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Brain, Behavior, and Immunity 94 (2021) 51–59

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Interplay between social isolation and loneliness and chronic systemic inflammation during the COVID-19 pandemic in Japan: Results from U-CORONA study

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ARTICLE INFO

Keywords:
Social relationship
Social isolation
Loneliness
Chronic inflammation
Neutrophil-to-lymphocyte ratio
C-reactive protein

ABSTRACT

In the face of the global coronavirus disease 2019 (COVID-19) pandemic, billions of people were forced to stay at home due to the implementation of social distancing and lockdown policies. As a result, individuals lost their social relationships, leading to social isolation and loneliness. Both social isolation and loneliness are major risk factors for poor physical and mental health status through enhanced chronic inflammation; however, there might be an interplay between social isolation and loneliness on the association with chronic inflammation. We aimed to clarify the link between social relationships and inflammation in the context of the COVID-19 pandemic by distinguishing whether social isolation only, loneliness only, or both were associated with chronic inflammation markers among community-dwelling adults. The data of 624 people (aged 18–92 years, mean 51.4) from the Utsunomiya COVID-19 seROprevalence Neighborhood Association (U-CORONA) study, which targeted randomly sampled households in Utsunomiya city, Japan, were analyzed. Social isolation was assessed as a structural social network by asking the number of social roles they have on a daily basis. Loneliness was measured with the UCLA loneliness scale. As chronic inflammation biomarkers, neutrophil-to-lymphocyte ratio (NLR) and the concentration of high-sensitivity C-reactive protein (CRP) were measured. Generalized estimating equations method was employed to take into account the correlations within households. Isolated-Lonely condition (i.e., being both socially isolated and feeling lonely) was associated with higher NLR among men (B = 0.141, 95%CI = −0.01 to 0.29). Interestingly, Nonisolated-Lonely condition (i.e., not socially isolated but feeling lonely) was associated with lower CRP among women (B = −0.462, 95%CI = −0.82 to −0.10) and among the working-age population (B = −0.495, 95%CI = −0.76 to −0.23). In conclusion, being both socially isolated and feeling lonely was associated with chronic inflammation. Assessing both social isolation and loneliness is critical for proper interventions to mitigate the impact of poor social relationships on health, especially in the context of the COVID-19 pandemic.

1. Introduction

In December 2019, the new coronavirus disease 2019 (COVID-19), which causes a highly infectious serious acute respiratory syndrome, emerged and has since spread all over the world (World Health Organization, 2020). One of the policies against COVID-19 were social distancing and lockdown to reduce physical contacts and prevent the spread of the virus from person to person, which was shown to be effective (Flaxman et al., 2020; Hsiang et al., 2020). However, due to the policy, many people have lost social connections and suffered from isolation and loneliness; 33% of people reported loneliness in the UK (Li and Wang, 2020) and Spain (Losada-Baltar et al., 2020), and 13.8% of people reported loneliness in the US (McGinty et al., 2020). Furthermore, 66% of the participants of an online survey in Israel indicated

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https://doi.org/10.1016/j.bbi.2021.03.007
Received 27 November 2020; Received in revised form 5 March 2021; Accepted 5 March 2021
Available online 9 March 2021

experience of loneliness (Elran-Barak and Mozeikov, 2020). These figures were significantly higher than those of pre-pandemic period (Killgore et al., 2020; Luchetti et al., 2020; McGinty et al., 2020; van Tilburg et al., 2020). Thus, it is important to elucidate the impact of disconnection and loneliness in the context of COVID-19 pandemic to address health status apart from the infection from COVID-19.

Social isolation and loneliness are associated with adverse health outcomes, such as all-cause mortality (Holt-Lunstad et al., 2015) and decline in mental health (Gariépy et al., 2016). Inflammation is considered to be one of the pathways for social isolation and loneliness to affect health (Audet et al., 2014; Cacioppo et al., 2011; Hawkley and Cacioppo, 2010; Kiecolt-Glaser et al., 2010). The associations of social isolation with interleukin-6 (IL-6), tumor necrosis factor, fibrinogen and C-reactive protein (CRP) (Smith et al., 2020; Uchino et al., 2018), as well as the associations between loneliness and IL-6 (Smith et al., 2020) have been consistently reported. Recently, as a convenient biomarker of chronic systemic inflammation for severity or prognosis of diseases such as cancer (Guthrie et al., 2013; Zhao et al., 2016), cardiovascular disease (Angkananard et al., 2018) and bipolar and major depressive disorders (Mazza et al., 2018), the neutrophil-to-lymphocyte ratio (NLR) has been used. This metric has the advantage of being obtained via a simple, inexpensive, reproducible and routinely preoperative blood test (Feng et al., 2020; Guthrie et al., 2013; Mazza et al., 2018). However, to the best of our knowledge, no studies have so far examined the association between social isolation or loneliness and NLR, except for one study (Cole, 2008) that examined the differences in glucocorticoid sensitivity of NLR by loneliness.

