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Original Article

C-reactive protein and telomerase reverse transcriptase (TERT) associate with chronic disease markers in a sample from low-income neighborhoods in Detroit, Michigan

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ABSTRACT

Racial and ethnic minorities in economically deprived inner cities experience high rates of chronic diseases compared to neighborhoods with higher socioeconomic status (SES). However, these economically deprived populations are understudied in terms of biomarkers associated with chronic disease risk which include C-reactive protein (CRP), telomerase reverse transcriptase (TERT), and glycosylated hemoglobin (A1C). We examined relationships between CRP and TERT and chronic disease indicators (body mass index [BMI] and A1C) in two low-income, predominantly African American (AA) neighborhoods in Detroit, Michigan. Sixty-nine adults (43 females, 26 males, mean age 46 years [y], standard deviation [*SD*] = 15.9) completed a health survey, anthropometry, and finger stick blood tests. A1C was measured using A1CNow test strips, and CRP and TERT levels were measured using enzyme-linked immunosorbent assay (ELISA) with samples extracted from dried blood spots. We examined CRP (mean = 4.9, *SD* = 3.1), TERT (mean = 32.5, *SD* = 15.1), and A1C (mean = 5.4, *SD* = 1.0) by BMI category. We fitted restricted maximum likelihood regression models to evaluate associations between CRP, TERT, BMI, and A1C, after adjustment for demographics and inclusion of a random effect for the neighborhood. In this predominantly AA sample (91%, 63/69), 68% had levels of CRP (means = 4.8 mg/L, *SD* = 3.0 for AAs; 6.4 mg/L, *SD* = 3.9 for all others) indicative of chronic inflammation (CRP greater than 3 mg/L). BMI was significantly associated with CRP ($p = 0.004$) and TERT ($p = 0.026$). TERT levels indicate that being overweight is associated with markers of chromosome remodeling, suggestive of chronic disease. CRP followed a similar trend with overweight individuals having higher inflammation and risk of chronic disease. Our findings warrant further exploration of additional factors that may influence CRP and TERT. Furthermore, examining populations in a more ethnically and/or economically diverse, yet still high proportion minority, sample will fill a knowledge gap in this understudied field.

Introduction

Racial and ethnic minorities who live in economically deprived inner cities experience poor mental and physical health.[1] As the global population increasingly becomes more urbanized,[2] it is important to understand the mechanisms leading to poorer mental and physical health in inner cities. One hypothesized mechanism is that low socioeconomic status (SES) leads to stress-induced inflammation and eventual development of negative health outcomes.[3–5] While acute stress responses may be protective, prolonged stress exposure can lead to chronic diseases including cancer, cardiovascular disease, depression, obesity, and type 2 diabetes as well as long -standing financial burdens.[6]

Measurement of biomarkers specific to a disease pathology guides risk assessment and treatment planning for these chronic diseases. For example, glycosylated hemoglobin (A1C) is a marker for blood glucose homeostasis,[7] diabetes severity, and risk of cardiovascular[8] and other noncommunicable diseases. A1C is the amount of glucose bound to hemoglobin within the last ~3 months and a valid measure of chronic glycemic homeostasis.[9] Similarly, C-reactive protein (CRP) is a blood

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Abbreviations

AA	African American
CRP	C-Reactive Protein
A1C	Glycosylated hemoglobin
ELISA	Enzyme-linked immunosorbent assay
SES	Socioeconomic Status
TERT	Telomerase reverse transcriptase
BMI	Body Mass Index
IRB	Institutional review board
DNA	Deoxyribonucleic acid
y	years
SD	standard deviation
SE	standard error
CV	coefficients of variation
β Coef.	Beta Coefficient
n	number
PBS	Phosphate Buffered Saline
RPM	Rotations Per Minute

marker for inflammation.[10] Chronic elevations in CRP (~3 mg/L) are associated with cardiometabolic disease,[11–13] while high levels of CRP (greater than 10 mg/L) are indicative of acute bacterial infections, tissue injury, and cell death.

