Prevalence and Correlates of Zinc Deficiency in Dengue Virus Infection

A Thesis

Presented to the Graduate School

Of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

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May 2020

ABSTRACT

Zinc is a known immunomodulator, yet there is limited literature on zinc deficiency or its role in the context of most arboviral infections, including Dengue virus infection (DENV). This study describes the prevalence of zinc deficiency and its associations with immune response markers in participants with and those exposed to DENV. We quantified the zinc status of a convenient subset of participants (n=71), who had available adequate sample volumes, from an arbovirus surveillance program in Ecuador. Data on anthropometry, demographics, clinical history, and immune response markers for participants was available through the parent surveillance program and earlier studies. Participants were categorized by the presence of nonstructural protein 1(NS1 antigen), IgM, and IgG for one of four DENV serotypes into one of the following categories: Healthy Controls, Non-Febrile DENV, Other Febrile Illness, and Apparent DENV. Associations between zinc status and covariates were explored using linear and logistic regression in SAS 9.4 (SAS Institute, Cary, NC). Significance testing to compare observations across illness categories was performed using ANOVA with a post hoc Tukey test, with results presented as mean and standard deviation. The average age of the sample population was 31.5 ± 15.0 years. The prevalence of zinc deficiency (serum zinc$\leq$ 65 µg/dL) in the study population was 30%. Using multivariate methods, CXCL10 concentrations, age, and sex were found to be significantly associated with serum zinc, with increasing levels of CXCL10 associated with greater odds of zinc deficiency. Serum zinc concentrations varied significantly across illness categories ($p<0.01$), with the symptomatic groups having the lowest mean serum zinc concentrations (SMD=1.27). This exploratory analysis adds evidence for a potential association between zinc status and expression of CXCL10 that needs to be investigated in longitudinal studies. Further, while the importance of zinc for optimal immune function has been well established, the effect of zinc status on the immune response and DENV pathogenesis remains to be fully described.

BIOGRAPHICAL SKETCH

Meghan Trumbull-Kennedy was born in Sayre, Pennsylvania. She completed her bachelor's degree in Biology at Alfred University, Alfred, NY. After earning her bachelor's degree, she spent one year working for the Cornell Environmental Health and Safety department and volunteering for the Biosafety division. In 2018 she enrolled in the Master of Science in the nutrition program at Cornell University, with the mentorship of Dr. Saurabh Mehta.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest appreciation to my committee chair, Dr. Saurabh Mehta. Without his assistance and dedicated involvement in the development of this thesis, this paper could not have been accomplished. I would like to sincerely thank him for his support and mentorship over these past two years.

I wish to extend my sincerest thanks to my minor committee member, Dr. Patricia Cassano, whose door was always open. I am gratefully indebted to her for her valuable comments on this thesis.

Furthermore, I would like to acknowledge Dr. Tolunay Beker Aydemir for her consistent support and guidance during the running of this project. Furthermore, I would like to thank the rest of the Aydemir research group for their support during data collection.

Lastly, I would like to express my deepest gratitude to my fellow members of the Mehta Research Group, for their support, thoughtful comments, and recommendations throughout this entire process. It has been a privilege to work alongside all of them for the last two years.

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DENV	Dengue virus
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
BMI	Body mass index
MUAC	Mid-upper arm circumference
PAHO	Pan American Health Organization
NF-κB	Nuclear factor-kappa beta
CRP	C-reactive protein
AGP	Alpha-1 acid glycoprotein

Introduction

Zinc is an essential micronutrient that is critical for optimal immune function. Research in the field of zinc metabolism in humans began with a series of reports describing Iranian and Egyptian patients, all presenting with what appeared to be a similar syndrome of dwarfism, hypogonadism, iron deficiency anemia, and hepatosplenomegaly[1]. Commonalities between patients included diets lacking in meat, sparse consumption of fresh fruit and vegetables, and geophagia. These reports were followed by descriptions of other patients across the world presenting with a similar collection of symptoms, including a report by Caggiano et al. describing a patient originally from Caguas, Puerto Rico with delayed growth, hypogonadism, hypogammaglobinemia, and chronic infections throughout childhood and early adulthood. Within the sixteen months following a 10-day course of oral zinc sulfate supplementation, an increase of 21 cm in height and 6 cm of linear growth was observed[2]. These reports and the hypothesized link between zinc deficiency, growth retardation, and hypogonadism led to the earliest zinc supplementation studies in humans, and due to observed recovery from zinc deficiency with supplementation, eventually, the establishment of zinc as an essential mineral by the National Academy of Sciences in 1974[3].