Social isolation and loneliness are two different concepts. Whereas social isolation is defined as inadequate quality and quantity of social relations with others and is mostly judged objectively, loneliness is a painful subjective emotional state that arises from a discrepancy between desired and achieved patterns of social interaction (Wang et al., 2017). Previous studies (Shankar et al., 2011; Walker et al., 2019) have examined the links of social isolation and loneliness simultaneously with various inflammatory markers, and reported different roles; social isolation was associated with elevated levels of fibrinogen, CRP and white blood cell (WBC) (Shankar et al., 2011; Walker et al., 2019), while loneliness was associated with insulin-like growth factor 1 (IGF-1) (Walker et al., 2019). Another study reported an interesting difference between experience of isolation and loneliness in transcription of genes expressed in circulating leukocytes; loneliness was associated with twice as many differentially expressed genes as isolation, and transcripts associated with loneliness originated from monocytes and dendritic cells, whereas those with isolation were derived from B lymphocytes (Cole et al., 2011). Although these findings suggested social isolation and loneliness can independently affect the levels of inflammation, no study has evaluated the synergistic effects. That is, some socially isolated individuals may not feel lonely; in contrast, there may be individuals who feel lonely even when they have a lot of social networks. The former is a condition of "desired solitude" (Campagne, 2019), and we hypothesized this state may have fewer negative effects on health than "undesired solitude", in which people experience fewer social connections than wanted. This classification of human according to the typology of social isolation and loneliness has been empirically confirmed in a population-based study that proposed three different groups: those with small social network and highest loneliness, those with moderate loneliness irrespective of social network size, and those with large social network and lowest loneliness (Capitanio et al., 2014).

Inflammation may affect sensitivity to social cues and increase the desire to get along with others (Eisenberger et al., 2017; Smith et al., 2020), thus the reverse causality cannot be ruled out. In the current study, we were able to overcome this limitation by using the COVID-19 pandemic as a social experiment since a change in frequency of social contact would occur irrespective of their wills. We aimed to reveal the impact of social isolation and loneliness on chronic inflammation during the COVID-19 outbreak in Japan. The country declared a proclamation of state of emergency in April and May 2020, under which people were required to stay home. Therefore, we examined the association of social isolation and loneliness, that is, four groups of social isolation and loneliness (socially connected and not lonely, socially isolated but not lonely, socially connected but lonely, and socially isolated and lonely) with chronic inflammatory markers (NLR and CRP). Considering that previous studies were biased towards older adults, and gender and age differences in inflammation levels by social relationships were reported (Eguchi et al., 2016; Loucks et al., 2006; Vingeliene et al., 2019), we also performed gender-stratified and age-stratified analyses.

2. Material and methods

2.1. Participants

The current study used data from the "Utsunomiya COVID-19 seROprevalence Neighborhood Association (U-CORONA)" study initiated to assess the seroprevalence of COVID-19 in Utsunomiya City, Japan (Nawa et al., 2020). The survey was conducted from 14th June 2020 to 5th July 2020, after the first but before the second wave of outbreak in Japan. The study invitations and questionnaires were sent to 2290 people (1973 adults aged 18 years or older; and 317 children aged below 18 years) in 1000 households randomly selected from the Utsunomiya City basic resident registry, and collected at the survey site along with written informed consent. A total of 649 adults and 104 children returned the questionnaire (response rate: 32.9%) and 644 adult and 100 child participants underwent blood test (participation rate: 32.5%). We included only adult participants, and excluded samples without data on both social isolation and loneliness (n = 20). Finally, 624 participants were analyzed. Comparing to the analytical sample with the excluded participants, the included samples were younger (mean age: 51.4 vs 63.0 years, p-value (p) < 0.01), had higher income (percentage of whose income below JPY3 million: 61.1 vs 22.9%, $p < 0.01$), and were highly educated (percentage of who finishied university or graduate school: 14.3 vs 42.6%, $p = 0.02$) (Supplementary Table 1). This study was approved by the research ethics committee at Tokyo Medical and Dental University.