Recently, the activity of the enzyme telomerase has emerged as a biomarker for age-related chronic disease development.[14] Telomerase activity is inhibited during periods of chronic stress contributing to cellular senescence, which can accelerate the aging of the immune system and eventually inhibit the body's ability to respond to immunological challenges, resulting in chronic inflammatory states and oxidative stress that will damage deoxyribonucleic acid (DNA) in chromosomes and potentially increase the risk for cancer development.[14]

Telomerase activity is difficult to measure in large epidemiological studies, but recent literature demonstrates that the abundance of telomerase reverse transcriptase (TERT) in human serum measured using enzyme-linked immunosorbent assays (ELISA) is closely associated with telomerase activity.[15] TERT is the catalytic subunit of the telomerase enzyme and is the rate-limiting component to maintaining the telomere repeat TTAGGG of the chromosome. Thus, alterations in TERT can be correlated with cellular senescence and DNA damage leading to chronic disease.[16,17]

Obtaining serum for biomarker analysis in large epidemiological studies presents a unique challenge, but dried blood spots are an ideal choice to evaluate circulating biomarkers.[18] Dried blood spots are simple to collect, minimally invasive, and easy to transport and store, all of which make their implementation appropriate to address the purpose of the present investigation.[19,20] Therefore, we used dried blood spots to collect whole blood samples from an economically deprived inner-city population. In addition to collecting dried blood spots, participants' body mass index (BMI) was calculated due to the positive associations between BMI and chronic disease risk.[21,22] Thereby, the purpose of the present investigation was to determine the abundance of biomarkers (A1C, CRP, and TERT) theoretically linked to stress, and chronic disease in an at-risk population of predominantly AAs and test for associations between CRP and TERT with two chronic disease indicators: BMI and A1C, adjusted for relevant demographics. The study was conducted using techniques that could be implemented in a future, large-scale epidemiological study.

Methods

Participant data collection

The study was approved by the Michigan State University Institutional Review Board. In 2018, we recruited 69 participants in two low-income, predominantly AA neighborhoods in Detroit, Michigan (Institutional Review Board [IRB] Approval #STUDY00003938) as a pilot study. The pilot project was a precursor study for a future study (IRB Approval #STUDY00000587).[23] In two low-income neighborhoods (> 40% of the population living 100% under the poverty line), we mailed postcards and staffed recruitment tables at prominent locations to recruit participants. We then visited households by going door-to-door to recruit participants. We included only one English-speaking adult (≥ 18y) without mobility issues per household.

Participants completed a university-approved informed consent document and then completed a health and demographic survey (included questions on age, sex, ethnicity, employment, length of residency, home ownership, children, income, chronic disease diagnoses), anthropometry, and a finger stick to measure A1C and collect dried blood spots. A1C[24,25] was analyzed with portable analyzers (A1CNow^{+}®, PTS Diagnostics) that are validated compared to laboratory-based analysis.[26] Height and weight were measured in duplicate, the respective measurements were averaged, and the resultant value was used to calculate BMI (kg/m^2). Height was measured using a portable stadiometer (SECA Corp); weight was measured using a digital scale (Tanita).

Dried blood spots for biomarkers of health

To characterize health and potential risk for chronic disease, we measured CRP and TERT from dried blood spots using commercially available ELISA kits which are marketed as appropriate to test the hypothesis in the present investigation. As a method of good practice, to establish the linearity of targeted protein extracted from dried blood spots versus serum separated from fresh whole blood, we conducted an in-house evaluation of the kits (described below) on a convenient sample of lab personnel. Briefly, five laboratory members had a finger pricked to collect blood on a Whatman 903 Protein Saver Card dried blood spot and in a 2 ml microfuge tube (whole blood).

Serum was extracted from fresh whole blood by allowing the sample to sit in the 2 ml microfuge tube for 30 min at room temperature. The sample was then spun at 2 000 g for 10 min and the serum was removed, frozen at −80 °C, and later analyzed by ELISA (described below). Correlations were run between serum extracted from fresh whole blood and dried blood spots for CRP and TERT. For CRP, the correlation between dried blood spots and serum was $r = 0.92$, and for TERT the correlation was $r = 0.94$. We acknowledge the sample size is small, but these data provide strong support for the use of samples derived from dried blood spots with the kits described below.