Zinc deficiency is classified as mild, moderate, or severe and is quantified through various methods including the assessment of dietary intake of zinc, determination of bioavailability through quantification of phytate consumption, and serum and plasma zinc. Historically, stunting has also been used as a proxy for estimating the zinc status and burden of deficiency in a population[4]. Due to the imprecision of current zinc

quantification methods, there is inadequate information on the global prevalence of zinc deficiency[5].

The International Zinc Nutrition Consultative Group categorizes a country as at high risk of zinc deficiency when greater than 20% of under-five children are stunted, along with at least 25% of the population estimated not to meet the recommended dietary intake of zinc[6]. For reference, recent surveys estimate the prevalence of stunting to be approximately 25.3% in Ecuador, an improvement from the previous estimate of 33.5%[7].

Globally the prevalence of zinc deficiency is likely widespread, with the highest-burden in low-income and resource-limited settings. This disparity is often attributed to high levels of phytate, a reservoir of phosphorus in plants, but a potent inhibitor of iron and zinc absorption in humans. Many populations living in resource-limited settings and low-income countries rely heavily on staple crops, such as rice, to make up the majority of their caloric intake. These staple crops are often rich in phytate and other potent inhibitors of zinc absorption. At the same time, foods rich in bioavailable zinc, such as animal products, are not readily available in populations that are at the highest risk of zinc deficiency[8].

There is no tissue-specific storage for zinc in the body; a constant supply is required to maintain normal intracellular function. Zinc status is tightly regulated through the regulation of endogenous losses and is not affected without extreme or prolonged changes in dietary intake. Mild to moderate deficiencies are the most common. Yet presently applied indicators of zinc status only begin to vary consistently with severe

zinc deficiency and are less reliable for the quantification of zinc status in cases of mild to moderate deficiency with high accuracy in individuals[9,3].

In Ecuador, the burden of zinc deficiency is relatively high; approximately 56.1% of reproductive-age women are at risk of insufficient zinc intake, based on a population level survey of dietary intake[10]. Dietary patterns in this region support this estimate; approximately 30% of the population consumes carbohydrates above recommended levels. Rice makes up the majority of daily caloric intake with the rest of the diet consisting of limited meat, fresh fruit, and vegetables[11].

Even mild deficiency of zinc has been associated with dysregulated immune function. It is estimated that globally, deficiency has a role in up to 16% of lower respiratory tract infections, 18% of malaria, and 10% of diarrheal diseases[3]. It is well documented that zinc has a role in the regulation of pro-inflammatory pathways and contributes to the downregulation of the pro-inflammatory immune response through modulation of signaling pathways[8]. Recently there has been evidence that zinc status before infection influences cytokine expression in response to an immune insult. For example, in cases of mild to moderate zinc deficiency IL-1β production was higher after an immune insult in mice on mouse chow lacking adequate zinc compared to mice consuming sufficient quantities of zinc[12].

Additionally, zinc has been found to target the expression of Nuclear Factor Kappa Beta (NF-κ β). This transcription factor is the master regulator of pro-inflammatory responses, further demonstrating the role of zinc in maintaining homeostasis and a balanced immune response to infection[13].

Dengue virus (DENV) is an arbovirus transmitted by the Aedes Aegypti mosquito. DENV is common in the Americas; with more than 70% of the global population at risk, the estimated incidence of DENV infection is 50 million new cases per year[14]. DENV belongs to the genus Flavivirus, along with other viruses such as Yellow Fever, Zika Virus, and Chikungunya. There are four antigenically distinct serotypes of DENV (DENV-1 to DENV-4), and the primary targets for dengue virus are innate immune cells such as monocytes, macrophages, and dendritic cells.

Many cases of DENV infection are asymptomatic, often resolving without the need for medical care. However, 1 out of every 20 asymptomatic cases progresses on to apparent or symptomatic DENV. With a wide range of clinical presentations, the differentiation of DENV infection and other cases of febrile illnesses is often challenging. Furthermore, while the incidence of severe DENV is relatively low, these cases can progress with little warning on to more severe disease. There are three categories of symptomatic DENV infection: undifferentiated fever, dengue fever (DF), and dengue hemorrhagic fever (DHF) [15].

