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Bilirubin concentration is positively associated with haemoglobin concentration and inversely associated with albumin to creatinine ratio among Indigenous Australians: eGFR Study

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Abstract

Low serum bilirubin concentrations are reported to be strongly associated with cardio-metabolic disease, but this relationship has not been reported among Indigenous Australian people who are known to be at high risk for diabetes and chronic kidney disease (CKD).

Hypothesis: serum bilirubin will be negatively associated with markers of chronic disease, including CKD and anaemia among Indigenous Australians.

Method: A cross-sectional analysis of 594 adult Aboriginal and Torres Strait Islander (TSI) people in good health or with diabetes and markers of CKD. Measures included urine albumin: creatinine ratio (ACR), estimated glomerular filtration rate (eGFR), haemoglobin (Hb) and glycated haemoglobin (HbA1c). Diabetes was defined by medical history, medications or HbA1c≥6.5% or ≥48mmol/mol. Anaemia was defined as Hb<130 g/L or <120 g/L in males and females respectively. A multivariate regression analysis examining factors independently associated with log-bilirubin was performed.

Results: Participants mean (SD) age was 45.1 (14.5) years, and included 62.5% females, 71.7% Aboriginal, 41.1% with diabetes, 16.7% with anaemia, 41% with ACR>3mg/mmol and 18.2% with eGFR<60ml/min/1.73m^2. Median bilirubin concentration was lower in females than males (6 v 8 µmol/L, p<0.001) and in Aboriginal than TSI participants (6 v 9.5 µmol/L, p<0.001). Six factors explained 35% of the variance of log-bilirubin; Hb and cholesterol (both positively related) and ACR, triglycerides, Aboriginal ethnicity and female gender (all inversely related).

Conclusion: Serum bilirubin concentrations were positively associated with Hb and total cholesterol, and inversely associated with ACR. Further research to determine reasons explaining lower bilirubin concentrations among Aboriginal compared with TSI participants are needed.

Introduction

Indigenous Australians have a higher burden of chronic non-communicable conditions, including anaemia, chronic kidney disease (CKD), diabetes and cardiovascular (CV) disease than other Australians [1]. Nationally reported statistics cannot adequately represent the variability within this population, including differences between the major indigenous ethnic groups (the Aboriginal and Torres Strait Islander peoples such as body composition or dyslipidaemia profile[2, 3]) or the geographical diversity with illness burden [4]. End stage chronic kidney disease is one chronic condition which disproportionately affects Indigenous Australian people than observed among non-Indigenous counterparts, occurring at a much younger age and for whom Indigenous females have almost twice the risk as Indigenous males [5]. It is recognised that additional mechanisms and markers of chronic disease risk are needed to explain the CV disease burden and risks within Indigenous Australian communities [6, 7].

We recently reported an inverse association of total serum bilirubin (inclusive of the pathological range >20 µmol/L) with albumin to creatinine ratio (ACR) among participants of the eGFR Study [8], an adult Indigenous population living in diverse regions of Northern and Central Australia, stratified for good health, diabetes and CKD. Serum bilirubin is the end product of haem degradation, has anti-oxidant properties, and in low concentration is associated with chronic cardio-metabolic disease risk [9]. Although our finding of an inverse association of ACR and bilirubin was consistent with reports of a cohort of adults with diabetes from Asia [10], it was in contrast to findings of a United States population-based health survey which reported a positive association between albuminuria and bilirubin [11].

The prevalence and severity of anaemia within populations is an important health indicator [12]. Anaemia is a condition frequently reported among sub-populations of Indigenous Australians [13] and is associated with diabetes and chronic kidney disease [14]. Factors contributing to anaemia thus include poor quality diet, micronutrient absorption, the impact of anaemia related to co-morbid chronic conditions and red cell destruction. We hypothesised that serum bilirubin will be

negatively associated with markers of cardio-metabolic disease in Indigenous Australians, a population known to have high risk for anaemia, diabetes, premature cardiovascular disease, and chronic kidney disease.

Methods

Participants

Participants were self-identifying adult Aboriginal and Torres Strait Islander (TSI) Australians from the baseline eGFR Study [15], recruited from more than 20 sites in urban, regional and remote areas known to have a high background prevalence of dialysis-dependent end stage kidney (ESKD) disease. All who expressed an interest to participate were able to be stratified for inclusion, including those with diabetes or CKD. We excluded pregnant or breast feeding women, or participants with rapidly changing kidney function. Participants provided written voluntary and informed consent for the eGFR Study, which received ethics approval and community support in each locality. Ethics approval was provided by the Northern Territory Department of Health and Families and Menzies School of Health Research Human Research Ethics Committee, including the Indigenous ethics subcommittee, which has the power of veto over studies involving Indigenous Australian peoples. Ethics approval for participants was provided in the other regions by the Cairns and Hinterland Health Services District Human Research Ethics Committee; Central Australian Human Research Ethics Committee; Western Australian Aboriginal Health Information and Ethics Committee; Royal Perth Hospital Ethics Committee.