Serum isolation from dried blood spots

Whole blood was extracted from the dried blood spots using previously established methods.[27,28] Briefly, a 0.64 cm hole was punched on the edge of the dried blood spot, and the hole was submerged in extraction buffer (4.995 mL 0.01 M Phosphate Buffered Saline [PBS], 5 μL Tween, 0.146 1 g NaCl, pH 7.2) and incubated overnight at 4 °C. Next, samples were centrifuged at 13 000 rotations per minute (RPM) for 15 min, to isolate serum from clotting factors. Aliquots of serum were separated for CRP and TERT analysis via the assays described below.

CRP and TERT assays

The present investigation used commercially available ELISA kits recommended for human plasma and serum to measure the abundance of CRP and TERT. CRP was measured using the CRP Human ELISA Kit (ab99995, Abcam). Following analysis of a dilution curve (data not shown), the samples were diluted 1:5 000 in assay buffer, loaded on plates in duplicate for processing, and read at 450 nm on a microplate reader (BioRad).

The concentration of TERT was measured using the Human

Telomerase ELISA Kit (Lifespan Biosciences, LS-F12713). Following analysis of a dilution curve (data not shown), samples were diluted at 1:40 000 and loaded onto plates in duplicate for processing. Plates were read at 450 nm on a microplate reader (BioRad).

For both CRP and TERT, concentration was determined by comparing the optical density of samples to those of the manufacturer-supplied standard. The limit of detection for the CRP assay was 34.29 pg/ml and 0.156 ng/ml for the TERT assay. For both, average concentrations and coefficients of variation (*CV*) were calculated for duplicate samples. For CRP, the *CV* for low (1.2 mg/L), medium (5.4 mg/L), and high (8.9 mg/L) concentration reference samples were 6.5%, 1.88%, and 0.64% respectively. For TERT, the CV for low (12 mg/L), medium (32 mg/L), and high (54 mg/L) concentration reference samples were 5.1%, 2.1%, and 1.21% respectively.

Statistics

Scatter plots were generated to visually inspect the relationship between CRP and TERT with A1C, age, BMI, and years of residency. We used chi-square tests for discrete variables and Kruskal-Wallis tests for continuous variables to assess differences by BMI categories: underweight (< 18.5), healthy weight (18.5–24.9), and overweight and obese (> 25). For regression analyses of CRP and TERT, we fitted mixed effect linear models by restricted maximum likelihood with a random effect of neighborhood and the following independent variables: BMI, BMI^2, A1C, sex, age, ethnicity, employment, years of residency (logged scale), home ownership, and children (binary).

We also explored the potential for recent, acute infection (via self-report on the survey) to affect CRP, yielding similar results (Table S1). Fitted models were assessed graphically for outliers and residual normality and were conducted for the 62 participants for whom we had complete data. Statistical analyses were conducted with Stata v16 (StataCorp, College Station, TX) and SAS version 9.4 (SAS Institute Inc, Cary, NC).

Results

Most participants were classified as overweight/obese (50.7%, Table 1), female (62.3%), and AA (91.3%). Non-African Americans in this sample were Hispanic ($n = 2$), White ($n = 2$) and Other ($n = 2$). Few participants were married/partnered (14.5%) or in the "underweight" category (4.4%) despite this group of married participants not being the youngest (44.8 years [y]). The average length of residency was > 6 y. The average A1C was 5.4% (standard deviation [*SD*] = 1.0%) and A1C did not differ by weight category. We detected significant differences in median CRP and TERT by BMI category (both $p < 0.01$, Table 1). Overweight/obese participants had higher CRP than the healthy weight group (all pairwise $p < 0.002$). Overweight/obese/healthy BMI participants had lower TERT than the underweight category (all pairwise $p < 0.02$).

Self-reported diagnoses of chronic disease were low (23%) but included incidence of hypertension ($n = 8$), mental health disorders ($n = 4$) and diabetes ($n = 3$) (data not shown in tabular form). Most participants (68%) had elevated CRP (Supplementary Fig. S1A), and the distributions of CRP and TERT were non-normal (Supplementary Fig. S1A&B). The negative correlation between CRP and TERT was statistically significant, yet weak ($r = -0.248$, $p = 0.044$; Fig. S2). We observed an average CRP of 4.9 mg/L (*SD* = 3.1) for all participants, 4.8 mg/L (*SD* = 3.0) for AAs, and 6.4 mg/L (*SD* = 3.9) for all others.