2.2. Social isolation and loneliness

Social isolation was evaluated with the number of social roles, which are only counted if the respondent interacted with at least one person regularly within that role during the pandemic (from March 2020 onward, that is, during the past four months). Based on the Cohen's Social Network Index (Cohen et al., 1997), the total number of types of social roles was assessed by asking "what kind of people do you meet and talk to on a regular basis? Please circle the appropriate social roles." with the following choices: spouse, child, parent, relative, neighbor, colleague, group member (e.g., club, gym, lesson, religious organizations), friend and other. The inversed total number of roles was calculated, which ranged from 0 to 9, with higher scores indicating severer social isolation. Loneliness was measured using the Japanese version of the 10-items UCLA Loneliness Scale Version 3 (Arimoto and Tadaka, 2019; Russell, 1996). The responses were deemed valid if the number of missing items was less than or equal to four. The Cronbach's alpha for the current population was 0.83. The scores ranged from 10 to 40, with higher scores indicating greater loneliness. The distribution of social isolation and loneliness scores are shown in Supplementary Fig. 1.

We dichotomized the population into socially isolated (social isolation score lies above the 50th percentile, i.e., higher than 6 (i.e., having 0, 1 or 2 social roles)) vs non-isolated people, and into lonely (loneliness score lies above the 80th percentile, i.e., score equals to or over 23) vs non-lonely people. Based on a previous definition (Smith et al., 2020), another cutoff was also applied as sensitivity analysis defining socially isolated people as those with social isolation score higher than 7 (i.e., having 0 or 1 social role). The cutoff of 80th percentile was defined

based on the report from Japanese version of Values in a Crisis Survey conducted in May 2020 showing 30% of participants felt lonely (Keio University, 2020). Further, participants were categorized into four groups: "Nonisolated-Nonlonely" (i.e., socially connected and non-lonely), "Isolated-Nonlonely" (i.e., socially isolated but non-lonely), "Nonisolated-Lonely" (i.e., socially connected but lonely), and "Isolated-Lonely" (i.e., socially isolated and lonely).

To assess whether social isolation and loneliness in this study were affected by the COVID-19 pandemic, we compared lifestyle change (working style, events related to COVID-19 among family members and frequency of contact) due to the pandemic and infection in the four groups described above (i.e., Nonisolated-Nonlonely, Isolated-Non-lonely, Nonisolated-Lonely, Isolated-Lonely) (Supplementary Table 2). We found that working style and frequency of contact were different across groups; most participants in Nonisolated-Nonlonely and Nonisolated-Lonely groups reported working in the office (53.0% and 55.1%) and relatively higher percentage of people of those in Nonisolated-Lonely and Isolated-Lonely groups tended to telework (13.5% and 15.2%, $p = 0.01$). The incidence of COVID-19 related events such as self-isolation due to symptoms among family members was very low, and did not differ among the four groups ($p = 0.44$). Isolated-Lonely participants perceived almost no changes in contact frequency, while participants in the other three groups reported much or slight decrease in frequency ($p = 0.022$).

2.3. Chronic inflammation markers

Blood samples were collected at the survey site and neutrophils and lymphocytes counts were measured using the automatic hematology analyzer Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan) (Aguadero et al., 2018). High-sensitivity CRP was measured using nephelometry on the Behring Nephelometer II analyzer (BN II; Siemens Healthcare Diagnostics, Tokyo, Japan) with a lower limit of detection of 0.05 mg/L. CRP levels of less than 0.05 mg/L were treated as 0.05 mg/L, and CRP values exceeding 10 mg/L, which is typically indicative of acute inflammation following active infection or injury (Pearson et al., 2003), were excluded from analysis since our focus is chronic inflammation. NLR was calculated by dividing the count of neutrophils by lymphocyte counts, and log-transformed to approximate to the normal distributions together with CRP concentration.

2.4. Covariates

We assessed the following variables in the questionnaire: age, sex, household income, educational attainment of the head of family, medical history (seasonal allergies (e.g., hay fever), asthma or other respiratory diseases, heart diseases, kidney diseases, immune diseases, diabetes or hyperglycemia, malignant tumor (e.g., cancer), arthritis, frequent and severe headaches, seizure disorders (e.g., epilepsy), diseases of stomach and duodenum, severe acne and other skin diseases, mental illnesses (e.g., depression, anxiety), alcohol or other drug problems, intellectual disability, autism spectrum disorder, learning disability, tuberculosis), body mass index (BMI), frequency of exercise, frequency of drinking, history of smoking habit and mental health (assessed with Kessler 6 scale (Furukawa et al., 2008; Kessler et al., 2002)).