Infection with one serotype does not confer immunity to other serotypes, with literature hypothesizing an association between secondary infection and Dengue Hemorrhagic Fever. Efforts to develop a vaccine have been hindered due to the potential increased risk of severe Dengue with each subsequent DENV infection[16].

The economic burden of DENV infection is substantial. Globally, the estimated annual total cost associated with Dengue virus infection was $587 million, based on officially reported cases from 2001-2005. Of the global estimated cost, the same data indicated

that about $340 million of that was from cases reported in the Americas. Throughout the region of the Americas, DENV infection negatively impacts work capacity across all age groups. Cases of DENV infection lead to increased absence from school, decreased work productivity, and often prolonged absence from work in cases requiring hospitalization. The same study by Suaya et al. found that after adjusting for the number of deaths and cases by setting, the estimated cost of DENV infection was an estimated $759 per case across the region of the Americas[17]. A similar study found that data from 2000-2007 indicated the economic burden of DENV infection in the Americas might be even higher, at an estimated $3.2 billion[18].

Thus, with the incidence of DENV infection on the rise, it is critical to identify potential risk modifiers of severe dengue infection outcomes[16]. Current evidence has shown that efficient viral replication within the host is necessary but not sufficient alone to lead to severe disease. Host factors such as nutrition, obesity, age, and an array of other factors modify the extent to which early viral replication leads to infection of new host cells and subsequently influences the resulting level of viremia[16].

Based on recent evidence, researchers have postulated that manifestations of severe Dengue may be due to increased viral replication rates in monocytes and macrophages', leading to what is commonly referred to as a cytokine storm[19]. Cytokine storms often lead to a life-threatening condition known as capillary leak syndrome, where overwhelming vasodilation leads to a dramatic decrease in blood pressure, among other potentially life-threatening symptoms[16].

This study focused on identifying the association of cytokines with zinc status, controlling for demographic and clinical covariates, and sought to elucidate further the potential association between zinc status and the response to dengue virus infection. Through this exploratory analysis, we quantified the prevalence of zinc deficiency in the study population and evaluated potential relationships between age, sex, and immune response markers in participants with and exposed to Dengue virus.

Methods:

Participants were recruited from an arbovirus surveillance study conducted in Machala, Ecuador[20]. Patients with suspected DENV infections, categorized as index cases, were referred to the study from five Ministry of Health Clinical Sites. Additional participants inhabiting the same household and nearby homes of the index cases, upon recruitment, were defined as associate cases.

Demographic data, anthropometric measurements, and venous blood samples were collected for both index and associate cases on enrollment into the study. The analysis described in the parent study was approved by the Cornell University Institutional Review Board[21]. For the present analysis, a subset (n=71) of participants with available data on immune response markers were selected for serum zinc quantification. $DENV^{+}$ cases were defined by a positive quantitative polymerase chain reaction (PCR) result. Participants were defined as $DENV^{-}$ based on negative results for PCR, NS1 antigen, and negative IgM and IgG determined by enzyme-linked immunosorbent assay (ELISA).

Immune response markers:

Acute-phase proteins, including C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP), were quantified by chemiluminescence and colorimetric assay, respectively. Concentrations of cytokines and chemokines (n=29) were measured by magnetic bead multiplex assay. Results are reported as median fluorescent intensity (MFI).

Quantification of serum zinc:

Serum zinc was assessed in triplicate by microwave plasma atomic absorption spectroscopy (MP-AES 4200, Agilent Technologies) with assessors blinded to the disease status of the study participants. Before the analysis, serum samples were diluted (1:5) with deionized water. A standard curve was established by using commercial zinc standards (Fischer Scientific) in concentrations of 0.031, 0.063, 0.125, 0.250, 0.500, 1.000, 2.000 and 5.000 mg/l. Serum zinc was calculated by multiplying parts per million zinc by the dilution factor, and then by a factor of 100 to reach zinc in µg/dL. After serum zinc was quantified, samples were re-identified before the statistical analysis.

