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Association between transferrin receptor and urine cobalt: NHANES 2015-2020

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Abstract. The transferrin receptor protein 1 (TfR1) is a critical cellular membrane protein. It orchestrates the internal transport and metabolism of iron, a vital process for intracellular functions. Additionally, TfR1 has the capacity to bind with metals beyond iron, including cobalt and manganese; however, the specific mechanisms of this binding and subsequent interactions with other proteins remain elusive. This study delves into the potential correlation between TfR1 levels in humans and the presence of cobalt in urine. Drawing on data from 3,424 participants across three survey cycles spanning two years each, this paper meticulously contrasts the clinical features of individuals across diverse TfR1 and urinary cobalt levels. Through the lens of both univariate and multivariate linear regression analyses, the research uncovers an inverse connection between TfR1 concentrations and urinary cobalt, post exclusion of participants with incomplete biochemical profiles. This inverse relationship could shed light on the complex interplay between TfR1 and metal ions within the body, providing a deeper understanding that may inform future studies and potential clinical applications. In the multivariate linear regression analysis, it is found that with the increasing of TFR1 quartiles, the proportion of obese participants ($P<0.01$), high level blood manganese, high cobalt level in urine and blood ($P<0.01$) increased gradually. This association remained significant in sensitivity analysis, while in the stratified analysis, above association was not significant in men with samples aged 65 and over. Transferrin receptor protein levels might be positively associated with urinary cobalt in the general U.S. crowd.

Keywords: Transferrin receptor protein (TfR1), Cobalt, The National Health and Nutrition Examination Survey (NHANES).

1. Introduction

In people's blood, most of the iron is carried by a protein called transferrin. This protein is especially important for the development of red blood cells in the bone marrow, as these cells heavily rely on iron that is attached to transferrin. They grab onto this iron using a special structure on their surface known as transferrin receptor 1 (TfR1), which red blood cells have in abundance [1]. The transferrin, with its iron cargo, binds to TfR1. TfR1 is quite the specialist; it is a protein built for the job, being a 97-kDa homodimer (a structure made of two identical subunits) that is particularly good at holding onto transferrin carrying two iron atoms [2]. After the iron is released inside the cell, it is transformed into a form that the cell can use, but TfR1 is not done yet, though; it is recycled back to the cell surface to fetch more iron [3]. An interesting point to note is the different forms iron takes inside versus outside of cells.

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Inside cells, iron is usually in the Fe^{2+} form, while outside, it is in the Fe^{3+} form [4]. This difference is likely to play a role in keeping the cell's internal environment stable and suitable for its many biochemical processes, reflecting the cell's ability to maintain a more reduced, or electron-rich, environment compared to the outside of the cell [5].

Cobalt, although only needed in tiny amounts, plays a crucial role in the human body's health and functionality [6]. One of its key jobs is to help stabilize proteins called hypoxia-inducible transcription factors [7]. When these factors are stable, people's bodies can produce more erythropoietin, a hormone that signals the production of red blood cells [8]. This process is essential for maintaining healthy oxygen levels in people's tissues and organs, showcasing cobalt's vital part in keeping people healthy and well-oxygenated.

For a metal to follow the iron acquisition pathway, many different conditions are required [9]. Among these metals, cobalt can be translocated by transferrin receptors, competing with iron [10]. The study compares the clinical attributes of subjects across varying groups categorized by transferrin receptor protein levels and urinary cobalt concentrations. Both univariate and multivariate linear regression analyses were utilized to assess the relationship between transferrin receptor protein levels and urinary cobalt concentrations. The specificity of the correlation between cobalt and transferrin receptor content is confirmed by analyzing the correlation between cobalt and manganese content in urine, blood, and transferrin receptor content.

This paper aims that the findings could enable the use of urinary cobalt levels as a diagnostic tool for anemia, neurodegenerative conditions, and other ailments associated with human transferrin receptor levels. The project anticipates that this data may offer valuable insights into the therapeutic strategies of these disorders.

2. Methods

2.1. Data source and study population

The Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) is a pivotal biennial assessment of America's health and nutrition trends. This systematic survey encompasses comprehensive interviews at participants' homes, followed by thorough health evaluations in mobile examination units. These units are fitted with the requisite medical apparatus to facilitate physical and laboratory examinations by proficient medical staff. Rigorous protocols for participant consent and data anonymity are strictly adhered to in accordance with ethical standards set by the National Center for Health Statistics.