The missing values in the covariates ranged from 0% for sex and medical history to 11.1% for household income ($n = 69$), and they were dealt with multilevel multiple imputation by chained equation using R package "mice" (van Buuren and Groothuis-Oudshoorn, 2010) since the data was clustered into the household level. With a maximum of 25 iterations, 100 imputed datasets were obtained. In the following analysis, parameters were obtained from each imputed dataset and aggregated into one estimate using the Rubin's rule (Rubin, 1987).

2.5. Analysis

We used multiple linear regression model, applying generalized estimating equation model to account for clustering at the household level (Hanley et al., 2003) with robust variance estimator and assumption that within household covariances were unstructured using R package "gee" (Halekoh et al., 2006) to examine each association of social isolation and loneliness with chronic inflammatory markers (NLR and CRP). Model 1 was adjusted for age, gender and household socioeconomic status (household income and educational attainment of the head of family) to consider the variability in values of inflammatory markers and confounding. Model 2 was adjusted for lifestyle factors (i.e., frequency of exercise and drinking, smoking habits, body mass index (BMI)) and the number of medical histories in addition to Model 1. Model 3 was further adjusted for depressive symptoms in addition to Model 2, all of whose confounders could be both confounders and mediators. In Model 4, social isolation and loneliness were mutually adjusted, i.e., loneliness was adjusted in addition to Model 3 for social isolation and social isolation was adjusted in addition to Model 3 for loneliness. Since there was some evidence on the interaction by gender in our sample, we further stratified by gender.

For sensitivity analysis, CRP values were dichotomized with a cutoff point of 3 mg/L according to the clinically relevant cut points (Ridker, 2003). Also, loneliness score was dichotomized with cutoffs of 80 and 90 percentiles of the population scores (i.e., 24 and 26 points, separately) and reanalyzed to check the linearity.

Then, multiple linear regression models with generalized estimating equation model to take into account clustering at the household level were also applied to examine the association between the four groups of social isolation and loneliness status (i.e., "Nonisolated-Nonlonely" vs "Isolated-Nonlonely" vs "Nonisolated-Lonely" vs "Isolated-Lonely") and chronic inflammatory markers. Model was adjusted for age, gender, household socioeconomic status, lifestyle factors, the number of medical histories and depressive symptoms. Further, based on evidence on interaction by age and gender, stratified analyses were conducted for men and women, and the working-aged (aged under 65 years) and older people (aged equal to or more than 65 years), separately. All the analyses were repeated with a sample excluding those who were diagnosed as COVID-19 positive using chemiluminescence immunoassay (CLIA) method (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China (Jin et al., 2020); the detailed description can be obtained from elsewhere (Nawa et al., 2020)) ($n = 3$), although they were not recognized as being infected before the study. Analyses were conducted using R version 4.0.2 (R core Team, 2020).

3. Results

Table 1 shows the sample characteristics categorized by social isolation and loneliness status. Briefly, participants under Isolated-Lonely condition were dominated by men (63.6%) and low-income households (household income below JPY6 million: 69.9%; over JPY10 million: 4.8%). Also, more participants in Isolated-Lonely group had experience of smoking (both in the past and currently: 38.5%) compared to the other group, especially Isolated-Nonlonely group (both in the past and currently: 21.7%). A total of 36.4% of Nonisolated-Lonely participants were overweight, accounting for the highest prevalence among four groups. Nonisolated-Lonely and Isolated-Lonely participants showed severer depressive symptoms (median K6 score: 5.0 and 3.0, respectively) compared to participants who were Nonisolated-Nonlonely and Isolated-Nonlonely (median K6 score: both 1.0). Correlations among social isolation score, loneliness score and demographics are presented in Supplementary Table 3. Social isolation and loneliness showed a weak correlation ($r = 0.08$, $p < 0.05$).

The association of social isolation and loneliness scores with NLR and CRP for total and stratified by gender are shown in Table 2. Social isolation score was not associated with NLR and CRP, but a higher

Table 1
Characteristics of analytical sample (n = 624).