Statistical Methods:

Descriptive statistics were calculated for the outcomes of interest (continuous serum zinc, zinc deficiency, and illness categories) and primary predictors (sex, age, immune function biomarkers). Descriptive statistics (mean and standard deviation) of zinc were calculated for each exposure variable of interest. A normality test (Q-Qplot) confirmed that continuous serum zinc followed a normal distribution. Linear regression models were used to analyze the association between each of the immune function biomarker exposure variables and continuous serum zinc (µg/dL). Logistic regression was

conducted for the binary outcome, zinc deficiency/sufficiency, with deficiency defined as serum zinc ≤ 65 µg/dL.

For univariate analysis, exposure variables were examined individually and then considered for inclusion in the multivariate model if there was an association between the exposure and serum zinc at $p<0.20$. Only the variables that were then significant at $p<0.05$ were retained in the final model.

Results

The characteristics of the study sample (n=71) overall and by zinc status are presented in Table 1 and Table 2. The average age of the study population was 31.5 years. The average weight, height, and BMI of the study population were 64.8 kg, 159.7cm, and 25.3 kg/m^2, respectively. Mid-upper arm circumference (MUAC) and waist circumference were measured for all participants by trained study staff, and the mean values were 28.7cm and 85.2 cm, respectively.

Table 1. Demographics and Medical History of Participants by Zinc Status

Characteristic, n (%)	Overall n=71	Serum Zinc ≤ 65 µg/dL n=32	Serum Zinc > 65 µg/dL n=39
Age(years)			
<19	18(25.4)	3(9.4)	16(41.0)
≥19	53(74.6)	29(90.6)	23(59.0)
Sex			
Male	36(50.7)	11(34.4)	25(64.1)
Female	35(49.3)	21(65.6)	14(35.9)
Medical History			
Hypertension	6(8.5)	3(9.4)	3(7.7)
BMI Category			

Underweight <18.5 kg/m^2	3(4.2)	2 (6.9)	1 (4.2)
Normal 18.5-25 kg/m^2	17(23.9)	11 (37.9)	6 (25.0)
Overweight 25-30 kg/m^2	20(28.2)	8(27.6)	12 (50.0)
Obese ≥30 kg/m^2	13(18.3)	8 (27.6)	5(20.8)

The prevalence of zinc deficiency (serum zinc ≤ 65 µg/dL) by different socio-demographic and anthropometric variables is presented in table 2. The prevalence of zinc deficiency in the study population was 45%, highest amongst females compared to males and adults over 19 compared to children 19 years of age and younger. Among participants identified as zinc deficient (serum zinc≤ 65 µg/dL) and zinc-sufficient (serum zinc > 65 µg/dL), there were no significant differences in weight, height, BMI, waist circumference, or mid-upper arm circumference(MUAC).

Table 2. Anthropometric Data by Zinc Status

Characteristic, mean ± SD or n(%)	Overall n=71	Serum Zinc ≤ 65 µg/dL n=32	Serum Zinc > 65 µg/dL n=39	[a]PValue
Age, years	31.5 ± 15.0	36.8 ± 15.2	27.1 ± 13.6	0.01
Weight, kg	64.8 ± 15.7	64.9 ± 14.5	64.6 ±16.9	0.93
Height, cm	159.7 ± 8.1	159.4 ± 6.6	160.0 ± 9.3	0.75
<150 cm	5(7.0)	1(3.1)	4 (10.3)	
BMI, kg/m^2	25.3± 5.8	25.6 ± 5.8	25.1 ± 5.8	0.73
Waist Circumference, cm	85.2± 14.2	86.9 ± 12.9	83.9 ± 15.3	0.39
Log MUAC, cm	3.3± 0.2	3.4 ± 0.2	3.3 ± 0.2	0.29
MUAC, cm	28.7± 4.7	29.3 ± 4.9	28.1± 4.4	0.28
Serum Zinc(ug/dL)	67.1± 14.2	54.4± 8.4	77.6± 8.0	<.0001
Male	36(51)	11(34)	25(64)	

[a]P values are from student's T-test comparing the zinc-deficient group to the zinc-sufficient group.