For the current study, the dataset was curated from three NHANES cycles spanning 2015–2016, 2016–2017, and up to March 2020. The cohort was carefully filtered to omit any individuals with incomplete blood and urine analyses or unavailable TFR1 data, culminating in a robust sample of 3,424 subjects for the analysis (see Figure 1).

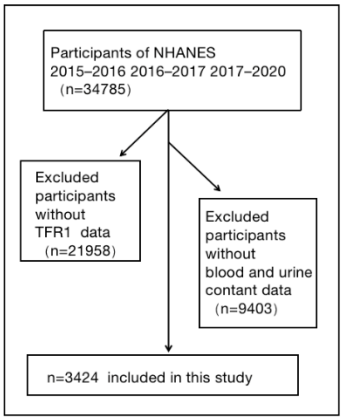

Figure 1. Flow chart of the screening process for the selection of eligible participants.

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2.2. Variables

The method principle for measurement of soluble transferrin receptor (sTfR) is a particle-enhanced immunoturbidimetric assay that uses Roche kits on the Cobas® c501 clinical analyzer. The lower limit of detection for the transferrin receptor is 0.500 mg/L. This method directly measures the cobalt level in urine specimens using mass spectrometry after a simple dilution sample preparation step. The lower limit of detection for urinary cobalt is 0.023 (ug/L). Analytes with analytic results below the lower limit of detection are divided by the square root of 2.

2.3. Covariates

Potential confounding factors in this study include age and BMI (normal: below 25 kg/m2; overweight: between 25 and 30 kg/m2; obesity: ≥30 kg/m2). The invasion of the hepatitis C virus into the human body is assisted by the transferrin receptor, taking as a covariate whether or not the subject has ever had hepatitis C. The transferrin receptor is closely related to blood erythrocytes, so this project took the patient's erythrocyte count and hemoglobin content. Previous studies have demonstrated that manganese is transported by transferrin receptors, so the project selected both urine and blood manganese levels as covariates with blood cobalt levels.

2.4. Statistical analysis

All statistical analyses are calculated using R (version 4.2.2). The normality of continuous variables is tested with the Kolmogorov-Smirnov normality test. Normally distributed variables are described as the mean ± standard deviation, and nonnormally distributed continuous variables are described as the median (interquartile range). The median values among different transferrin receptor and urinary cobalt groups were compared with the Mann-Whitney U test and Kruskal. Wallis test. The chi-square test was adopted to compare the percentages of categorical variables among different transferrin receptor and urinary cobalt groups. The Bonferroni test is used for the intergroup comparison. This project evaluates the correlation between each independent variable and urinary cobalt by using univariate and multivariate linear regression models. Because the distribution of the transferrin receptor is skewed right, it is log-transformed in the regression analysis. With the transferrin receptor as the dependent variable, regression coefficients, standard errors, and P values are determined.

In addition, stratified analyses by age (group 1: 18≤age<45, group 2: 45≤age<65, group 3: age≥65), BMI (normal, overweight, and obesity), history of Hepatitis C are performed to examine the association between urinary cobalt and log-transformed transferrin receptor. Urinary cobalt distribution is categorized into normal and excessive groups based on 0.1 (1ug/l). This project analyzes the multicollinearity of the multivariate linear regression. A two-sided $P<0.05$ is considered statistically significant.

3. Results

A total of 3424 participants are included in this study. The mean age is 38.57, the mean transferrin receptor level is 42.18 nmol/L, and the mean blood cobalt level is 0.68 ug/L. As seen in Table 1, transferrin receptor levels are categorized based on transferrin receptor quartiles: Q1 (TFR1≤29.9 nmol/L), Q2 (29.9<TFR1≤36.3 nmol/L), Q3 (36.3<TFR1≤45.0 nmol/L), and Q4 (TFR1>45.0 nmol/L). A Bonferroni test is adopted for intergroup comparison, and this project finds that the proportion of obese participants ($P<0.01$), the level of blood manganese, and the cobalt level in urine and blood ($P<0.01$) increase gradually with the increasing of TFR1 quartiles. It is interesting that the levels of cobalt and manganese in urine and blood are different in relation to the transferrin receptor composition, even though both of them have been shown to be transported by the transferrin receptor.

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Table 1. Clinical characteristics of the study population disaggregated by quartiles of the transferrin receptor level.