	Total (n = 624)		Nonisolated-Nonlonely (n = 304)		Isolated-Nonlonely (n = 165)		Nonisolated-Lonely (n = 89)		Isolated-Lonely (n = 66)		P-value [e]
	Mean/N	SD/% [a]	Mean/N	SD/% [a]	Mean/N	SD/% [a]	Mean/N	SD/% [a]	Mean/N	SD/% [a]	
Age											<0.01
18-<40	162	26.0	74	24.6	48	29.4	23	26.1	17	25.8	
40-<65	290	46.5	157	52.2	49	30.1	50	56.8	34	51.5	
65+	166	26.6	70	23.3	66	40.5	15	17.0	15	22.7	
Missing	6	1.0	3	1.0	2	1.2	1	1.1	0	0.0	
Gender											0.02
Men	293	47.0	133	43.8	73	44.2	45	50.6	42	63.6	
Women	331	53.0	171	56.2	92	55.8	44	49.4	24	36.4	
Household income (JPY)											0.01
0 - <3 M	127	22.9	47	17.5	44	29.9	17	22.4	19	30.2	
3 - <6 M	181	32.6	98	36.4	37	25.2	21	27.6	25	39.7	
6 - <10 M	170	30.6	83	30.9	42	28.6	29	38.2	16	25.4	
+10 M	77	13.9	41	15.2	24	16.3	9	11.8	3	4.8	
Missing	69	11.1	35	11.5	18	10.9	13	14.6	3	4.5	
Household education level [b]											0.54
Junior/ high	243	40.8	125	43.3	61	38.1	35	41.7	22	34.9	
Vocational	99	16.6	50	17.3	25	15.6	16	19.0	8	12.7	
University/ graduate	254	42.6	114	39.4	74	46.3	33	39.3	33	52.4	
Missing	28	4.5	15	4.9	5	3.0	5	5.6	3	4.5	
Frequency of exercise											0.31
No exercise	305	49.3	149	49.8	72	43.6	52	58.4	32	48.5	
1–2 days/ w	157	25.4	72	24.1	49	29.7	23	25.8	13	19.7	
3–4 days/ w	74	12.0	37	12.4	22	13.3	5	5.6	10	15.2	
5–7 days/ w	83	13.4	41	13.7	22	13.3	9	10.1	11	16.7	
Missing	5	0.8	5	1.6	0	0.0	0	0.0	0	0.0	
Frequency of drinking											0.75
No/ rarely	306	49.7	140	46.8	81	50.0	48	53.9	37	56.1	
1-few times/ m	49	8.0	24	8.0	14	8.6	7	7.9	4	6.1	
1-few times/ w	131	21.3	61	20.4	37	22.8	18	20.2	15	22.7	
+1 times/ d	130	21.1	74	24.7	30	18.5	16	18.0	10	15.2	
Missing	8	1.3	5	1.6	3	1.8	0	0.0	0	0.0	
Smoking experience											0.02
Never	425	69.8	201	68.1	126	78.3	58	65.9	40	61.5	
Yes, in the past	107	17.6	61	20.7	19	11.8	17	19.3	10	15.4	
Yes, currently	77	12.6	33	11.2	16	9.9	13	14.8	15	23.1	
Missing	15	2.4	9	3.0	4	2.4	1	1.1	1	1.5	
Number of past medical history [c,d]	1.0	1.0	0.9	1.0	0.9	1.0	1.2	2.0	1.0	1.8	0.15
BMI											0.13
Underweight	36	5.8	22	7.6	9	5.7	3	3.4	2	3.0	
Normal	409	65.5	204	70.6	105	66.0	53	60.2	47	71.2	
Overweight	157	25.2	63	21.8	45	28.3	32	36.4	17	25.8	
Missing	22	3.5	15	4.9	6	3.6	1	1.1	0	0.0	
Depressive symptoms (K6 score) [d]	2.0	5.0	1.0	4.0	1.0	3.0	5.0	7.0	3.0	5.8	<0.01
Missing	8	1.3	4	1.3	3	1.8	1	1.1	0	0.0	

a: % of missing was calculated comparing to total sample size of each group; % of other categories was calculated comparing to number of available data.
b: defined as educational attainment of head of household.
c: assessed medical conditions were hay fever, asthma, cardiological disease, renal disease, immune disease, diabetes, cancer, arthritis, epilepsy/ convulsion, gastroenteric disease, dermatological disease, mental disease, alcohol/ drug abuse, intellectual disabilities, autism spectrum disorder, learning disabilities, tuberculosis.
d: median and interquartile range are shown due to non-normal distribution.
e: statistical tests performed: Fisher's Exact test for categorical data with simulated p-value; Kruskal-Wallis test for continuous data.
Abbreviations: BMI, body mass index; JPY, Japanese yen.