BMI was significantly associated with CRP. For every half standard deviation increase in BMI from the mean, we would expect an increase in mean CRP of 0.52 mg/L (standard error [*SE*] = 0.201, $p = 0.021$) (Table 2, Model A). While only approaching statistical significance, we also found that AA participants had significantly lower CRP (decrease in CRP of 2.3 mg/L), compared to participants of other races/ethnicities. We observed non-significant, negative associations between CRP and those identifying as female, AA, married, owning a home, and having children.

When evaluating associations with TERT, only BMI was significantly associated (Table 2, Model B). For every half standard deviation increase in BMI from the mean, we would expect a decrease in mean TERT of −0.19 mg/dL (*SE* = 0.108, $p = 0.091$) (Table 2, Model B). We also observed non-significant, positive associations between TERT, and years living at the residence and being married. We observed non-significant, negative associations between TERT and those identifying as female, employed, AA, owning a home, having children, A1C and age.

Discussion

AA have an increased risk of chronic disease which is partially due to disparities in SES.[1] However, the pathway through which various factors influence BMI, inflammation, and chronic disease development remains unclear. Stress may induce changes in the nervous, endocrine, and immune systems that may cause obesity and chronic disease development.[4,29,30] Some studies have shown that perceived poorer neighborhood conditions were associated with higher stress and

Table 1
Demographic characteristics of the sample, stratified by weight status.

Characteristic		Under weight	Healthy weight	Over weight	Obese	Total[a]	*p*-value[b]
Weight category, *n* (%)		3 (4.4)	15 (21.7)	16 (23.2)	35 (50.7)	69 (100)	
% Female		66.7	46.7	50.0	74.3	62.3	0.202
% African American		100	93.3	87.5	91.4	91.3	> 0.999
% Married/Partnered		33.3	6.7	18.8	14.3	14.5	0.664
% Employed		…	40.0	37.5	31.4	33.3	0.653
% Owned home		33.3	26.7	31.3	34.3	31.9	0.972
% Income < $30 k		0.0	50.0	60.0	52.2	50.0	0.651
% Children 1 or more		66.7	33.3	25.0	45.7	39.1	0.365
Age, years	mean	44.8	42.7	51.2	45.2	46.0	0.465
	SD	14.3	15.3	16.2	16.3	15.9	
Years resident	mean	12.3	3.3	4.5	7.6	6.2	0.064
	SD	4.9	3.5	6.1	10.2	8.3	
CRP, mg/L	mean	0.58	2.5	6.2	5.7	4.9	< 0.001
	SD	0.60	2.6	2.9	2.6	3.1	
TERT, mg/L	mean	53.1	40.7	27.6	29.5	32.5	0.011
	SD	3.2	14.2	12.4	14.8	15.1	
A1C, %	mean	5.7	5.1	5.5	5.5	5.4	0.388
	SD	1.1	0.5	1.2	1.0	1.0	

n – number; CRP- C-Reactive Protein; A1C – glycosylated hemoglobin; TERT-telomerase reverse transcriptase; *SD* – standard deviation.
[a] Distribution of characteristic in sample.
[b] *p*-value for three group differences from Kruskal-Wallis test for continuous variables, exact chi-square test for categorical variables.

Table 2
Regression analyses for associations with CRP (Model A) and TERT (Model B).

	Model A: CRP, mg/L			Model B: TERT, mg/dL		
	β Coef.	*SE*	*p*-value	*β Coef.*	*SE*	*p*-value
A1C	0.63	0.40	0.121	−0.15	0.22	0.499
BMI	1.32	0.43	**0.004**	−0.49	0.21	**0.026**
BMI^2	−0.56	0.35	0.123	0.24	0.17	0.170
Age	0.04	0.03	0.182	−0.02	0.02	0.245
Female	−0.07	0.86	0.927	−0.05	0.48	0.924
Years resident, log	0.03	0.33	0.919	0.09	0.18	0.610
African American	−2.31	1.49	0.127	−0.19	0.80	0.817
Employed	0.90	0.87	0.308	−0.43	0.49	0.386
Married/partnered	−0.35	1.43	0.807	0.42	0.66	0.522
Own home	−1.19	0.87	0.175	−0.27	0.48	0.570
Children, binary	−0.30	0.98	0.760	−0.79	0.52	0.136

