmental abnormality caused due to incomplete cleavage of the rostral neural tube (basal forebrain) structures during early embryogenesis. This defect causes incomplete separation of the right and left cerebral hemispheres. Children manifest a wide spectrum of clinical manifestations, the extent of which depends upon the degree of hemispheric nonseparation. We describe an infant with midline cleft referred for preoperative evaluation in whom, asymptomatic electrolyte abnormalities and holoprosencephaly were identified. On further evaluation, the infant was diagnosed to have isolated central diabetes insipidus and she responded well to oral desmopressin therapy.

Cleft lip and palate is one of the commonest congenital malformations and midline clefts are likely to be associated with significant pituitary abnormalities. Awareness about the syndromic associations with

clefts and the associated anomalies are important for early diagnosis and intervention in these children.

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INTRODUCTION

Holoprosencephaly (HPE) is a developmental malformation of the forebrain with defective midline cleavage of the cerebral hemispheres. The incidence of HPE is estimated to be 1 in 250 during early embryonic development, and 1 in 16,000 live births [1]. Midline craniofacial clefts and hypothalamic/pituitary dysfunction are commonly associated with HPE [2]. We report a 4½ months-old girl, with a midline cleft, who was found to have severe hypernatremia and polyuria on pre-operative evaluation. Investigations revealed semi-lobar HPE and central diabetes insipidus (DI). She responded well to oral desmopressin.

CASE DETAILS

A 4½ months-old girl with midline cleft lip and palate was referred to Pediatrics for preoperative evaluation prior to corrective surgery. She was the second child of a non-consanguineous marriage with an uneventful antenatal history. She was delivered by caesarean section at 36th week of gestation with a birth weight of 2 kg. In view of the midline cleft, she was started on oro-gastric feeding (through Ryle's tube), with expressed breast milk and preterm infant formula. There was no family history of a similar illness.

On examination, she had median cleft lip and palate, depressed nasal bridge, cranio-synostosis, microcephaly (31.5cm), hypotonia of all four limbs and global developmental delay with a developmental age of only 6 weeks. Her length (54 cm) and weight (4 Kg) for age Z scores were less than -3 (WHO 2006 charts).

On investigation, her complete blood count, blood sugar, renal and liver function tests were normal. Serum electrolytes which were done as a part of routine preoperative evaluation revealed hypernatremia (158mmol/L) and hyperchloremia (129mmol/L). She was on preterm formula feeds (34mg/dL sodium in 100ml feed) and on further probing, mother revealed that she was adding a pinch of salt with each feed. She was asked to stop adding salt. The baby was initiated on a term infant milk formula with a lower sodium content (16mg/dL sodium in 100ml feed).

In spite of these changes the child had worsening of hypernatremia (167mmol/L) and there was no weight gain. On questioning, the mother also reported frequent passage of urine but there were no obvious signs of dehydration. Diabetes mellitus, urinary tract infection, hypercalcaemia, hypokalaemia, renal failure and thyrotoxicosis were ruled out. Diabetes insipidus was suspected. Urine output was monitored by catheterization and it ranged from 6 ml/kg/hr to 9 ml/kg/hr. Increased serum osmolality of 308mOsmol/kg (normal: 285-295mOsm/kg) with a low urine osmolality 160 mOsmol/kg (50-1200mOsm/kg) was noted.

The serum and urine osmolality were calculated by the laboratory analyzer which is preprogrammed with the formula.

$$\text{Serum osmolality} = \text{Serum sodium} \times 2 + \frac{\text{Blood glucose}}{18} + \frac{\text{Blood urea nitrogen}}{2.8}$$

$$\text{Urine osmolality} = \text{Urine potassium} \times 2 + \frac{\text{Urine glucose}}{18} + \frac{\text{Urine urea nitrogen}}{2.8}$$

Serum electrolytes were measured by indirect ion selective electrodes, blood urea by urease UV, and blood glucose by hexokinase method.

As the serum osmolality was >300mOsm/kg and urine osmolality less than the serum osmolality, water deprivation test was deferred and

subcutaneous vasopressin was given at a dose of 2 microgram. Doubling of urine osmolality (160mOsmol/kg to 369mOsmol/kg), fall in serum sodium from 168 to 160mmol/L and reduction in urine output from 9ml/kg/hour to 4ml/kg/hour were observed (Table 1).

MRI brain and pituitary revealed "semi-lobar holoprosencephaly", anatomically normal anterior pituitary with "absent pituitary bright spot" and "olfactory bulb agenesis" (Fig 1). Anterior pituitary hormonal evaluations were within normal limits. Echocardiogram and ultrasound abdomen were normal.

The child was started on oral desmopressin 10 mcg per day. Oral feeds were initiated and gradually shifted to semisolid feeds (Energy–531kcals and Protein 11.5g/ day). Eventually, serum sodium normalized and she started gaining weight. Her discharge weight was 4.35kg and serum sodium was 145 mmol/L. Genetic testing has been suggested on follow up.

DISCUSSION

Holoprosencephaly (HPE) is a complex developmental anomaly of the brain characterized by failure of the embryonic forebrain (prosencephalon) to divide into two distinct cerebral hemispheres, a process normally completed by 5th week of gestation. The etiology is multifactorial and includes genetic and environmental factors. Chromosomal abnormalities (cytogenetic abnormality of chromosome 13 and 18) and single gene disorders have been identified [3] and the common environmental risk factors include maternal smoking, alcoholism, maternal diabetes, and prenatal exposure to certain drugs [4].