The characteristics of the study sample (n=71) across four illness categories: Healthy Controls, Non-Febrile DENV infection, Other Febrile Illness, and Apparent DENV infection, are presented in Table 3. Illness categories were assigned by the parent trial[20]. Study participants were defined as $DENV^-$ based on the following criteria; negative for nonstructural protein 1(NS1) antigen based on the results of a rapid test or reverse transcription-polymerase chain reaction(RT-PCR), negative for anti-DENV immunoglobulin(Ig)M enzyme-linked immunosorbent assay (ELISA) results, and negative for anti-DENV IgG, the presence of which was also determined by ELISA. Cases of confirmed DENV infection were defined as a positive NS1, RT, NS1 ELISA, or RT-PCR test result. Participants were further classified by fever, which was self-reported and defined as fever within the 7 days or upon entering the study. $DENV^-$ participants without fever identified as healthy controls. Cases of confirmed DENV that did not report a fever were classified as cases of non-febrile DENV infection. Cases classified as other febrile illness were confirmed $DENV^-$ participants that had reported or had a fever on enrollment in the parent trial. Lastly, participants who had both a fever and tested positive for DENV infection were identified as cases of apparent DENV infection.

Anthropometry data was available for all 71 participants included in this analysis. Serum zinc concentrations varied significantly across illness categories, with the highest concentrations in the healthy controls and non-febrile DENV cases with a standardized mean difference of 1.27. Furthermore, mean serum zinc concentrations of $DENV^+$ cases differed significantly from the mean serum zinc concentrations of participants identified as healthy controls. Significance testing was done by ANOVA and a post hoc

Tukey test. There were no significant differences in participant weight, height, or age across illness categories ($p>0.05$). Furthermore, there was no significant difference in the prevalence of zinc deficiency across illness categories.

In addition to demographic data, cytokine and chemokine biomarkers are described in Table 3. Data were available for all 71 included study participants. Biomarker concentrations were assessed using a 29-panel biomarker assay. Levels of 8 markers, as well as AGP and CRP, differed significantly across illness categories ($p<0.05$)

Table 3. Demographic Data and Concentrations of Immune Function Markers by Infection Status of Study Population

	Healthy Controls	Non-Febrile DENV	Other Febrile Illness	Apparent DENV	
Characteristic, mean (SD) or n(%)	Fever$^-$/DENV$^-$ (n=44)	Fever$^-$/DENV$^+$(n=12)	Fever$^+$/DENV$^-$(n=4)	Fever$^+$/DENV$^+$(n=11)	[b]PValue
Serum Zinc (µg/dL)	68.14 (15.32)	70.00(10.00)	56.25(20.57)	63.94(9.62)	0.0012
Zinc deficiency (≤0.65µg/dL)	(18)40.9%	(5)41.7%	(2)50%	(7)63.6%	0.60
Age (years)	32.36(15.56)	33.08(13.61)	31.00(13.34)	25.46(14.48)	0.55
Weight	62.71(16.03)	71.76(15.77)	71.05(16.65)	60.42(14.99)	0.24
Height	157.37(12.27)	160.30(5.12)	165.63(11.59)	160.91(6.58)	0.37
Male	22(50.00)	4(33.33)	3(75.00)	7(64.00)	
CRP(mg/L)	3.98(5.54)	2.96(3.30)	25.08(22.09)	14.81(12.57)	<.0001
AGP(g/L)	0.72(0.18)	0.69(0.15)	1.12(0.21)	0.94(0.25)	<.0001
EGF	182.8(243.0)	197.0(224.2)	65.6(83.3)	143.4(223.2)	0.74
Eotaxin	598.3(430.6)	435.1(328.4)	227.1(119.8)	669.5(888.2)	0.36
G-CSF	42.5(30.9)	42.9(32.5)	28.6(1.5)	48.7(24.9)	0.72
GM-CSF	22.4(16.2)	19.6(5.3)	31.3(21.9)	27.9(9.6)	0.36
INFα2	19.0(20.2)	14.5(2.5)	15.9(3.3)	29.2(19.0)	0.24
IFNγ	67.3(188.0)	36.0(25.1)	2436.3(4752.2)	179.1(153.4)	<.001
IL-10	64.8(153.8)	36.1(51.7)	5298.3(10540.0)	1562.52(2370.99)	<.001
IL-12P40	27.7(28.2)	23.2(10.7)	20.1(4.1)	32.3(9.5)	0.73
IL12-P70	41.7(105.6)	20.6(18.8)	33.1(18.0)	19.6(4.7)	0.81
IL-13	42.4(57.2)	28.6(9.4)	22.8(4.2)	32.8(16.3)	0.69