Measures

Detailed descriptions of the methods have been previously described. In brief, diabetes status was confirmed by the medical record or glycated haemoglobin (HbA1c) defined as ≥6.5% or ≥48mmol/mol [16]. Current smoking status was self-reported and categorically described. The remoteness index score (ARIA) >10.53 indicated a community/town in very remote Australia [17]. Medication prescription was recorded from participants and confirmed by the medical record. Measurements of resting blood pressure, height, weight, waist and hip

circumferences were collected [18]; waist to hip ratio (WHR) and body mass index (BMI) were calculated. Laboratories local to each recruitment site measured (non-fasting) serum high density lipoprotein (HDL)-cholesterol, C-reactive protein (CRP), HbA1c, urine albumin, urine creatinine and serum creatinine was measured using an isotope dilution mass spectrometry traceable standardised assay. During the period of this study, all Australasian laboratories achieved a coefficient of variation <15% for urine albumin (including 92% of participating laboratories achieving <10% . These methods support less variation, and thus agreement of results across different platforms in urine albumin and creatinine (serum and urine) results. Cystatin C was measured in a central laboratory as previously described [15, 19]. Estimated GFR (eGFR) was calculated by the chronic kidney disease epidemiology collaboration formula, without the African American correction factor [20]. Albuminuria was defined as ACR ≥3.0 mg/mmol [21]. Anaemia was defined as Hb<130 g/L in males and <120 g/L in females [12].

Liver function tests including total serum bilirubin and haemoglobin were also performed in local laboratories. Twelve laboratories used five individual methods for bilirubin across the recruitment sites (Ortho Clinical Diagnostics Fusion 5.1; Roche Cobas Integra 800; Roche Cobas Integra 400; Beckman Unicel DXc600; Ortho Clinical Diagnostics Vitros 250). Detailed methods at each laboratory for these measures including total serum bilirubin have been previously described [15]. The total bilirubin method principle is diazo method having result traceability to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 916 across all the platforms, which minimises the variation of results between laboratories. Standardisation of haemoglobin measurements are important [22], with methods standardised in Australia which minimises the variation of results between laboratories. In this study, haemoglobin was analysed on the Beckman-Coulter analyser (HmX, MaxM, LH75, LH500), and Sysmex XE500, Abbott, Cell Dyne Saphire analysers.

In this analysis, elevated serum total bilirubin was defined as >20µmol/L [23], and may represent concurrent liver disease. In this analysis, suspected liver disease

was categorically scored based on criteria described by Lee et al. [24] who defined liver disease as at least one of the following: alcohol excess, low serum albumin (<35 g/L), serum alanine transferase (ALT) twice the upper normal reference range (normal range: <44 mmol/L), serum total bilirubin twice the upper normal reference range (normal range <20 µmol/L). Alcohol use was categorically recorded in the eGFR Study as follows: first, participants were asked about the frequency of alcohol consumed (daily, weekly, monthly and never), and second, on quantity of alcohol per reported frequency (1-7, 8-19, >20 units). In this analysis alcohol excess was described if participants recorded daily alcohol consumption (no alcohol free-days)[25] or if weekly alcohol users consumed more than 20 units.

This analysis describes 594 participants who had both a serum bilirubin and haemoglobin test result of the 653 participants enrolled at baseline, accounting for the following exclusions: age <18 years (n=13); missing data for haemoglobin (n=40), bilirubin (n=6) (Supplementary Figure S1). Of the 600 participants with a Hb result, the first 29/600 participants only had a Hb result requested, whilst subsequent participants also had a mean corpuscle volume (MCV) available (n=568). Using our crude criteria, 86/600 (14.3%) participants were identified with suspected liver disease as follows: daily alcohol consumption, high weekly alcohol consumers, serum ALT more than two times the upper limit of normal of 44 u/L, serum total bilirubin more than two times the upper normal limit of 20 µmol/L, and serum albumin less than 35g/L (n=16, 33, 10, 2 and 25 respectively).

Statistical Analyses

Descriptive characteristics of participants were reported by Indigenous status (Aboriginal or TSI) and, within each indigenous group, across tertiles of bilirubin. Categorical variables were described with a percentage and continuous variables, when normally distributed, with mean and standard deviation (SD). Continuous variables with a skewed distribution were described with median and interquartile range (IQR). Serum bilirubin was also log-transformed to achieve a normal distribution in values. In order to minimise the impact of multiple comparisons we

presented data for males and females separately with p values for trend in difference across tertiles of bilirubin by chi-squared test for categorical variables, (using heterogeneity test for continuous normally distributed variables and Kruskill Wallis test for continuous non-normally distributed variables. Associations between participants' characteristics and the outcome log-bilirubin were assessed, by first exploring the association of each independent factor with log-bilirubin. Subsequently we built multivariable models that included all the potentially significant factors ($p<0.1$ on linear regression models) and assessed the extent to which each model was effective at explaining the variability of the log-bilirubin by reporting the coefficient of determination (R^2). Sensitivity analyses exploring the impact of suspected liver disease was also explored. Statistical analyses were performed using Stata v14 (Stata Corporation, College Station, TX).

Results

Diabetes, albuminuria (without significantly impaired eGFR) and hypertension were prominent findings observed among the 594 participants (Table 1). Aboriginal participants, who comprised 71% of the study cohort, had a higher frequency of anaemia, albuminuria and GFR<60 ml/min/1.73m^2 than TSI participants.

Bilirubin

Serum total bilirubin had a non-normal distribution (skewed to the right), with a median (IQR); range) concentration of 6 (2, 29; range 2, 61) µmol/L. Aboriginal participants had lower bilirubin concentrations than TSI participants (median (IQR): 6 (4, 8) µmol/L v 9.5 (7, 12) µmol/L, p=0.001). Bilirubin concentrations were also lower in female than male participants (median (IQR): 6 (4, 8) µmol/L v 8 (6, 11) µmol/L, p<0.001). Logarithmic transformation of total serum bilirubin produced a normal distribution in values (Figure 1), with one unit change of log-bilirubin in our data representing a serum total bilirubin range of 7-20 µmol/L.