	Transferrin Receptor Level (by quartile)					
	Q1	**Q2**	**Q3**	**Q4**	**p**	**test**
n	866	857	852	849		
age (median [IQR])	37.00 [25.00, 51.00]	35.00 [20.00, 53.00]	37.00 [21.00, 55.25]	36.00 [19.00, 48.00]	0.028	nonnorm
BMI (mean (SD))	3.00 (0.00)	3.05 (0.23)	3.08 (0.79)	3.06 (0.24)	0.909	
URDUPBLC (median [IQR])	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.423	nonnorm
blood manganese (median [IQR])	167.09 [137.24, 204.68]	174.19 [140.88, 216.42]	180.75 [146.16, 226.80]	210.96 [168.00, 275.21]	<0.001	nonnorm
urine volume(median [IQR])	79.00 [44.00, 133.75]	84.00 [47.00, 148.00]	75.00 [43.00, 135.00]	81.00 [47.00, 139.00]	0.013	nonnorm
Transferrin receptor content(mean (SD))	2.19 (0.25)	2.81 (0.16)	3.40 (0.21)	5.94 (3.61)	<0.001	
Manganese in urine(mean (SD))	0.18 (0.60)	0.15 (0.21)	0.17 (0.28)	0.19 (0.60)	0.324	
Lead in urine(mean (SD))	0.43 (0.62)	0.41 (1.01)	0.40 (0.50)	0.40 (0.43)	0.715	
Cobalt in urine (mean (SD))	0.49 (0.63)	0.60 (1.34)	0.61 (0.69)	1.02 (1.50)	<0.001	
Red blood cell content(median [IQR])	4.58 [4.30, 4.84]	4.66 [4.38, 4.96]	4.66 [4.39, 4.98]	4.70 [4.36, 4.96]	<0.001	nonnorm
hemoglobin content(median [IQR])	13.80 [13.10, 14.70]	13.70 [13.10, 14.80]	13.60 [12.80, 14.50]	12.80 [11.50, 13.70]	<0.001	nonnorm
BMI_category (%)					<0.001	
<25	358 (41.3)	328 (38.3)	275 (32.4)	252 (29.7)		
25-30	243 (28.1)	243 (28.4)	232 (27.3)	211 (24.9)		
>=30	265 (30.6)	286 (33.4)	343 (40.4)	386 (45.5)		
HCV history = Yes (%)	8 (0.9)	11 (1.3)	3 (0.4)	10 (1.2)	0.188	
UA_quantile (%)					<0.001	
Q1	866 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Q2	0 (0.0)	857 (100.0)	0 (0.0)	0 (0.0)		
Q3	0 (0.0)	0 (0.0)	852 (100.0)	0 (0.0)		
Q4	0 (0.0)	0 (0.0)	0 (0.0)	849 (100.0)		
age_cat (%)					0.013	
<45	564 (65.1)	541 (63.1)	532 (62.4)	579 (68.2)		
45-65	213 (24.6)	206 (24.0)	190 (22.3)	175 (20.6)		
>=65	89 (10.3)	110 (12.8)	130 (15.3)	95 (11.2)		

The clinical characteristics of participants with different urinary cobalt levels are shown in Table 2. It is found that there is a strong linear relationship between the transferrin receptor and urinary cobalt ($P<0.001$), and a higher level of urinary cobalt is associated with a higher hemoglobin and red cell level in the blood ($P<0.01$), which is closely associated with the presence of transferrin receptors. Besides,

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there is a strong correlation between urine and blood cobalt levels (P<0.001). Samples with low blood cobalt levels are older (38 versus 29 years old) (P<0.01), and there is no strong linear relationship between obesity and urinary cobalt levels.

Table 2. The clinical characteristics of participants with different urinary cobalt levels.