IL-15	43.35(13.36)	35.52(7.64)	47.94(26.68)	90.14(35.16)	<.0001
IL-17α	71.7(166.9)	49.6(45.6)	61.00(38.8)	50.3(30.2)	0.94
IL-1Rα	63.1(190.1)	28.2(10.8)	276.0(490.4)	126.4(108.2)	0.11
IL-1α	35.8(27.8)	52.8(61.9)	56.5(42.7)	60.7(39.3)	0.16
IL-1β	25.6(16.2)	30.9(26.6)	29.8(11.2)	28.5(7.5)	0.79
IL-2	46.3(36.6)	46.3(29.2)	42.3(14.8)	55.7(35.6)	0.85
IL-3	14.5(5.2)	13.5(1.9)	15.0(4.9)	19.2(8.4)	0.06
IL-4	23.0(31.3)	14.5(4.0)	18.8(8.0)	18.9(6.5)	0.76
IL-5	30.3(22.9)	49.3(101.9)	23.6(8.3)	36.2(32.1)	0.62
IL-6	55.08(91.18)	32.94(22.93)	126.88(128.33)	92.52(116.95)	0.21
IL-7	24.7(12.5)	19.2(3.6)	24.3(5.7)	45.1(47.5)	0.02
IL-8	302.7(293.0)	193.6(82.1)	405.5(258.7)	479.7(236.4)	0.06
IP-10	2141.31(2500.3)	2099.4(2755.7)	12310.8(12770.6)	22555.7(4475.4)	<.0001
VEGF	46.3(50.2)	33.1(16)	46.5(45.5)	31.5(2.18)	0.35
TNFα	109.6(57.4)	133.38(133.4)	109.5(16.3)	317.42(146.6)	<.0001
TNF β	33.2(64.5)	17.0(6.7)	21.6(4.9)	22.9(9.9)	0.77
MIP-1α	106.6(187.1)	165.2(335.4)	107.2(16.1)	435.5(238.5)	0.01
MIP-1β	93.6(93.7)	104.5(125.1)	105.8(62.2)	198.8(193.5)	0.65
MCP-1	5362.0(3134.1)	7292.8(6042.2)	8523.9(1818.6)	23002.0(13633.7)	<.0001

[b]P values from ANOVA

Presented in table 4 are the results of a multivariate linear regression model used to explore relationships between the outcome, continuous serum zinc (µg/dL), with a panel of immune response biomarkers (n=71) as predictors. For this exploratory analysis, individual linear regressions were run for each predictor variable with the outcome of continuous serum zinc. All significant variables were included in a multivariate model. Initially, fifteen variables were significantly associated with serum zinc ($p<0.20$). Then one by one, the least significant covariate was removed from the multivariate model until only covariates that were significantly associated with serum zinc ($p<0.05$) remained in the final multivariate linear regression model. After adjusting for covariates age and sex, immune function markers IFN-γ ($p = 0.0050$), IL12-P70 ($p = 0.0101$), and

CXCL10 (p = 0.0409) remained in the final model. Every 100 unit increase in IFN-γ was associated with a <0.01 µg/dL reduction in serum zinc. A one-unit increase in IL-12P70 was associated with a 0.05 µg/dL higher concentration of serum zinc. Every 100 unit increase in CXCL10 was associated with a <0.001 µg/dL decrease in serum zinc**.** Every year increase in age was associated with a 0.35 µg/dL decrease in serum zinc, and females had 7.90 µg/dL less serum zinc than males.

Table 4. Multivariate Linear Regression

Variable		Beta	SE	T value	p-value
IFN-γ*		-0.39	0.13	-2.92	0.01
IL-12P70		0.05	0.02	2.64	0.01
CXCL10*		-0.04	0.02	-2.03	0.03
Age		-0.34	0.10	-3.54	<0.001
Sex					
Female	Reference				
Male		-7.75	2.87	-2.71	0.01

* Results are presented as the effect of a 100 unit increase in median fluorescence intensity (MFI).
Table 4: P-values are the result of multivariate linear regression with the outcome continuous serum zinc.

