

Outcome of analysis on haemoglobin derivatives and CD4 count of inmates in congested custodial center Rivers state, Nigeria

Abstract

Introduction: Overcrowding promotes poor hygiene which may possibly create a medium for the growth and fast spread of infections and diseases, this can further compromise the immune system of the affected individuals. Wherever there is overcrowding, oxygen in circulation reduces while carbon dioxide increases, when carbon dioxide attaches to hemoglobin, it produces carbaminohemoglobin, which reduces hemoglobin's oxygen affinity through the Bohr effect. This study carried out laboratory analysis on HbO₂-oxyhemoglobin, CO₂Hb-carbaminohemoglobin and CD4-clusters of differentiation 4, of male inmates living in congested and non-congested cells of maximum-security custodial center Port Harcourt. Examining the biomarkers above, may provide an understanding of the health challenges faced by inmates living in congested correctional centers. It may also assist in the development of targeted strategies for addressing overcrowding in the Nigerian Correctional Centres.

Methods: A total of two hundred and forty (240) subjects aged between of 18 to 65 were recruited and categorized into three groups which includes; 80 male inmates from congested cells, 80 male inmates from non-congested cells and 80 male non-inmates as control living in Port Harcourt. Five milliliters (5ml) of venous blood was withdrawn from each participants aseptically and dispensed into Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant bottle for the assay of CD4, oxyhemoglobin and carbaminohemoglobin. Automated flowcytometry and spectrophotometric methods were used for the assay respectively. Data generated were analyzed using Graph-pad Prism 11.0 The mean values of HbO₂ for control, non-congested and congested subjects were (15.0±1.3, 13.8±1.0 and 10.9±2.4) respectively, CO₂Hb for the three groups were (0.3±0.1, 0.3±0.1 and 0.7±0.2) respectively while mean values of CD4 cells for control, non-congested and congested subjects were (1042±148.5, 776.4±159.2 and 718.2±336.0) respectively.

Conclusion: Mean values of congested subjects in some of the parameters were statistically significantly lower than normal, this explains that congestion had a great effect on some of these parameters thereby exposing the inmates to reduced immune system activation in case of any infection. This could possibly lead to a health risk capable of susceptibility to diseases conditions.

Keywords: overcrowding, immune system, oxyhemoglobin, carbaminohemoglobin and clusters of differentiation 4.

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Introduction

One of problems of Correctional Centres is the problem of overcrowding. Overcrowding occurs in Correctional Centres when the number of inmates exceeds the capacity of the facility, making it impossible for inmates to be housed in a humane, safe and psychologically appropriate manner. This poses a serious problem in Correctional Centres, especially those located in the country's major cities. Sometimes, in a country, Cell facilities in Correctional Centres are left to accommodate up to three times their capacity. In such jails, inmates have very little freedom of movement of their bodies and limbs hence each inmate is assigned a "post", which is about a foot and a half in length.¹ Congestion in Nigerian Correctional Centres accounted for the appalling living conditions in some of the prisons causing damages to the physical and mental well-being of inmates.² Research carried out by Jacob *et al.*, 2020 showed that overcrowding in Port Harcourt Maximum Security Custodial has effects on some of the haematological parameters where some of the haematological parameters are either increased or decreased.

Haemoglobin (Hb) is a tetrameric protein in the blood cells that binds oxygen and transports it from the lungs to the body's tissues releasing in exchange for carbon dioxide, which is then transported back to the lung for exhalation.³ Haemoglobin in red blood cell binds to carbon dioxide, forming a compound called carbaminohemoglobin, Carbaminohaemoglobin is a key transport mechanism for carbon dioxide in the blood, accounting for nearly quarter of the total carbon dioxide carried in the blood. The distribution of carbon dioxide in the blood is as follows; 23% is transported as carbaminohaemoglobin, 70% is converted to bicarbonate and carried in plasma and 7% is dissolved in plasma as free carbon dioxide.⁴ In the lungs, oxygen binds to haemoglobin in red blood cells, the binding of oxygen to haemoglobin is reversible, allowing for efficient oxygen transport. Haemoglobin which is a respiratory pigment in red blood cells, plays a crucial role in transporting oxygen from the lungs to various tissues. The formation of oxyhaemoglobin is dependent on the partial of oxygen (pO₂), which is high in the alveoli, favoring the binding of oxygen to haemoglobin.