Anaemia

Male participants, compared to females, had a higher mean (SD) Hb concentration (147 (15.7) g/L v 129.5 (15.6) g/L, p<0.001), and higher mean corpuscle volume (MCV) [86.5 (5.6) v 84.5 (6.6) fl, p=0.001]. Low MCV (<80 fl) was observed in 17.2% of participants. When anaemia was observed (in 100/600 participants), this was more frequently observed in females than males (17.9% v 12%, p=0.02), and was associated with a normal MCV (80-100 fl) in 58% and low MCV in 37% participants respectively. The frequency of anaemia was almost twice as high in participants with diabetes than without diabetes (23.2% v 11.9%, p<0.001) and participants with lower eGFR [healthy with eGFR>90 ml/min/1.73m^2 v Diabetes with eGFR≥90 ml/min/1.73m^2 v GFR 60-89 ml/min/1.73m^2 v eGFR 30-59 ml/min/1.73m^2 v eGFR<30 ml/min/1.73m^2: 6.3 v 9.4 v 12.6 v 38.7 v 66.6%, p<0.001] respectively.

We examined the characteristics of males and females separately by tertile of bilirubin (Table 2, 3). Characteristics of males and females within tertile-1 included a higher WHR, ACR, triglycerides, GGT and ALP (features of the metabolic syndrome) and lower Hb, lower MCV and lower serum albumin. There was a positive gradient of total cholesterol with tertile of bilirubin in both males and females. Some differences in characteristics were noted between Aboriginal and TSI participants, and these are presented separately in each gender by tertile of bilirubin in supplementary Table S1 and S2.

Regression modelling

In unadjusted linear regression modelling among all participants, log-bilirubin was significantly and positively associated with: total cholesterol, Hb, MCV, ALT, cystatin C, triglycerides, remoteness index, and inversely associated with: ALP, albumin, ACE inhibitor-ARB use, statin use, urea, creatinine, ACR, diabetes, triglycerides, microscopic haemoglobinuria, WHR, total protein and aspirin use. In the final multivariate regression model (Table 4), serum log-bilirubin was positively associated with Hb and total cholesterol and inversely associated with ACR and triglycerides, even after excluding participants with suspected liver disease (Figure 2). Furthermore, of the variables inversely associated with log-bilirubin, Aboriginal

ethnicity and serum triglycerides explained a higher proportion of the variance than ACR (12.6%, 2.3%, 1.1%). Conversely, haemoglobin concentration and total cholesterol were each positively associated with log-bilirubin (each explaining 4.5% and 1.2%) of the explained variance of log-bilirubin.

Discussion

We report four key findings in this analysis of associations between bilirubin and the chronic conditions of diabetes, CKD and anaemia, in a cohort of 594 Indigenous Australians recruited from more than twenty sites. First serum log-bilirubin was positively associated with haemoglobin concentration, and anaemia was also commonly observed in this cohort. Second, log-bilirubin concentrations was positively associated with total cholesterol and inversely associated with triglycerides. Third, the association between serum log-bilirubin and markers of chronic kidney disease was only observed for ACR, and not associated with other measures of kidney function that we assessed. Fourth, Aboriginal ethnicity was inversely associated with log-bilirubin, and explained the largest variance in the multivariate model, but the reasons for the lower bilirubin concentration than TSI participants remain unclear. Low serum bilirubin concentration is a reported marker of oxidative stress and CV disease risk, and anaemia an important health indicator across populations [12]. Our findings demonstrate a strong association of bilirubin with heightened chronic disease risk in participants (particularly Aboriginal participants), though the causal link between bilirubin concentration and cardiovascular disease and other conditions is presently unknown in this population.

Bilirubin is proportional to haemoglobin concentration

Serum bilirubin was positively associated with haemoglobin concentration among participants, explaining 4.5% of the total variance in the multivariate regression model, and anaemia was also commonly observed in this cohort. Anaemia was more frequently observed in participants with diabetes (in 23%) and lower eGFR (66% of adults with eGFR<30 ml/min/1.73m^2), which is a relationship that is consistent with other studies [14]. In the present analysis, the normal MCV result

was suggestive of either anaemia of chronic disease, whilst the low MCV result was consistent with iron deficiency or potentially a thalassaemia trait [26]. It was not possible to further examine this without iron studies, dietary information, Hb electrophoresis or examine for intestinal parasites including strongyloidiasis [27], which is a prevalent condition associated with iron deficiency anaemia in this population, without information on the presence or absence of eosinophilia. Lack of access to daily fruit and vegetables is an important modifiable contributor to cardiovascular disease in many populations [28], and in adults with CKD, uncorrected anaemia is strongly linked with the development of cardiovascular disease outcomes [29]. Our findings highlight the association between anaemia and adverse health among a cohort of Aboriginal and Torres Strait Islander adults recruited across a range of good health, diabetes and chronic kidney disease.

Bilirubin & chronic disease risk associations

Dyslipidaemia (including hyper-triglyceridaemia, low HDL cholesterol and high non-HDL cholesterol) is an important cardiovascular disease risk marker [2, 9, 30] documented in both developed and developing countries internationally [28]. Serum bilirubin levels have been associated with cardiovascular risk; hyper-bilirubinaemia, such as occurs in Gilbert's syndrome (a genetic mutation in the enzyme UGT1A which metabolises bilirubin) has been associated with lower cardiovascular disease risk, independent of the lipoprotein profile [31], and lower serum bilirubin concentrations are recognised as a marker of cardiovascular disease risk [9]. We hypothesised that serum bilirubin was associated with markers of chronic disease, including CV disease, CKD and anaemia among Indigenous Australians. We have shown bilirubin was inversely associated with triglycerides, but bilirubin was also positively association with total cholesterol. We adjusted for numerous indicators of CV risk in the multivariate regression model by inclusion of age, blood pressure, adiposity, HbA1c, ACEI-ARB use, serum CRP and serum albumin. Our finding of an inverse association between serum log-bilirubin and triglycerides is consistent with other health screening surveys [32]. Cardiovascular disease risk management guidelines promote reduction in total cholesterol using statins as a first agent [33]. HMG-CoA reductase inhibitors

(statins) are reported to have a low negative effect on total bilirubin concentrations in individuals [34], however the final multivariate regression model was strengthened when limited to participants who were prescribed a statin. We suggest reverse causality may be one explanation for the positive association between bilirubin and total cholesterol in this cross-sectional study. Our findings remain unique, as other studies have not examined the association of bilirubin when adjusted for both Hb concentration and markers of CKD.