Presented in table 5 are the results of a multivariate logistic regression model used to estimate the odds of zinc deficiency using observations from participants with available data on immune function markers(n=71); odds ratios (ORs) and confidence intervals (CIs) are reported. At risk of zinc deficiency was defined as serum zinc ≤ 65 µg/dL, and at risk of zinc sufficiency was defined as serum zinc > 65 µg/dL. Variables with a strong association (p<0.05) with the outcome of zinc deficiency were retained in the final model. Statistical analysis was conducted using SAS 9.4(SAS Institute, Cary, NC).

Table 5. Multivariate Logistic Regression

Variable	OR	95% CI		Wald Chi-Square	Pr>Chi^2
CXCL10*	1.01	1.00	1.02	6.99	0.01
Age	1.06	1.01	1.10	6.78	0.01
Sex					
Females	Reference				
Males	4.24	1.35	13.4	6.08	0.01

* Results are presented as the effect of a 100 unit increase in median fluorescence intensity (MFI).
Table 4: P-values are the result of multivariate logistic regression with the outcome of zinc deficiency (serum zinc ≤ 65 µg/dL).

Individual linear regressions were run for each of the predictor variables and the binary outcome of zinc deficiency. All significant variables (p<0.20) were included in a multivariate model. Initially, eleven variables were significantly associated with serum zinc. Variables with a strong association (p<0.05) with the outcome of zinc deficiency were retained in the final model. Statistical analyses were conducted using SAS 9.4(SAS Institute, Cary, NC). After adjusting for covariates age and sex, CXCL10 was the only predictor variable that remained significant and was therefore included in the final model. When compared to females, males had a significantly lower level of serum zinc ($\beta - 7.75, p = 0.009$) and higher odds of zinc deficiency ($OR: 4.24, 95\%\ CI: 1.345: 13.360$). Regarding age, for every year increase in age, there was a significantly lower level of serum zinc ($\beta - 0.34, p = 0.0007$), with increased odds of zinc deficiency for every year increase in age ($OR: 1.06, 95\%\ CI: 1.014: 1.106$). Lastly, regarding the chemokine, CXCL10, for every 100 units increase in CXCL10, there was a significantly lower level of serum zinc ($\beta - 0.04, p = 0.0339$), with increased odds of zinc deficiency for every 100 units increase in CXCL10 ($OR: 1.01, 95\%\ CI: 1.002: 1.017$).

Discussion:

In a cross-sectional analysis, we assessed zinc status and immune response markers among participants in a febrile surveillance study. Participants had been grouped into four illness categories (Healthy control, Nonfebrile DENV, Other Febrile Illness, and Apparent DENV). Mean serum zinc concentrations significantly differed across illness categories, lowest in cases of apparent DENV infection, and other febrile illnesses. Males made up the majority of zinc-deficient participants, an interesting finding given we expected a higher prevalence of zinc deficiency amongst female participants. A potential explanation for this observation could be the higher proportion of males with cases of other febrile illness and apparent DENV infection. Decreased serum zinc concentrations in symptomatic cases of febrile illness would be expected, given the relatively high level of resulting inflammation.

In multivariate models adjusted for age and sex, elevated concentrations of CXCL10 were associated with higher odds of zinc deficiency and correlated significantly with lower serum zinc concentrations. While in another multivariate model, IFN-γ, IL12-P70, and CXCL10 were significantly associated with levels of serum zinc. The release of IFN-γ and IL12-P70 often precedes the release of CXCL10; this finding may provide insight for future interventions that aim to evaluate the relationship between serum zinc concentrations and cytokine expression throughout DENV infection**.** While IFN-γ is a common target of available commercial treatments, there is a gap in the research on the efficacy of treatments targeting its downstream effects. CXCL10 is an inflammatory chemokine whose expression is regulated by the production of IFN-γ[22] and has been associated with increased morbidity in patients with viral infections[23]. A better

understanding of the relationship between zinc and cytokines in Dengue virus infection may provide evidence of the role of zinc status in the etiology of severe Dengue outcomes. Longitudinal studies are needed to further explore the potentially modifiable relationship between zinc status and cytokines such as CXCL10, in cases of viral infection, specifically DENV infection.