CD4 cells, also known as T helper cells, play a central role in immunity by; Activating immune responses; CD4 cells recognize antigens presenting by antigen-presenting cells (APCs) and activates immune responses by releasing cytokines.⁵ Coordinating immune cell interactions: CD4 cells facilitates communication between immune cells such as T cells and B cells, to mount effective immune responses.⁶ Regulating immune tolerance; CD4 cells helps maintain tolerance by suppressing autoimmune responses and preventing excessive inflammation.⁷

The populations of the correctional facilities are disproportionately affected by infectious diseases, malnutrition and inadequate healthcare, leading to a heightened risk of morbidity and mortality. CD4 cell count and haemoglobin derivatives, serves as crucial indicators of immune, nutritional and overall health. This study aimed at analysing some hemoglobin derivatives such as Carbaminohaemoglobin and Oxyhaemoglobin and CD4 cells of inmates in Port Harcourt Maximum Security Custodial Centre. Examining these biomarkers, may provide better understanding of the health challenges faced by inmates living in congested correctional centres. It may also give useful information on how to develop targeted strategies to address them, ultimately reducing health risk and the dangers associated with overcrowding in the Nigerian Correctional Centre.

Materials and methods

Experimental design

Simple random sampling technique was employed to analyse HbO₂-oxyhemoglobin, CO₂Hb-carbamino hemoglobin and CD4-clusters of differentiation 4, of male inmates living in congested and non-congested cells of maximum-security custodial center Port Harcourt.

Study area

Subjects were recruited into this study from Maximum Security Custodial Centre located in Borokiri, Port Harcourt, Rivers state. Port Harcourt is the capital city of Rivers State, a major hub of oil exploration activity at the heart of Niger Delta, Nigeria. The Maximum-Security Custodial Centre, Borokiri is located at Latitude 4.7490N and Longitude 7.0350E Bundu Road. The Port Harcourt correctional center is a maximum-security custodial center because offenders of all categories of crime, including awaiting trial, convicts, and condemned criminals of both sexes are kept there. It is one of the earliest remand institutions in Nigeria. It was established by the British colonialists in 1918. The institution has a holding capacity of 804 inmates. However, according to National Bureau of Statistics, 2017, Port Harcourt prison has a total of 3,824 inmates (3,422 awaiting trials, 402 convicted). Out of the total prison population of 3,824 inmates, 3,747 (98%) were males, while 77(2%) were females which confirms an earlier observation that "Nigerian prison has less than 4% of total prison admissions to be females, about 80% serve short-term imprisonment of less than two years, and more than 50% are between the ages of 28 and 50 years."⁴

Study population

This study was carried out among male inmates of Port Harcourt Maximum Security Custodial Center, Rivers State Command. A total of 240 participants were recruited into the study and grouped into three which includes; eighty (80) male inmates living in the congested cells, eighty (80) male inmates living in non-congested cells and eighty (80) apparently healthy non-male inmates living in Port Harcourt. All the subjects were within the age of eighteen (18) to sixty-five (65) years old. Duration of stay of the individuals in the custody was also taken into considerations.

Sample size

Sample size was determined using G. Power 3.1.9.2 at probability error 0.05 and power of 0.95. This gave a total sample size of 177 subjects but this study adopted a sample of 240 to accommodate more persons for the study and account for errors that may result from sample handling.

Eligibility criteria

Inclusion criteria

Male inmates living in the congested and non- congested cells and non-inmates who were between the age of 18 - 65 years, showing no sign of illness and who voluntarily gave consent at the point of blood sample collection. The inmates were either serving jail term, awaiting trial or those on death sentence.

Exclusion criteria

Male inmates and non-mates who were below 18 - 65 years, who were showing signs of illness, those on any medication or admitted into the sick cells. Those who declined either informed or verbal consent. Moreover, newly admitted in-mates into any of the cells and female in-mates were all excluded from the study.

Blood sample collection and processing

Five milliliters (5ml) of venous blood was collected aseptically from each participant and dispensed into an Ethylene Diamine Tetra acetic Acid (EDTA) anticoagulant bottle assay of CD4, oxyhemoglobin and carbamino hemoglobin using their different preparation procedures and methods.