loneliness score was associated with higher NLR among men in Model 1 (i.e., adjusted for age and household socioeconomic status) (B = 0.009, 95%CI = 0.001 to 0.02), which was attenuated after adjusted for current lifestyle, past medical history, BMI and depressive symptoms (B = 0.007, 95%CI = −0.002 to 0.02). Interestingly, higher loneliness score was associated with lower CRP in total sample (B = −0.019, 95%CI = −0.04 to −0.001), and this directional association was significant only among women (B = −0.028, 95%CI = −0.08 to −0.004) but not men (B = −0.015, 95%CI = −0.04 to 0.01). Further, social isolation and loneliness were mutually adjusted as Model 4, in which we found loneliness remained significantly inversely associated with CRP in total and women after adjustment for social isolation. The directions and significances of association did not change when using dichotomized loneliness score as independent variable.

Table 3 shows the association between the four groups of social isolation and loneliness and NLR for men and women, and the working-age and older people, respectively. We found Isolated-Lonely condition was associated with higher NLR among men (B = 0.141, 95%CI = −0.01 to 0.29) compared to Nonisolated-Nonlonely men, but not among women. The association was also stratified by age group, where we found no significant association. The associations of four groups of social isolation and loneliness status and CRP by gender and age group are shown in Table 4. Nonisolated-Lonely condition was associated with lower CRP level among women (B = −0.462, 95%CI = −0.82 to −0.10) and the working-age population (B = −0.495, 95%CI = −0.76 to −0.23) compared to their Nonisolated-Nonlonely counterparts. These

Table 2
Association of social isolation and loneliness with chronic inflammation markers in total and stratified by gender.

		NLR			CRP [a]			CRP (<3 vs 3–10 mg/l) [b]		
		B	95%CI	P-value	B	95%CI	P-value	B	95%CI	P-value
Total (n = 624)										
Social isolation	Model 1	−0.001	−0.03 to 0.03	0.968	0.007	−0.07 to 0.08	0.851	−0.243	−0.55 to 0.06	0.117
	Model 2	−0.004	−0.03 to 0.02	0.769	0.010	−0.05 to 0.07	0.771			
	Model 3	−0.003	−0.03 to 0.02	0.824	0.011	−0.05 to 0.07	0.743			
	Model 4	−0.004	−0.03 to 0.02	0.765	0.016	−0.05 to 0.08	0.624			
Loneliness	Model 1	0.006	−0.001 to 0.01	0.077	−0.002	−0.02 to 0.02	0.811	−0.043	−0.14 to 0.05	0.359
	Model 2	0.005	−0.001 to 0.01	0.105	−0.013	−0.03 to 0.005	0.155			
	Model 3	0.003	−0.004 to 0.01	0.382	−0.019	−0.04 to −0.001	0.041			
	Model 4	0.003	−0.004 to 0.01	0.364	−0.019	−0.04 to −0.001	0.039			
Men (n = 293)										
Social isolation	Model 1	0.032	−0.003 to 0.07	0.076	0.045	−0.06 to 0.15	0.401	−0.003	−0.43 to 0.42	0.991
	Model 2	0.023	−0.01 to 0.06	0.201	0.049	−0.04 to 0.14	0.289			
	Model 3	0.022	−0.01 to 0.06	0.222	0.047	−0.04 to 0.14	0.311			
	Model 4	0.020	−0.02 to 0.05	0.264	0.053	−0.04 to 0.14	0.256			
Loneliness	Model 1	0.009	0.001 to 0.02	0.031	−0.003	−0.03 to 0.02	0.855	−0.077	−0.20 to 0.04	0.212
	Model 2	0.007	−0.001 to 0.02	0.091	−0.012	−0.04 to 0.01	0.354			
	Model 3	0.007	−0.002 to 0.02	0.132	−0.015	−0.04 to 0.01	0.243			
	Model 4	0.006	−0.002 to 0.01	0.156	−0.016	−0.04 to 0.01	0.208			
Women (n = 331)										
Social isolation	Model 1	−0.025	−0.06 to 0.01	0.156	−0.031	−0.13 to 0.07	0.557	−0.609	−1.07 to −0.15	0.010
	Model 2	−0.025	−0.06 to 0.01	0.146	−0.020	−0.11 to 0.07	0.674			
	Model 3	−0.022	−0.05 to 0.01	0.200	−0.012	−0.10 to 0.08	0.793			
	Model 4	−0.021	−0.05 to 0.01	0.213	−0.002	−0.10 to 0.09	0.972			
Loneliness	Model 1	0.000	−0.01 to 0.01	0.945	−0.005	−0.03 to 0.02	0.722	−0.005	−0.14 to 0.13	0.946
	Model 2	0.000	−0.01 to 0.01	0.928	−0.017	−0.04 to 0.01	0.145			
	Model 3	−0.003	−0.01 to 0.01	0.604	−0.028	−0.05 to −0.004	0.022			
	Model 4	−0.002	−0.01 to 0.01	0.696	−0.028	−0.05 to −0.004	0.024			