Bilirubin and markers of chronic kidney disease

This analysis explored more deeply the associations of albuminuria as part of its cardio-metabolic risk in this cohort [8]. Log-bilirubin was inversely associated with ACR in the multivariate regression analysis (coefficient -0.004, p=0.001), where 1 mg/mmol higher ACR was associated with 0.004 units lower log-bilirubin, or 1.00 µmol/L lower serum total bilirubin. In this cohort a wide range of ACR was observed (normo to macroalbuminuic range), thus ACR in the macroalbuminuria range is associated with lower bilirubin, and likely to be clinically meaningful. Our finding of an inverse association of bilirubin and ACR was consistent with reports from Korea among adults with and without diabetes [35] and Taiwanese adults [24]. In contrast to our findings, we note the positive association between bilirubin and albuminuria among a United States study [11]. It is possible that these different associations between bilirubin and albuminuria may be explained by the clinical and biochemical characteristics of the cohorts, including differences in median bilirubin concentrations observed (United States cohort v our cohort: (median (IQR): 12(10.3-14.7) v 6(5, 9)µmol/L).

In this analysis we have shown that bilirubin was not significantly explained by other measures of kidney function (Cystatin C, serum creatinine or eGFR). Thus the association of low bilirubin was not likely to be explained by inhibiting effects of Cystatin C which has been shown to attenuate anti-inflammatory processes in the setting of low GFR [37]. We suggest the inverse association of serum total bilirubin and ACR more strongly reflects cardio-metabolic risk, since bilirubin was not explained by other measures of kidney function.

Riphagen et al. reported baseline bilirubin levels were inversely associated with both ACR and eGFR [38], however our analysis showed bilirubin was only inversely associated with ACR. Our study findings remain important, since Riphagen et al. further reported low bilirubin concentration was associated with progression of diabetic kidney disease to ESKD, which has important implications for CKD and CV risk progression among Aboriginal and Torres Strait Islander peoples. We acknowledge there are several factors of interest to bilirubin production in adults with chronic kidney disease (erythrocyte lifespan [39], albumin production, albumin-bilirubin binding, enzymatic degradation of haemoglobin in adults with renal disease [40]), which are unlikely to significantly explain low bilirubin concentrations, since our participants were recruited from the community without acute illness and had stable kidney function.

Bilirubin concentrations between populations

Our data shows Aboriginal participants had lower bilirubin values than TSI participants. Reference ranges for bilirubin have not been developed from a healthy population of Aboriginal and Torres Strait Islander people, and there are very limited studies which describe the biochemical profiles including serum bilirubin of adult Indigenous Australians in very good health. O'Dea et al. reported the following profile among a semi-traditionally living Aboriginal family group of 18 adults in North-East Arnhem Land of the Northern Territory of Australia [41]: mean ((SD), range) serum bilirubin 5.6 ((0.3), 4-9) µmol/L, cholesterol 3.9 ((0.2), 2.1-5.6) mmol/L, creatinine 80 ((10), 50-120) µmol/L and haematocrit 45.7 ((1.1), 39-54) %, lean adult physique (BMI<17 kg/m^2) [41]. Thus these bilirubin levels were not too dissimilar to values we herein report amongst a cohort with concurrent cardio-metabolic risks.

It is unclear to what extent the different associations with bilirubin concentration and cardiovascular diseases reported across studies internationally (such as reported by Lee et al. [24] and Shin et al. [35]) were driven by ethnic population differences or study design. Although genetics can influence the intrinsic activity

of bilirubin metabolism (haem-oxygenase, HO-1, and biliverdin reductase), genetic assessments were not measured in these studies. Genetic variation studies (including mendelian randomisation) are one approach which may assist in addressing study confounding, to elucidate the causal relationships between gene products and outcomes. A recent meta-analysis confirmed differences in HO-1 genotype length between Asian and Caucasian populations, which the authors suggest are likely to impact on the interpretation of cardiovascular disease risk in large multiethnic studies[42]. Furthermore, by modelling the bilirubin gene UGT1A1*28, it was recently shown that bilirubin affects the cardiovascular system through vasomotor tone and artery reactivity [43].

Weaknesses and Strengths

We acknowledge some limitations in this study. First we used a cross-sectional study design with volunteering participants, which introduces potential for bias and explores associations of low serum bilirubin and not causality. Only serum total bilirubin was used in this analysis as we recognise that fractionated bilirubin measures are less clinically useful at low concentration of bilirubin. Urine ACR was based on the assessment of a single urine sample. Although all laboratories were nationally accredited, we acknowledge that differences in analytical methods for serum total bilirubin may make comparisons between laboratories difficult. We did not undertake a sensitivity analysis for bilirubin concentration by laboratory or region as participants were not representative of all strata (or population representative) in all study sites, and thus not strata-representative in every laboratory catchment area. In the multivariate regression model, the association of total bilirubin and total cholesterol (p=0.038) is regarded as borderline statistical significance given the number of tests performed. Classification of adults with suspected liver disease may be enhanced by additional medical history (cirrhosis, viral hepatitis) and a detailed alcohol record [24]. We were unable to determine corroborating details of other factors which contribute to the balance of anti-oxidant and oxidant stress among participants including poor quality atherogenic diets low in micronutrients [28], physical inactivity, a comprehensive assessment of tobacco and alcohol use, or presence and cause of chronic low

grade inflammation beyond the serum C-reactive protein [44]. These are often manifestations of communities in significant lifestyle transition and or poverty, thus disproportionately affecting minority and indigenous peoples in modernising countries [45, 46]. However, this study is unique as the only report describing the associations of serum bilirubin with metabolic risks in a community-based cohort of Aboriginal and or TSI people, and highlights modifiable associations of low serum bilirubin concentrations with ACR and low Hb concentration.