Laboratory analysis

Oxyhaemoglobin levels were determined using spectrophotometric method at the wavelength of 540nm with a light-path of 1cm and Carbaminohaemoglobin levels were determined using spectrophotometric method at the wavelength of 538nm and 578nm while CD4 cell count were determined using flow cytometer.

Procedure for oxyhaemoglobin using spectrophotometer as described by khan et al.⁸: Ammoniated water was prepared fresh by adding 0.04ml of ammonia to 100ml of distilled water and 4ml of ammoniated was pipetted into the test tube. The sample were mixed well and 20mml (0.02ml) was added to the test tube and stopper it with rubber bung, then it was mixed properly by inversion and the standard solution was read as well as the test solution in the colorimeter using light-path of 1cm and wavelength of 540nm against the ammoniated water (blank), using the formula to find the concentration of test

$$\text{Conc. Of test} = \text{O.D of Test (gm/dl)}$$

$$\text{O.D of Std} \times \text{Conc. of Std.}$$

Procedure for carbaminohaemoglobin using spectrophotometer as described by khan et al.⁹: 0.1ml of test was added to 20ml of 0.4ml/l ammonia solution and mixed. 20mg of sodium dithionite was added and the absorbance measured at 538nm and 578nm with a spectrophotometer within 10 minutes. The percentage of the carbamino hemoglobin was read from the calibration curve. The results were calculated by dividing the absorbance of test at 538nm wavelength by the absorbance of test at 578nm wavelength which is the quotient D^{538}/D^{578}

Procedure for CD4 cell count using flow cytometer as described by Schechter et al.¹⁰: Blood was collected in EDTA tubes and processed with 24 hours. The sample was prepared by centrifuging the blood

sample, removing plasma, and washing cells with PBS. The cells were then stained using a CD3-FITC/CD4-PE/CD45-PerCP antibody cocktail and incubated for 20 minutes. Flow cytometer with two lasers and at least 10,000 events were acquired in the lymphocyte gate. The data was analyzed using a CD3/CD4 plot to identify CD4+ T-cells and the percentage of CD4+ T-cells among total lymphocytes was calculated. Finally, the CD4 count was calculated by multiplying the percentage of CD4+ T-cells by the total of lymphocytes count from a haematology analyzer.

Statistical analysis

Data were analyzed using Graph-Pad Prism version 9.0.4 of Apple Machine HD Big Sur (Version 11.0) statistical package was used for data analysis. Descriptive statistical tools such as mean and SD were used. Students independent sample t-test and ANOVA were respectively used to compare means of two and more groups for inferential evaluation. Tukey's multiple comparison means of in-between groups. Pearson correlation was used to determine the linear relationships between two sets of data. The probability (p) value less than 0.05 ($P < 0.05$) was used and considered statistically significant.

Results

The concentration of oxyhemoglobin (HbO_2), Carbaminohemoglobin (CO_2Hb), CD4 and Full Blood Count (FBC) was determined among 80 inmates in congested cells, 80 inmates in non-congested cells from Custodial Centre and 80 non-inmates living in Port Harcourt. Classification of the subjects by age range 20-29 years were 83 subjects which included 35 in the congested cells, 16 in non-congested cells and 32 were non-inmates, those between 30-39 years old included 31 inmates from the congested cells, 34 from non-congested and 32 were non-inmates giving a total of 97 subjects. Subjects between 40-49 years old had 11 from congested cells, 19 from non-congested cells and 13 non-inmates, giving a total of 43 subjects. Those of 50-59 years, were 2 from congested cells, 8 from non-congested cell, 2 non-inmates and the total number was 12 subjects. Results of subjects above 60 years of age were, 1 subject from the congested cell, 3 from the non-congested cell, 1 control and this group totaled 5 subjects. This result is shown in Table 1 below

Table 1 The Demographic characteristics of participants in the study

Age range/group	20-29	30-39	40-49	50-59	Above 60 N
Congested group	35	31	11	2	1 80
Non-congested	16	34	19	8	3 80
Control	32	32	13	2	1 80
Total	83	97	43	12	5 240

Comparison of Mean $\pm$ SD of CD4, HbO_2 , & CO_2Hb of the three groups

The mean CD4 count (cell/mm^3) for control subjects, non-congested subjects and congested subjects were 1144 ± 906.6 , 887.1 ± 932.1 and 759.5 ± 264.9 respectively. The mean HbO_2 (g/dl) for control subjects, non-congested subjects and congested subjects were 14.4 ± 1.0 , 14.1 ± 1.2 and 12.1 ± 2.0 respectively. The mean CO_2Hb (%) for control subjects, non-congested subjects and congested subjects were 0.4 ± 0.7 , 0.3 ± 0.1 and 0.6 ± 0.2 respectively.