Abbreviations; NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein.
NLR and continuous CRP were transformed to log scale.
Social isolation score ranged from 0 to 9, and loneliness score ranged from 10 to 40.
Model 1 adjusted for age, gender (only model for all participants) and household socioeconomic status (household income and educational attainment of head of family).
Model 2 adjusted for model 1 + current lifestyle (frequency of exercise, drinking alcohol and smoking), medical history and BMI.
Model 3 adjusted for model 2 + depressive symptoms.
Model 4 adjusted for model 3 + loneliness (model for social isolation) or social isolation (model for loneliness).
The analysis was repeated across imputed datasets and aggregated using the Rubin's rule.
a: 17 and 7 participants were excluded due to missing CRP data and high CRP values (>10 mg/L), respectively.
b: Result for dichotomized CRP adjusted for age, sex and household socioeconomic status due to violation of positivity.

association did not change after excluding participants diagnosed as COVID-19 positive. In a sensitivity analysis that defined socially isolated as those with 0 or 1 social role, the associations with CRP remained similar but associations with NLR, especially in Isolated-Lonely status among men, were attenuated and not reached significance although their directional associations were similar to those of the median-split definition of social isolation (Supplementary Table 4).

4. Discussion

Although the association of social isolation and loneliness with chronic inflammation has been reported, this study was the first to examine the association of four groups based on social isolation and loneliness statuses with chronic inflammation during the COVID-19 pandemic. We showed experience of Isolated-Lonely (both socially isolated and feeling lonely) was associated with higher NLR among men while experience of Nonisolated-Lonely (not socially isolated but feeling lonely) was associated with lower CRP level among women and the working-age population. Beyond the previous studies showing the link between social isolation and loneliness and chronic inflammation (Shankar et al., 2011; Smith et al., 2020; Uchino et al., 2018; Walker et al., 2017), we demonstrated novel evidence on the interplay between social isolation and loneliness, which differs by gender and age in our sample.

To the best of our knowledge, the current study is the first to investigate the differences in NLR among the four groups based on social isolation and loneliness experiences. NLR is the ratio between neutrophil and lymphocyte. During inflammation, neutrophils are the first white blood cells to be recruited and their numbers are increased by pro-inflammatory cytokines. In contrast, lymphocytes do not show significant changes in numbers in early-stage inflammation, but are decreased or exhausted as a result of cell damage and speed-up of apoptosis (Actor, 2012; Feng et al., 2020; Mazza et al., 2018). Therefore, higher NLR indicates chronic systemic inflammation (Feng et al., 2020; Guthrie et al., 2013). We showed Isolated-Lonely was associated with higher inflammation level, which mainly appeared among men. Although we did not have enough evidence on older people due to insufficient sample size, Isolated-Lonely was possibly associated with higher NLR in older people as well.

The underlying mechanisms on the positive association of social isolation and loneliness with chronic inflammation level have been considered as a change in health behaviors such as sleep and physical activities (Hawkley and Cacioppo, 2010, 2003; Kiecolt-Glaser et al., 2010), altered autonomic and neuroendocrine systems due to chronic stress (Cacioppo et al., 2011; Hänsel et al., 2010; Hawkley and Cacioppo, 2010, 2003; Kiecolt-Glaser et al., 2010; McCray and Agarwal, 2011), and promotion of inflammation-related gene encoding (Cole et al., 2011; Hawkley and Cacioppo, 2010; Miller, 2011). Additionally, from an evolutionary perspective, social isolation and loneliness are dangerous states, and evolutionary reaction to increase the chance of survival without turning to anyone for help may manifest as a highly inflammatory state (Leschak and Eisenberger, 2019). As for the first and second

Table 3
Association between social isolation/ loneliness status and NLR stratified by gender and age groups.