Cobalt Levels (high/normal)				
	high	**normal**	**p**	**test**
n	589	2835		
age (median [IQR])	29.00 [18.00, 43.00]	38.00 [22.50, 55.00]	<0.001	nonnorm
BMI (mean (SD))	3.08 (0.28)	3.04 (0.35)	0.734	
BMXHT (median [IQR])	162.80 [157.50, 168.50]	163.40 [157.55, 170.20]	0.048	nonnorm
Manganese in blood (median [IQR])	216.97 [169.46, 279.22]	175.83 [141.43, 219.88]	<0.001	nonnorm
cobalt in blood (median [IQR])	6.62 [4.24, 9.41]	2.55 [2.21, 3.05]	<0.001	nonnorm
urine volume (median [IQR])	73.00 [45.00, 116.00]	82.00 [45.00, 142.00]	0.001	nonnorm
Transferrin receptor content(mean (SD))	5.00 (4.08)	3.28 (1.55)	<0.001	
Manganese in urine (mean (SD))	0.21 (0.30)	0.16 (0.49)	0.023	
URXUPB (mean (SD))	0.66 (1.29)	0.36 (0.44)	<0.001	
cobalt in urine(mean (SD))	1.95 (2.27)	0.41 (0.24)	<0.001	

Then this project makes more exploration of the differences between manganese and cobalt. Clinical characteristics of the study population in different urinary manganese level groups (0.1ug/l) and blood manganese level groups (200nmol/L) are shown in Table 3. Interestingly, the project finds that there is no significant correlation between the urinary manganese level and the red blood cell and hemoglobin level (P<0.01), and there is a positive correlation between blood manganese levels and hemoglobin levels (P<0.01). However, there is a linear relationship between manganese levels in urine and blood.

Table 3. Clinical characteristics of the study population in different urinary manganese level groups (0.1ug/l) and blood manganese level groups (200nmol/L).

Urinary Manganese Level Groups (0.1ug/l)				
	high	**normal**	**p**	**test**
n	1176	2248		
age(median [IQR])	35[19.00,52.00]	37[22.75,52.00]	0.057	nonnorm
Transferrin receptor content(mean (SD)	43.64(28.83)	41.42(26.13)	0.012	
Manganese in blood (median [IQR])	186.02[148.53,238.72]	179.65[144.66,227.53]	0.023	nonnorm
cobalt in urine(mean (SD))	0.93(1.25)	0.55(1.03)	P<0.001	
hemoglobin content(median [IQR])	13.50[12.80,14.50]	13.50[12.70,14.50]	0.51	nonnorm
Red blood cell content(median [IQR])	4.66[4.38,4.94]	4.62[4.34,4.94]	0.115	nornorm
blood manganese level groups (200nmol/L)				
	high	**normal**	**p**	**test**
n	1176	2248		
age(median [IQR])	34.00[20.00,46.00]	37.50[22.00,58.00]	P<0.001	nonnorm
Transferrin receptor content(mean (SD)	50.77(38.63)	36.84(13.75)	P<0.001	

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Table 3. (continued)

cobalt in urine(mean (SD))	0.83(1.21)	0.59(1.06)	P<0.001	
hemoglobin content(median [IQR])	13.30[12.40,14.30]	13.70[12.90,14.60]	P<0.001	nonnorm
Red blood cell content(median [IQR])	4.65[4.38,4.92]	4.63[4.34,4.95]	0.411	nornorm

Back to the linear relationship between transferrin receptors and urinary cobalt levels, this project predicts the log-transformed transferrin receptor level through univariable and multivariate linear regression analysis (see Table 4). Both in univariable and multivariate linear regression, it is found that the increasing age (P<0.01) is associated with the declining transferrin receptor level while the manganese level in blood (P<0.01), the cobalt level in urine and blood (P<0.01), and the red blood cell and hemoglobin level (P<0.01) are associated with increasing transferrin receptor levels.

Table 4. Univariable and multivariate linear regression analysis predicting log-transformed transferrin receptor level.

Variable	**Univariable Linear Regression Analysis**				**Multivariable Linear Regression Analysis**			
	R2	B	SE	P value		B	SE	value
Manganese level in urine	-0.0003	-0.0036	0.0145	0.802		-0.0207	0.0107	0.0535
lead level in urine	-0.0001	-0.007	0.0098	0.475		0.0063	0.0074	0.3966
Arsenic level in urine	0.0001	0.0001	0.0001	0.286		0.0002	0.0001	0.0049
Cobalt level in urine	0.0419	0.0712	0.0058	<0.001		0.0315	0.0045	<0.001
hemoglobin level	0.0024	0.044	0.0145	0.0024	Adj.R2	-0.1746	0.0045	<0.001
Red blood cell count	0.0441	0.044	0.01447	0.0023	0.4492	0.3807	0.0139	<0.001
Urine volume	0.0003	0.0001	0.0001	0.15		0.0001	0.0001	0.0779
Manganese level in blood	0.1744	0.0021	0.0001	<0.001		0.0011	0.0001	<0.001
HCV	-0.0003	-0.0107	0.0694	0.878		0.0214	0.0514	0.6771
BMXBMI	0.0124	0.0057	0.0008	<0.001		0.0019	0.0007	0.0054
RIDAGEYR	0.0015	-0.0008	0.0003	0.0128		0.0018	0.0003	<0.001