There was a significant difference in CD4 cell count ($p = 0.0065$),

HbO_2 ($p < 0.0001$) and CO_2Hb ($p = 0.0018$) between the control, non-congested, and congested subjects as shown Table 2 below.

Table 2 Comparison of Mean $\pm$ SD of CD4, HbO_2 , & CO_2Hb of the three groups

Study groups	N	Parameters		
		CD4 (cell/mm^3)	HbO_2 (g/dl)	CO_2Hb (%)
Control	80	1144 ± 906.6	14.4 ± 1.0	0.4 ± 0.7^c
Non-Congested cells	80	887.1 ± 732.1	14.1 ± 1.2^c	0.3 ± 0.1
Congested cells	80	759.5 ± 264.9^a	12.1 ± 2.0^a	0.6 ± 0.2^b
F-value		5.142	54.4	6.514
P-value		0.0065	< 0.0001	0.0018
Remark		S	S	S

Key: N, number of subjects; S, significant; NS, not significant at $P < 0.05$; HbO_2 -oxyhemoglobin, CD4-clusters of differentiation 4; CO_2Hb , carbaminohemoglobin

The different alphabets a, b and c in superscript within each parameter indicates statistically significant difference at $P < 0.05$

Comparison of Mean $\pm$ SD of CD4, HbO_2 & CO_2Hb for 20-29 age group

The mean CD4 (cell/mm^3) count for control subjects, non-congested subjects and congested subjects were 1304.0 ± 1411.0 , 829.0 ± 120.5 and 806.3 ± 199.7 respectively. The mean HbO_2 (g/dl) value for control subjects, non-congested subjects and congested subjects were 14.1 ± 0.8 , 14.38 ± 0.8 and 12.5 ± 1.7 respectively. The mean CO_2Hb (%) count for control subjects, non-congested subjects and congested subjects were 0.5 ± 1.0 , 0.3 ± 0.1 and 0.5 ± 0.2 respectively. There was a statistically significant difference in Mean $\pm$ SD of HbO_2 ($p = < 0.0001$). There was no statistically significant difference in Mean $\pm$ SD of CD4 and CO_2Hb between the control, non-congested and congested subjects as shown in Table 3.

Table 3 Comparison of Mean $\pm$ SD of CD4, HbO_2 , & CO_2Hb for 20-29 age group

Study groups	N	Parameters		
		CD4 (cell/mm^3)	HbO_2 (g/dl)	CO_2Hb (%)
Control	80	1304.0 ± 1411.0	14.1 ± 0.8^c	0.5 ± 1.0
Non-Congested cells	80	829.0 ± 120.5	14.38 ± 0.8	0.3 ± 0.1
Congested cells	80	806.3 ± 199.7	12.5 ± 1.7^b	0.5 ± 0.2
F-value		2.923	16.95	0.5582
P-value		0.0597	< 0.0001	0.5745
Remark		NS	S	NS

N, number of subjects; S, significant; NS, not significant at $P < 0.05$; HbO_2 -oxyhemoglobin, CD4-clusters of differentiation 4; CO_2Hb , carbaminohemoglobin

The different alphabets a, b and c in superscript within each parameter indicates statistically significant difference at $P < 0.05$

Comparison of Mean $\pm$ SD Values of CD4, HbO_2 , & CO_2Hb for 30-39 age group

The mean CD4 (cell/mm^3) count for control subjects, non-congested and congested subjects were 1042.0 ± 148.5 , 776.4 ± 159.2 and 718.2 ± 336.0 respectively. The mean HbO_2 (g/dl) value for control subjects, non-congested subjects and congested subjects were

14.4±0.8, 14.1±1.5 and 11.9±2.3 respectively. The mean CO₂Hb (%) count for control subjects, non-congested subjects and congested subjects were 0.3±0.1, 0.4±0.1 and 0.6±0.2 respectively. There was a statistically significant difference in Mean ± SD of HbO₂ (P = <0.0001), CD4 (P = <0.0001) and CO₂Hb (P = <0.0001) between the control, non-congested and congested subjects as shown in Table 4.