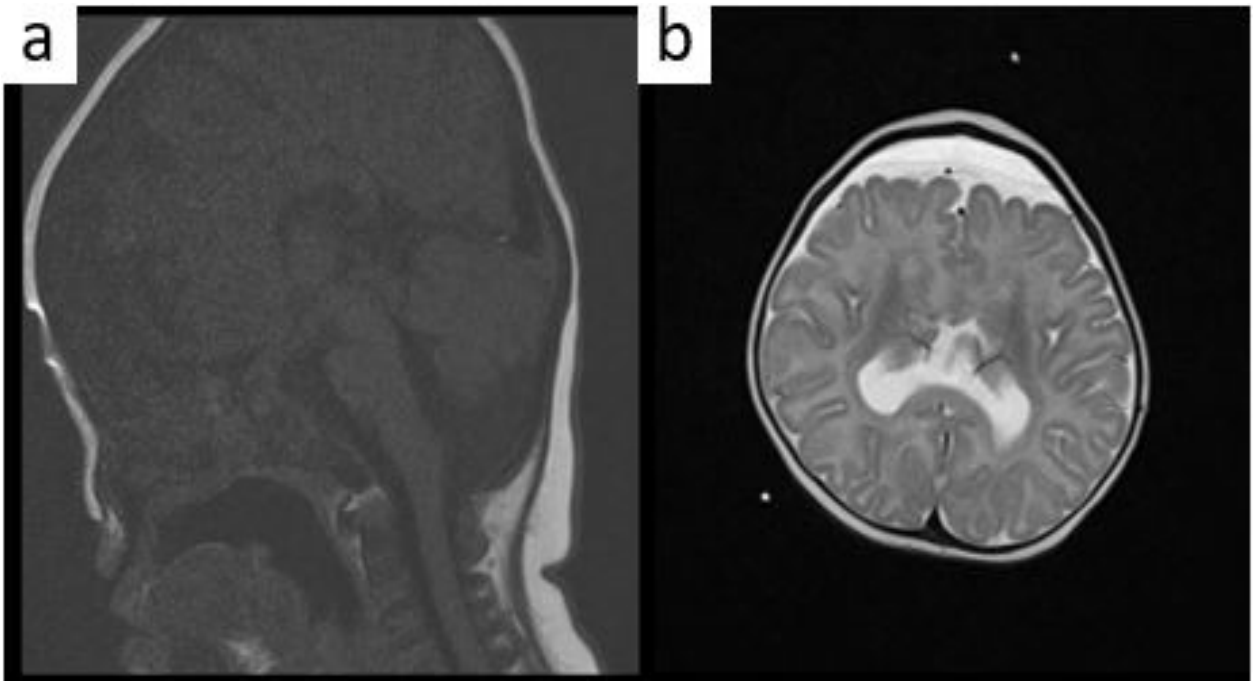
Holoprosencephaly is frequently associated with craniofacial (80%) and hypothalamic/pituitary abnormalities causing central DI in 70% [5,6].

Diabetes insipidus is characterized by polyuria (urine output >4ml/kg/hour in children and >6ml/kg/hour in infancy) and polydipsia.

Table 1 Serial investigations during the hospitalization

	Baseline	Post vasopressin challenge	At discharge
Fluid intake	1150ml/day	Nil oral	90ml/3rd hourly through nasogastric feed with demand paladai feeds
Urine output	9ml/kg/hr	4ml/kg/hr	3.2ml/kg/hr
Serum sodium	158mmol/L	154mmol/L	148
Serum chloride	121mmol/L	117mmol/L	110
Serum osmolality	308mOsm/kg	298mOsm/kg	-
Urine osmolality	160mOsm/kg	369mOsm/kg	-
Weight	4 kg	-	4.35 Kg

Figure 1 Panel a: MRI brain showing absent pituitary bright spot
Panel b: Semi-lobar holoprosencephaly



Extreme dehydration can result in hypernatremia, which can lead to neurological symptoms such as seizures, encephalopathy and coma [7,8]. In our case, despite severe hypernatremia (167 mmol/L), the child was asymptomatic.

Central DI (vasopressin-dependent) occurs due to deficiency of arginine vasopressin (AVP) in the hypothalamus or the pituitary gland, whereas nephrogenic DI (vasopressin-independent) occurs due to abnormal tubular response to AVP in the kidneys [7].

Water deprivation test with vasopressin challenge helps to differentiate central DI from nephrogenic DI; Performing water deprivation could be challenging in a young infant and may not be required in children with simultaneous hypernatremia and urine osmolality lower than serum osmolality. Hence a response to vasopressin challenge could be an easy alternative. Vasopressin will increase the urine osmolality above the serum osmolality though maximum osmolality may not be documented immediately because of chronic lack of AVP action on the renal medulla [9].

Diagnosis of holoprosencephaly is confirmed by brain computed tomography (CT) or magnetic resonance imaging (MRI). Serum electrolytes, thyroid-stimulating hormone, free T4, cortisol, adrenocorticotropin hormone, and insulin-like growth factor 1 should be analyzed for detecting hypothalamic/pituitary defects in patients with HPE [1,2,4].

Management of children with HPE is symptomatic and supportive. Coordinated multi-disciplinary care is recommended, which may include hormone replacement therapy for pituitary dysfunction, gastrostomy tube for feeding difficulties, surgical repair of cleft lip and/or palate with special attention towards fluid and electrolyte balance during surgery [2].

Chromosomal aberrations are seen in approximately 25% to 50% of patients with HPE [2]. Hence, genetic testing may help to counsel families about the nature of this condition, probability of recurrence and prenatal detection of HPE.

LEARNING POINT

Midline cleft lip/palate is frequently associated with hypothalamic/pituitary dysfunction and hence serum electrolytes should be tested as a part of preoperative evaluation.

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Compliance with ethical standards

Conflict of interests: The authors have declared that no Conflict of interest exists.

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