BMI-Body Mass Index; CRP- C-Reactive Protein; A1C – glycosylated hemoglobin; TERT-telomerase reverse transcriptase; *β Coef.* – Beta coefficient; *SE* – standard error.
Bolded font $p \leq 0.05$, BMI centered at the mean (30.68 kg/m^2) and scaled by the standard deviation (*SD*) (7.47 kg/m^2). Although not significant, a single random intercept for the neighborhood is allowed for Model A, but omitted for Model B due to non-convergence issues.

inflammation, which were both associated with increased BMI.[31] Other studies report that differences in BMI appear to explain much of the relationship between CRP and ethnicity.[5,32] In the present study, we found significant associations of BMI with both CRP and TERT levels.

In the absence of acute infection, CRP varies from 0 to 10 mg/L. Concentrations between 0 and 3 mg/L are indicative of low disease risk, those between 3 and 10 mg/L are indicative of increased risk of chronic disease and values greater than 10 mg/L are indicative of acute infection.[25,26] In the latest NHANES survey that included CRP data (2009–2010), the average CRP level for adults was 0.41 mg/L (*SD* = 0.75) overall and 0.50 mg/L (*SD* = 0.87) for AA. The mean level of CRP for all participants in the current study was 4.9 mg/L, a concentration that is associated with chronic psychosocial/physiological stress and elevated risk of chronic disease.[12,33] Our observation is consistent with published literature on the effects of race, SES, stress on immune function, and long-term health outcomes.[34,35] It is possible that the sample in the present investigation contained individuals who presented with acute inflammation from infection or an undiagnosed acute condition. We assessed this possibility by re-running all analyses after removing data for individuals with CRP levels greater than 10 mg/L or who had reported experiencing an infection within the last two weeks. The relationships with BMI, age, and AA ethnicity were strengthened when individuals suspected of experiencing an acute infection were removed from the analysis (Table S1).

Although AA ethnicity was associated with lower CRP relative to other ethnicities (4.8 mg/L vs 6.4 mg/L), the levels for AA participants were still indicative of chronic low-grade inflammation. Interestingly, there were no significant associations between CRP or TERT and the socioeconomic variables (home ownership, employment status), which may be an artifact of low variability in these factors in our sampled population.

A higher BMI and CRP concentration were associated with lower concentrations of TERT. The role of telomeres and TERT in cellular senescence and chronic disease is an emerging science with existing literature demonstrating diminished telomerase activity associated with telomere shortening and cellular senescence.[14] Although TERT increases in response to acute infection; chronic infection and inflammation may decrease TERT concentration, accelerating telomere shortening.[14] The negative relationship between TERT and CRP (Fig. S3) and the positive (non-significant) association between CRP and A1C (Table 2 and Table S1) in the present investigation are consistent with the hypothesis that chronic stress has negative impacts on health in low-income neighborhoods. Future studies to explore the relationship between high CRP and low TERT in this population must be conducted to elucidate biological mechanisms.

Conclusions

We observed a positive and significant increase in CRP levels with increasing BMI, indicative of increased risk for chronic disease. Similarly, we observed a negative and significant relationship between TERT and BMI, suggesting that being overweight is associated with chromosome remodeling that may contribute to the risk of chronic diseases. These findings warrant further exploration of cultural, behavioral, or lifestyle factors in a more ethnically and/or economically diverse (yet still minority) sample or matched study design. Additionally, these results suggest that further investigation of the role of serum levels of TERT as a biomarker of chronic disease risk is warranted.

Submission statement

The results described have not been published previously and is not under consideration for publication elsewhere. The manuscript has been approved by all authors and will not be published elsewhere without the consent of the copyright holder.

Authors' contributions

AP and DF conceived of the study. AP led data collection, assisted by KP and TH. EL led laboratory analyses, with supervision of the molecular analysis by DF. AP ran statistical analyses with help from JG. DF, TH, EL and EL, DF, and AP drafted the manuscript. All authors read and approved the manuscript.

Ethic approval statement

The study was approved by the Michigan State University Institutional Review Board. (IRB Approval #STUDY00003938). This project was a precursor study for a future study (IRB Approval #STUDY00000587). Participants completed a university approved informed consent document.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.smhs.2022.07.002.

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