Table 4 Comparison of Mean ± SD of CD4, HbO₂, & CO₂Hb for 30-39 age group

Study groups	N	CD4 (cell/mm ³)	Parameters	
			HbO ₂ (g/dl)	CO ₂ Hb (%)
Control	80	1042.0±148.5 ^c	14.4±0.8	0.3±0.1 ^c
Non-Congested cells	80	776.4±159.2 ^b	14.1±1.5 ^c	0.4±0.1
Congested cells	80	718.2±336.0	11.9±2.3 ^a	0.6±0.2 ^b
F-value		18.23	22.74	28.49
P-value		<0.0001	<0.0001	<0.0001
Remark		S	S	S

N, number of subjects; S, significant; NS, not significant at P < 0.05; HbO₂-oxyhemoglobin, CD4-clusters of differentiation 4; CO₂Hb, carbaminohemoglobin

The different alphabets a, b and c in superscript within each parameter indicates statistically significant difference at P < 0.05

Comparison of Mean ± SD values of CD4, HbO₂ & CO₂Hb for 40-49 age range

The mean CD4 (cell/mm³) count for control subjects, non-congested and congested subjects were 1032.0±141.8, 733.4±150.6 and 718.2±336.0 respectively. The mean HbO₂ (g/dl) value for control subjects, non-congested and congested subjects were 15.0±1.3, 13.8±1.0 and 10.9±2.4 respectively. The mean CO₂Hb (%) count for control subjects, non-congested and congested subjects were 0.3±0.1, 0.3±0.1 and 0.7±0.2 respectively. There was a significant difference in CD4 (p = <0.0001), HbO₂ (p = <0.0001) and CO₂Hb (p = <0.0001) between the control, non-congested and congested subjects as shown in Table 5.

Table 5 Comparison of Mean ± SD of CD4, HbO₂, & CO₂Hb for 40-49 age group

Study Groups	N	CD4 (cell/mm ³)	Parameters	
			HbO ₂ (g/dl)	CO ₂ Hb (%)
Control	80	1032.0±141.8 ^c	15.0±1.3	0.3±0.1 ^c
Non-Congested cells	80	733.4±150.6 ^a	13.8±1.0 ^c	0.3±0.1
Congested cells	80	718.2±336.0	10.9±2.4 ^a	0.7±0.2 ^b
F-value		9.976	19.73	28.06
P-value		0.0003	<0.0001	<0.0001
Remark		S	S	S

N, number of subjects; S, significant; NS, not significant at P < 0.05; HbO₂-oxyhemoglobin, CD4-clusters of differentiation 4; CO₂Hb, carbaminohemoglobin

The different alphabets a, b and c in superscript within each parameter indicates statistically significant difference at P < 0.05

Comparison of Mean ± SD of CD4, HbO₂ & CO₂Hb for 50 years and above age group

The mean CD4 count (cell/mm³) for control subjects, non-

congested and congested subjects were 927.0±143.8, 1405.0±2291.0 and 709.0±168.2 respectively. The mean HbO₂ (g/dl) value for control subjects, non-congested and congested subjects were 15.5±1.0, 14.0±0.9 and 13.3±1.5 respectively. The mean CO₂Hb (%) count for control subjects, non-congested and congested subjects were 0.4±0.0, 0.3±0.1 and 0.5±0.2 respectively. There was a significant difference in HbO₂ (p = 0.0376) and CO₂Hb (p = <0.0100) between the control, non-congested and congested subjects whereas there was no statistically significant difference in Mean ± SD of CD4 as shown in Table 6.