Conclusions

Our findings show that serum bilirubin is associated with markers of chronic disease, including CKD, dyslipidaemia, albuminuria and anaemia among Indigenous Australians. Further work in this population is required to determine the normal range of bilirubin, including ascertainment of factors which may explain the lower bilirubin concentrations among Aboriginal participants; and prospectively determine any causal relationship of low serum bilirubin with chronic disease progression and evaluate bilirubin variance in response to targeted anaemia management.

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Conflict of Interest Statement:

All authors have no conflicts to declare.

List of Abbreviations

ACEI-ARB	Angiotensin converting enzyme inhibitor-angiotensin receptor blocker
ACR	Albumin to creatinine ratio
ALP	Alkaline phosphatase
ALT	Alanine transferase
BMI	Body mass index
BP	Blood pressure
CKD	Chronic kidney disease
CRP	C-reactive protein
CV	Cardiovascular
eGFR	Estimated glomerular filtration rate
ESKD	End stage kidney disease
GGT	γ glutamyl transferase
Hb	Haemoglobin
HBA1c	Glycated haemoglobin
Hct	Haematocrit
HDL	High density lipoprotein
IDMS	Isotope dilution mass spectrometry
IQR	Interquartile range
MCV	Mean corpuscle volume
SD	Standard deviation
TSI	Torres Strait Islander
WHR	Waist to hip ratio

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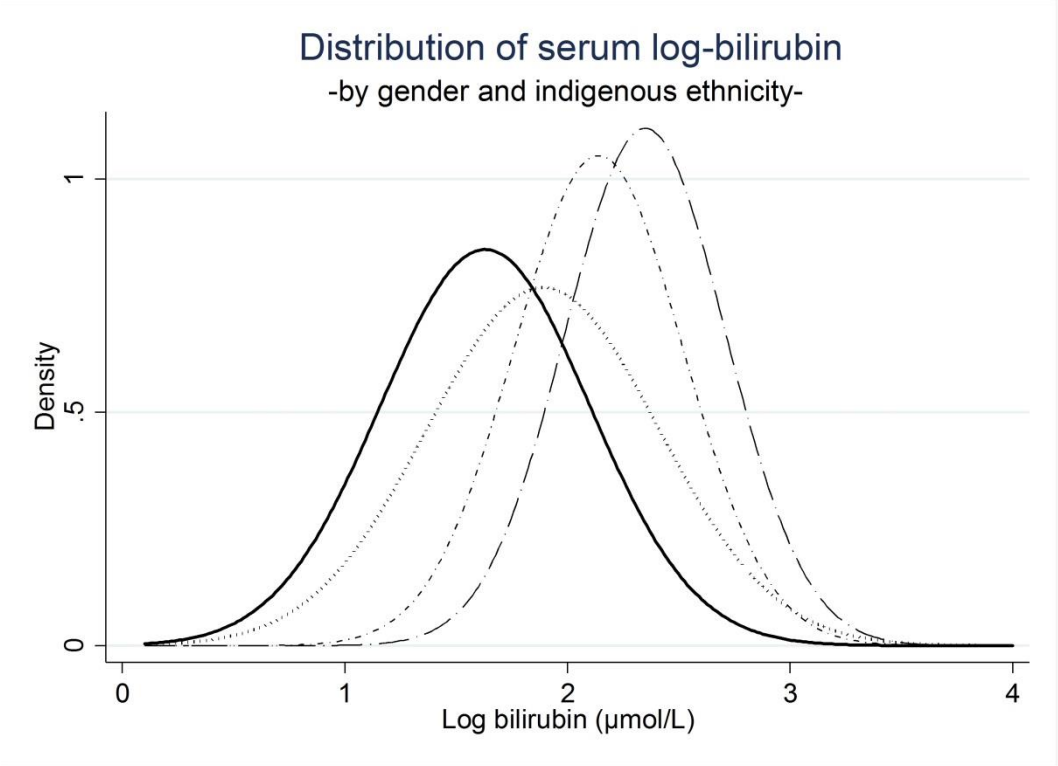

Figure 1 Normal distribution curve of log bilirubin by gender and ethnicity. Curves indicate (from left to right) Aboriginal female (solid), Aboriginal male (dot), TSI female (dash-dot), TSI male (long dash-dot).