The motivation for this research was to explore the relationship between DENV infection and serum zinc. At present, there is a lack of information on factors associated with better outcomes of DENV infection. While we do not yet have a clear understanding of how cytokine storms occur, patients may benefit from efforts to mitigate the effects of resulting pro-inflammatory pathways; one such method may be zinc supplementation for those found to be at increased risk of DENV infection. For this analysis, we evaluated associations between serum zinc and immune function markers to explore the relationship between serum zinc and cytokine expression. We found that cytokines IFN-γ, IL12-P70, and CXCL10 correlated strongly with serum zinc in our multivariate model of continuous serum zinc, evidence for a potential association between zinc status and expression of CXCL10 that needs to be investigated in longitudinal studies. This finding is supported by recent research on the role of nutrition in response to infection. Both CXCL10 and IFN-γ have been identified as plasma markers associated with severe dengue[16]. Another study noted that IFN-γ levels were significantly higher in severe vs. nonsevere dengue[24]. The results of cell culture studies modeling the effects of zinc on cytokine production have been mixed. One study found no significant effects of zinc on PHA-induced release of IL-1 β, IL-2, or IFN-γ[25]. Another study using cell lines derived

from zinc supplemented subjects observed higher levels of TNFα, IL-1 β, and IFN-γ compared to cells from subjects on placebo[26].

Research on associations between the severity of dengue virus infection and serum zinc is limited. Through our analysis, we found that serum zinc was lowest in cases of non-DENV febrile illness and Apparent DENV infection. Similar findings have been reported in other research on the role of zinc status in outcomes of DENV infection. One study found that children with higher grades of dengue disease had lower plasma zinc values during the first toxic phase, with nearly all participants' plasma zinc values averaging below 70 μg/dL. The same study reported that the length of hospital stay was significantly for participants with zinc values below 40 μg/dL[27].

Altering host zinc status may be an antiviral strategy depending on the timing of supplementation. The importance of zinc for proper barrier function, including junctions between epithelial and endothelial cells, is well understood. Some experiments using a cellular model of zinc deficiency have demonstrated that DENV replication may be inhibited when the virus is deprived of zinc[28]. However, while zinc is a critical component of host antiviral responses, it is also required for viral replication. This dichotomy has hindered the development of zinc supplementation strategies, particularly in the setting of DENV infection, as little is known about the optimal timing of zinc sufficiency and deprivation throughout Dengue virus infection, especially when cases have progressed to severe Dengue.

There have been few trials that have studied the effects of zinc supplementation in cases of Dengue viral infection. One randomized controlled trial found that the mean

duration of defervescence, or abatement of fever, was not affected by zinc supplementation. However, the length of hospital stay was significantly lower in patients receiving zinc supplementation, 3 times per day for 5 days[29].

This study had several limitations. Current methods to quantify zinc status include the assessment of dietary intake of zinc, determination of bioavailability through quantification of phytate consumption, and serum and plasma zinc; in some cases, stunting has been used as a proxy for estimating zinc status. However, these methods are confounded by the influence of infection, inflammation, obesity, age, and sex.

As this was a cross-sectional study design, the assessment of DENV infection, serum zinc concentrations, and immune function markers from samples taken at a single time point, which prevents any interpretation of causation between serum zinc and immune function. Furthermore, the subset of participants available for serum zinc quantification may not be representative of the population with limited observations for children under 10 years of age and adults over 50 years of age. The heterogeneity of this study population may have hindered the ability to detect relationships between our predictor variables with the outcome of serum zinc. Furthermore, serum zinc assessment was limited to one point in time, with limited information on dietary intake and time since the last meal. It has been well established that serum zinc is not a robust indicator of individual zinc status as it is altered by many factors including infection, inflammation, and time since the last meal and is not a reliable indicator of individual zinc status[30]. However, at present, serum zinc and dietary recall are the primary methods for estimating the burden of zinc deficiency. Thus there is a need for more reliable

indicators of individual zinc status for future studies to make more accurate estimates of the relationship between zinc and the immune response.

Conclusion:

Zinc is a known immunomodulator, yet there is limited literature on zinc deficiency or its role in the context of most arboviral infections, including Dengue virus infection (DENV). Through multivariate methods, we found that concentrations of CXCL10 were significantly associated with greater odds of zinc deficiency. This finding supports current evidence that zinc is associated with the regulation and production of pro-inflammatory cytokines, and should be further explored in future longitudinal studies. Identifying protective factors that may modify the risk of severe DENV infection outcomes may lead to decreased incidence of dengue hemorrhagic fever and therefore reduce the burden of DENV on populations with the potential to benefit from nutrition interventions.

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