Table 6 Comparison of Mean ± SD of CD4, HbO₂ & CO₂Hb for 50 years and above age group

Study Groups	N	CD4 (cell/mm ³)	Parameters	
			HbO ₂ (g/dl)	CO ₂ Hb (%)
Control	80	927.0±143.8	15.5±1.0	0.4±0.0
Non-Congested cells	80	1405.0±2291.0	14.0±0.9	0.3±0.1 ^c
Congested cells	80	709.0±168.2	13.3±1.5 ^a	0.5±0.2
F-value		0.2338	4.003	6.116
P-value		0.794	0.0376	0.0100
Remark		NS	S	S

N, number of subjects; S, significant; NS, not significant at P < 0.05; HbO₂-oxyhemoglobin, CD4-clusters of differentiation 4; CO₂Hb, carbaminohemoglobin

The different alphabets a, b and c in superscript within each parameter indicates statistically significant difference at P < 0.05

Discussion

From comparison of CD4 cell counts among control, inmates living in non-congested and congested cells, the provided data reveals significant differences in CD4 cell counts between the three groups at P-value < 0.05. The significant decrease in CD4 cell counts for congested cell inmates suggests: Increase susceptibility to infections: CD4 cells play a crucial role in immune response, reduced counts increases the risk of opportunistic infections particularly tuberculosis, which is prevalent in correcting setting.¹¹ Chronic stress and immune suppression: Overcrowding can lead to chronic stress, suppressing immune function and reducing CD4 cells counts.¹² Poor living conditions: Congested cells may have inadequate sanitation, ventilation and nutrition, contributing to immune system weakening.¹³

HIV progression: Lower CD4 cell counts can accelerate HIV disease progression, emphasizing the need for target intervention.¹⁴ From comparison of oxyhaemoglobin levels among control, inmates living in non-congested and congested cells, the provided data reveals significant differences in oxyhaemoglobin levels between the three groups at P-value < 0.05. The significant decrease in oxyhaemoglobin levels in congested cell inmates suggests hypoxia. Reduced oxyhaemoglobin indicates decreased oxygen delivery to tissues, potentially leading to hypoxia. Hypoxia can impair immune function, increasing susceptibility to infection.¹⁵ Decrease oxyhaemoglobin can also suggest poor ventilation; Congested cells may have inadequate ventilation, contributing to reduced oxygen levels. Overcrowding can lead to chronic stress, affecting oxygenation and overall health.¹⁶ These findings highlight the importance of addressing overcrowding and improving ventilation in correctional facilities to mitigate hypoxia and related health risk. The comparison of carbaminohaemoglobin levels among control, inmates living in non-congested and congested cells, reveals significant differences in carbaminohaemoglobin levels

between the three groups at P-value < 0.05. A significant increase in carbaminohaemoglobin level was observed in congested cell inmates and this typically indicates increased carbon dioxide (CO₂) binding to haemoglobin suggesting a range of health complications: Respiratory acidosis: elevated carbaminohaemoglobin (CO₂Hb) may reflect impaired CO₂ elimination, leading acid-base imbalance. Respiratory disease: Increased carbaminohaemoglobin may be associated with chronic obstructive pulmonary disorder (COPD), asthma or other respiratory conditions.¹⁵ Ventilation-perfusion mismatch: Elevated carbaminohaemoglobin suggests inadequate gas exchange, potentially due to lung disease or cardiovascular conditions. Increased oxygen affinity: carbaminohaemoglobin can increase haemoglobin's oxygen affinity, potentially affecting oxygen delivery tissues.

Conclusion

From this comparative study of CD4 cell counts, Oxyhaemoglobin and Carbaminohaemoglobin levels among inmates living in congested and non-congested cells, there was significantly lower CD4 cell counts in congested cell inmates, indicating compromised immune function. Reduced oxyhaemoglobin levels in congested cell inmates, suggesting hypoxia and poor ventilation. Elevated Carbaminohaemoglobin levels in congested cell inmates was also observed, indicating increased carbon dioxide binding to haemoglobin and potential respiratory acidosis.

Recommendation

To improve the health of inmates in correctional facilities, regular inmates health screening, ventilation upgrade, overcrowding mitigation, health education programs and collaboration between healthcare providers and administration is recommended.

Consent

Oral informed consent were obtained from participants before blood collection. Participants were made to understand the nature of the study and the fact that the participation is voluntary with confidentiality of recovered data maintained at all times during and after the study.

Ethical approval

For the purpose of this study ethical approval was obtained from Rivers State University Teaching Hospital Health Research Ethics Committee on the 14th June, 2023 with number NHREC/21/03/2022.

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