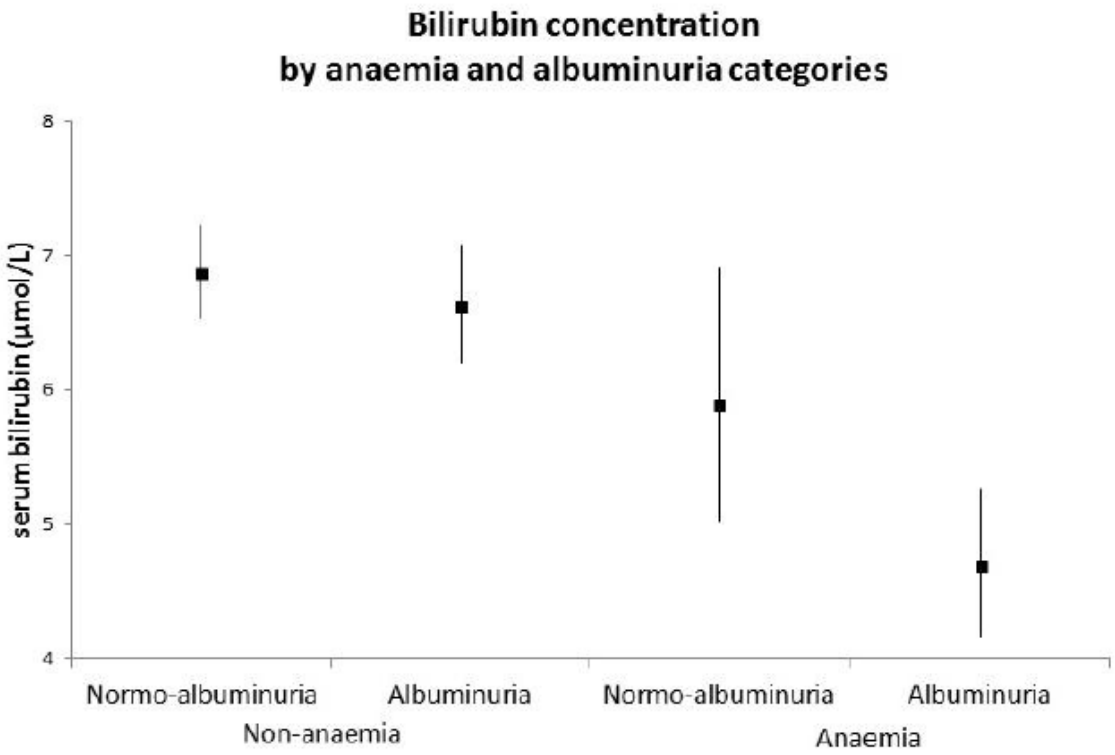

Figure 2. Bilirubin concentration by anaemia and albuminuria group categories, which are fully adjusted for independent variables identified in the multivariate regression model. Bilirubin expressed as geometric mean (95% confidence intervals).

Table 1: Participant characteristics

Aboriginal Ethnicity	72.1%
Female sex	62.6%
Diabetes	40.8% *
Hypertension	42.3% *
Anaemia	16.7%
Albuminuria ǂ	41.0 % *
eGFR<60 ml/min/1.73m^2 ǂǂ	18.0%
ACEI-ARB use	34.3%
Statin therapy use	25.6%
Current Smoking	41.9% *
Alcohol excess	33.9% *
BMI >25 kg/m^2	75.5% *
Age, years	45.1 (14.5)
eGFR⌈, ml/min/1.73m^2	99.1 (79.5, 112.3)
Cystatin C⌈ (mg/L)	0.85 (0.75, 1.01) *
Urea⌈, mmol/L	4.8 (3.9, 6.5)
ACR⌈, mg/mmol	1.8 (0.7, 19.6) *
HbA1c⌈, mmol/mol	42.1 (37.7, 55.2) *
Serum bilirubin⌈, µmol/L	6 (5,9)
Serum albumin, g/L	42 (4)
Hb, g/L	136 (18)
MCV, fl	85.3 (6.3) *
SBP, mmHg	118 (18) *
DBP, mmHg	75 (10) *
BMI, kg/m^2	30.0 (7.2) *
WHR	0.94 (0.09) *

N=594. Data are percentage, mean (standard deviation) or median (inter-quartile range)⌈. Data missing for* diabetes (n=593); hypertension (n=589); albuminuria (n=571); current smoking (n=585); alcohol excess (n=454); BMI (n=591); Cystatin C (n=545); ACR (n=571); HbA1c (n=588); MCV (n=562); blood pressure (n=588); BMI (n=591); WHR (n=566).