Revisiting the analysis on the correlation between transferrin receptors and urinary cobalt concentrations, the study applies both univariate and multivariate linear regression models to forecast the levels of the log-transformed transferrin receptor. As shown in Table 5, the findings consistently indicate that an increase in age is linked to a decrease in transferrin receptor levels (P<0.01), while higher concentrations of manganese in the blood (P<0.01), as well as elevated cobalt levels in both urine and blood (P<0.01), along with increased red blood cell and hemoglobin levels (P<0.01), are associated with an upsurge in transferrin receptor levels.

In detailed subgroup analyses tailored for multivariate linear regression, specifically predicting the log-transformed levels of transferrin receptor, the study observes that the connection between transferrin receptor levels and cobalt concentrations remains statistically significant across different BMI categories and histories of Hepatitis C Virus (HCV) infection. However, this association does not hold statistical significance in the subgroup of participants aged 65 and above, indicating potential age-related disparities in the biochemical interactions between transferrin receptors and cobalt levels.

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Table 5. Stratified analyses by selected characteristic, for multivariate linear regression analysis predicting the log-transformed transferrin receptor level.

Variable		Multivariable Linear Regression Analysis		
		B(Cobalt level)	**SE**	**p value**
Age (years)				
	18	0.2356	0.0115	<0.001
	45	0.0435	0.011	0.0001
	Age>65	0.0012	0.0067	0.8564
BMI(kg/m2)				
	BMI<25	0.2189	0.0169	<0.001
	25	0.0354	0.0074	0.0001
	BMI>30	0.0879	0.0106	<0.001
Suffered HCV				
	Yes	0.0338	0.0138	0.0213
	No	0.0782	0.0063	<0.001

4. Conclusion

This investigation synthesizes the findings from three biennial survey cycles spanning from 2015 to 2020, involving 3,424 participants, to explore the dynamics of transferrin receptor (TfR) levels in relation to urinary cobalt concentration. Utilizing both univariate and multivariate linear regression analyses, the study identifies a positive correlation between TfR levels and urinary cobalt. However, when dissecting the data further, this association does not hold for participants aged 65 and above. Additionally, the research underscores a strong linkage between urinary cobalt levels and hemoglobin and red blood cell counts, indicators that are closely tied to TfR presence. In contrast, while no significant relationship is found between urinary manganese levels and hemoglobin or red blood cell counts, an affirmative correlation emerges between blood manganese levels and hemoglobin, offering insights into the nuanced interplay of metal ions and biomarkers within the human body. Transferrin receptors have received much attention in popular fields such as Ferroptosis and iron metabolism. However, the relationship between the processes of iron metabolism and the total body substance metabolism is still unclear. Through the project, this correlation relationship can be indicative of human transferrin receptor levels in terms of urine biopsy data. It also indicates directions for the relationship between iron metabolism and human substance metabolism: the bioactive role of metallic elements.

The work also raises the question of why cobalt levels in urine correlate with transferrin receptor levels, while manganese, for example, does not. This may possibly related to the competition between cobalt and iron, resulting in spurious increasing transferrin receptor levels. Metal elements interact with the transferrin receptor through different mechanisms.

However, based on a cross-sectional design, this study can only explore the association between urine cobalt levels and transferrin receptor levels, and it cannot verify causality. The association the project investigated in this study is biologically plausible, and further large-scale prospective studies are required to confirm the causal relationship. There are some questions to be solved in the future, for instance, why there is a strong correlation between urinary cobalt levels and transferrin receptor levels. Further exploration of the causes of the differences in different metallic elements and mechanism studies are needed.

In sum, this research underscores a positive link between transferrin receptor levels and urinary cobalt concentrations within the U.S. populace. This finding holds promise for leveraging urinary cobalt as a biomarker for diagnosing conditions such as anemia, neurodegenerative diseases, or other disorders associated with transferrin receptor fluctuations. Ultimately, it is hoped that these insights can inform and enhance therapeutic strategies for these ailments.

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