Table 2 Descriptive characteristics of male participants by bilirubin tertile

Bilirubin (µmol/L)	N=222	Tertile 1: 2- 6 µmol/L n=86	Tertile 2: 7-10 µmol/L n=72	Tertile 3: 11-61 µmol/L n=64	p
		Percentage (%)			
Aboriginal ethnicity	222	89.6	72.2	39.0	**<0.001**
Very remote residence	217	54.8 ǂ	60.6 ǂ	64.5 ǂ	0.48
Diabetes	222	51.2	30.6	29.7	**0.007**
Current smoking	219	60.0 ǂ	35.2 ǂ	38.1 ǂ	**0.003**
Micro-haemoglobinuria	212	14.8 ǂ	10.3 ǂ	14.3 ǂ	0.69
Alcohol excess	222	3.5	15.3	20.3	**0.005**
Medication Prescription					
Statin	222	33.7	19.4	17.2	**0.03**
Aspirin	222	38.4	13.9	17.2	**<0.001**
ACEI-ARB	222	46.5	26.4	26.6	**0.009**
		Mean (standard deviation)			
Age (years)	222	44.3 (13.6)	45.2 (16.6)	42.8 (16.0)	0.65
BMI (kg/m^2)	220	27.5 (6.0) ǂ	30.2 (8.2) ǂ	30.0 (7.0)	**0.03**
Waist-hip ratio	217	1.00 (0.08) ǂ	0.99 (0.10) ǂ	0.94 (0.09) ǂ	**0.003**
SBP (mmHg)	219	119 (19) ǂ	123 (17)	122 (16) ǂ	0.43
DBP (mmHg)	219	74 (12) ǂ	76 (11) ǂ	76 (11) ǂ	0.31
Total Cholesterol (mmol/L)	217	4.5 (0.9) ǂ	5.0 (1.1) ǂ	5.0 (1.0) ǂ	**0.003**
HDL-Cholesterol (mmol/L)	213	0.98 (0.28) ǂ	1.07 (0.27) ǂ	1.06 (0.32) ǂ	0.17
Total Protein (g/L)	219	79 (6) ǂ	78 (7) ǂ	78 (5) ǂ	0.68
Alkaline phosphate (U/L)	221	109 (31)	97 (31) ǂ	90 (33)	**0.001**
Serum albumin (g/L)	222	42 (5)	43 (4)	43 (5)	0.27
Hb (g/L)	222	143 (17)	148 (14)	152 (14)	**0.001**
Hct (%)	215	0.43 (0.05) ǂ	0.44 (0.04) ǂ	0.45 (0.04) ǂ	**0.007**
MCV (fl)	215	86.2 (5.5) ǂ	87.1 (4.6) ǂ	86.5 (6.6) ǂ	0.60
		Median (interquartile range)			
Urea (µmol/L)	222	5.3 (4.4, 7.7)	5.6 (4.5, 7.2)	5.0 (4.2, 6.7)	0.51
Creatinine (µmol/L)	222	86 (75, 116)	88 (73, 97)	82 (74, 93)	0.34
eGFR (ml/min/1.73 m^2)	222	96 (62, 109)	94 (78, 110)	100 (84, 114)	0.28
Cystatin C (mg/L)	204	0.92 (0.78, 1.24) ǂ	0.86 (0.77, 1.07) ǂ	0.85 (0.75, 0.98) ǂ	0.08
ACR (mg/mmol)	217	4.2 (1.0, 73.0) ǂ	1.4 (0.5, 15.0) ǂ	1.1 (0.6, 4.5) ǂ	**0.001**
GGT (U/L)	222	51 (32, 81)	39 (27, 70)	30 (23, 50)	**0.01**
ALT (U/L)	221	30 (22, 39)	33 (23, 43) ǂ	31 (22, 49)	0.45
HbA1c (mmol/mol)	221	44.3 (39.9, 63.9) ǂ	41.5 (38.8, 47.5)	40.4 (36.6, 48.6)	**0.009**
Triglycerides (mmol/L)	217	2.2 (1.5, 3.3) ǂ	1.8 (1.5, 2.4) ǂ	1.6 (1.1, 2.3) ǂ	**0.001**
CRP (mg/L)	215	5 (3, 10)	4 (2, 8) ǂ	3 (2, 8) ǂ	**0.02**

ǂ Data incomplete as follows: Tertile 1, n=85 [smoking , BMI, WHR, SBP, DBP, total protein, HbA1c]; n=84 [Very remote residence, ACR, CRP]; n=83 [total cholesterol, Hct, MCV, triglycerides]; n=82 [HDL-cholesterol]; N=81 [urine microhaemoglobinuria]; N=78 [Cystatin C]. Tertile 2, n=71 [Very remote residence, smoking, BMI, SBP, DBP, total cholesterol, HDL-cholesterol, ALP, ACR, ALT, total protein, triglycerides]; n=70 [WHR, CRP]; n=69 [Hct, MCV]; n=68 [urine

micro-haemoglobinuria]; n=65 [Cystatin C]. Tertile 3, n=63 [smoking, urine microhaemoglobinuria, total cholesterol, Hct, MCV, triglycerides]; n=62 [Very remote residence, WHR, SBP, DBP, total protein, ACR]; n=61 [Cystatin C, CRP]; n=60 [HDL-cholesterol].

Table 3 Descriptive characteristics of female participants by bilirubin tertile

Bilirubin (µmol/L)	N=372	Tertile 1: 2- 5 µmol/L n=163	Tertile 2: 6-7 µmol/L n=91	Tertile 3: 8-29 µmol/L n=118	p
		Percentage (%)			
Aboriginal	372	92.6	73.6	47.5	**<0.001**
Very remote residence	363	51.6 ǂ	51.1 ǂ	72.4 ǂ	**0.001**
Diabetes	371	49.1	41.8	33.3 ǂ	**0.03**
Current smoking	366	38.3 ǂ	47.7 ǂ	35.3 ǂ	**0.18**
Micro-haemoglobinuria	347	26.7 ǂ	17.2 ǂ	18.2 ǂ	0.14
Excess alcohol	372	2.5	4.4	11.0	0.008
Medication Prescription					
Statin	372	35.6	13.2	23.7	**<0.001**
Aspirin	372	24.5	17.6	22.0	0.44
ACEI-ARB	372	42.3	31.9	25.4	**0.01**
		Mean (standard deviation)			
Age (years)	372	45.3 (14.6)	46.5 (12.8)	44.8 (14.3)	0.70
BMI (kg/m^2)	371	30.9 (7.4) ǂ	29.9 (6.6)	30.4 (7.6)	0.58
Waist-hip ratio	349	0.93 (0.09) ǂ	0.92 (0.08) ǂ	0.90 (0.08) ǂ	**0.03**
SBP (mmHg)	369	118 (18) ǂ	118 (17)	113 (18) ǂ	0.07
DBP (mmHg)	369	75 (9) ǂ	76 (10)	72 (9) ǂ	**0.005**
Total Cholesterol (mmol/L)	363	4.5 (1.0) ǂ	4.8 (1.0) ǂ	5.0 (1.2) ǂ	**0.001**
HDL-Cholesterol (mmol/L)	353	1.08 (0.34) ǂ	1.15 (0.34) ǂ	1.16 (0.35) ǂ	0.13
Total Protein (g/L)	370	80 (8)	79 (8) ǂ	78 (7) ǂ	0.08
Alkaline phosphate (U/L)	372	115 (42)	102 (34)	100 (45)	**0.004**
Serum albumin (g/L)	372	41 (4)	42 (4)	42 (4)	**0.02**
Hb (g/L)	372	125 (17)	131 (13)	135 (13)	**<0.001**
Hct (%)	347	0.38 (0.05) ǂ	0.40 (0.04) ǂ	0.41 (0.03) ǂ	**<0.001**
MCV (fl)	347	83.0 (6.9) ǂ	84.3 (6.3) ǂ	86.5 (5.7) ǂ	**<0.001**
		Median (interquartile range)			
Urea (µmol/L)	372	4.6 (3.7, 9.0)	4.6 (3.7, 5.9)	4.8 (3.9, 5.7)	0.51
Creatinine (µmol/L)	372	63 (54, 97)	57 (52, 66)	62 (56, 72)	**<0.001**
eGFR (ml/min/1.73 m^2)	372	97 (55, 115)	106 (94, 114)	99 (87, 112)	**0.03**
Cystatin C (mg/L)	341	0.85 (0.73, 1.44) ǂ	0.82 (0.72, 0.97) ǂ	0.82 (0.76, 0.92) ǂ	0.09
ACR (mg/mmol)	354	2.9 (0.9, 53.5) ǂ	2.2 (0.9, 13.5) ǂ	1.1 (0.6, 4.2) ǂ	**<0.001**
GGT (U/L)	372	33 (23, 62) ǂ	31 (25, 46)	26 (19, 41)	**0.03**
ALT (U/L)	371	23 (17, 30) ǂ	23 (17, 30)	24 (18, 37)	0.24
HbA1c (mmol/mol)	367	43.2 (38.3, 158.5) ǂ	43.2 (38.8, 55.2) ǂ	39.9 (36.6, 49.7) ǂ	**0.03**
Triglycerides (mmol/L)	363	2.1 (1.4, 2.7) 162	1.7 (1.3, 2.3) ǂ	1.5 (1.0, 1.9) ǂ	**<0.001**
CRP (mg/L)	364	7 (4, 15) 159	6 (3, 12) ǂ	5 (2.3, 10) ǂ	**0.01**

For current smokers; bilirubin data is expressed as geometric mean (95% confidence interval). ǂ Data incomplete as follows: Tertile 1, n=162 [current smoking, BMI, total cholesterol, triglycerides, ALT]; n=161 [SBP, DBP]; n=160 [HbA1c]; n=159 [Very remote residence, CRP]; 158 [HDL-cholesterol]; n=151 [Hct, MCV, ACR]; n=150 [WHR, urine microhaemoglobinuria]; n=144 [Cystatin C]. Tertile 2, n=90 [total protein, HbA1c, CRP]; n=88 [Very remote residence, current smoking, ACR]; n=87 [urine micro-haemoglobinuria, total cholesterol, triglycerides]; n=86 [Hct, MCV]; n=84 [WHR]; n=83 [HDL-cholesterol]; n=81 [Cystatin C]. Tertile 3, n=117 [diabetes, SBP,

DBP, HbA1c]; n=116 [Very remote residence, current smoking, Cystatin C]; n=115 [WHR, ACR, CRP]; n=114 [total cholesterol, triglycerides]; n=112 [HDL-cholesterol]; n=110 [urine microhaemoglobinuria, Hct, MCV].

Table 4 Multivariate regression model of log-bilirubin

	Model A: All Participants				Model B: Participants without suspected liver disease			
	β Coefficient	95% Confidence Interval		p	β Coefficient	95% Confidence Interval		p
Aboriginal ethnicity	-0.43	-0.51	-0.35	**<0.001**	-0.42	-0.50	-0.33	**<0.001**
Female	-0.10	-0.18	-0.02	**0.02**	-0.12	-0.21	-0.03	**0.011**
Hb (g/L)	0.010	0.007	0.012	**<0.001**	0.008	0.005	0.011	**<0.001**
ACR (mg/mmol)	-0.0004	-0.0007	-0.0002	**0.001**	-0.0004	-0.0007	-0.0001	**0.004**
Triglycerides (mmol/L)	-0.06	-0.09	-0.04	**<0.001**	-0.07	-0.10	-0.03	**<0.001**
Cholesterol	0.04	0.002	0.072	**0.038**	0.055	0.018	0.092	**0.004**
ALT	0.002	0.0001	0.004	**0.038**	-	-	-	-
Constant	0.89	0.53	1.25	**<0.001**	1.02	0.63	1.41	**<0.001**
	Model R^2 0.39, n=556				Model R^2 0.35, n=479			

The initial model included categorical variables (1=Yes, 0=No) [Female sex, Aboriginal ethnicity, Diabetes, very remote residence, microscopic haem, medications (statin, aspirin, ACEI-ARB)] and continuous variables [WHR, triglycerides, total cholesterol, total protein, alkaline phosphatase, alanine transferase, serum albumin, haemoglobin, mean cell volume, urea, creatinine, eGFR, cystatin C, ACR]. Log-bilirubin was converted to bilirubin (in µmol/L) using bilirubin =exp(equation).

Model B: Thus bilirubin = exp[(1.02 -0.42(if Aboriginal) -0.12 (if female) + 0.008 (Hb) -0.0004 (ACR) -0.07 (triglycerides) + 0.055 (cholesterol)] µmol/L

Highlights

- Bilirubin was positively associated with Hb and inversely associated with ACR
- Bilirubin was not associated with other markers of kidney function apart from ACR
- Bilirubin was lower in Aboriginal than Torres Strait Islander participants
- Factors explaining this lower concentration requires further investigation