	N (%)	Median NLR	B	95%CI	P-value
Total (n = 624)					
Nonisolated-Nonlonely	304 (48.7)	1.63	0	ref.	
Isolated-Nonlonely	165 (26.4)	1.66	−0.005	−0.08 to 0.07	0.893
Nonisolated-Lonely	89 (14.3)	1.66	−0.020	−0.12 to 0.08	0.708
Isolated-Lonely	66 (10.6)	1.85	0.086	−0.02 to 0.19	0.115
Men (n = 293)					
Nonisolated-Nonlonely	133 (45.4)	1.53	0	ref.	
Isolated-Nonlonely	73 (24.9)	1.62	−0.005	−0.10 to 0.10	0.919
Nonisolated-Lonely	45 (15.4)	1.63	−0.029	−0.16 to 0.10	0.657
Isolated-Lonely	42 (14.3)	1.91	0.141	−0.01 to 0.29	0.061
Women (n = 331)					
Nonisolated-Nonlonely	171 (51.7)	1.73	0	ref.	
Isolated-Nonlonely	92 (27.8)	1.70	−0.013	−0.12 to 0.09	0.795
Nonisolated-Lonely	44 (13.3)	1.88	−0.031	−0.19 to 0.13	0.706
Isolated-Lonely	24 (7.3)	1.74	−0.004	−0.15 to 0.15	0.959
Working-age (<65YO) (n = 457)					
Nonisolated-Nonlonely	234 (51.2)	1.67	0	ref.	
Isolated-Nonlonely	98 (21.4)	1.66	−0.024	−0.12 to 0.07	0.618
Nonisolated-Lonely	74 (16.2)	1.65	−0.044	−0.16 to 0.07	0.458
Isolated-Lonely	51 (11.2)	1.89	0.050	−0.07 to 0.17	0.396
Older adults (65YO +) (n = 167)					
Nonisolated-Nonlonely	70 (41.9)	1.50	0	ref.	
Isolated-Nonlonely	67 (40.1)	1.66	0.102	−0.02 to 0.23	0.112
Nonisolated-Lonely	15 (9.0)	1.78	0.014	−0.25 to 0.28	0.913
Isolated-Lonely	15 (9.0)	1.81	0.131	−0.14 to 0.41	0.349

Abbreviations; NLR, neutrophil-to-lymphocyte ratio.
NLR was transformed to log scale for statistical analysis.
Isolated was defined as having 0, 1 or 2 social roles, and nonisolated was defined as having 3 or more social roles.
Model adjusted for age, gender (only model for all, working-age and older people participants), household socioeconomic status (household income and educational attainment of head of family), current lifestyle (frequency of exercise, drinking alcohol and smoking), medical history, BMI and depressive symptoms.
The analysis was repeated across imputed datasets and aggregated using the Rubin's rule.
84% confidence intervals of the coefficients of the association with NLR among males: −0.07 to 0.07; −0.05 to 0.15; 0.09 to 0.29 for Isolated-Nonlonely, Nonisolated-Lonely and Isolated-Lonely, respectively; indicating significant difference between Isolated-Nonlonely and Isolated-Lonely.

Table 4
Association between social isolation/ loneliness status and CRP stratified by gender and age groups.

	N (%)	Median CRP (mg/L)	B	95%CI	P-value
Total (n = 624)					
Nonisolated-Nonlonely	304 (48.7)	0.30	0	ref.	
Isolated-Nonlonely	165 (26.4)	0.32	0.001	−0.19 to 0.19	0.994
Nonisolated-Lonely	89 (14.3)	0.33	−0.276	−0.53 to −0.03	0.031
Isolated-Lonely	66 (10.6)	0.38	−0.029	−0.33 to 0.28	0.852
Men (n = 293)					
Nonisolated-Nonlonely	133 (45.4)	0.38	0	ref.	
Isolated-Nonlonely	73 (24.9)	0.47	0.071	−0.24 to 0.38	0.647
Nonisolated-Lonely	45 (15.4)	0.43	−0.117	−0.46 to 0.23	0.509
Isolated-Lonely	42 (14.3)	0.43	0.056	−0.32 to 0.43	0.767
Women (n = 331)					
Nonisolated-Nonlonely	171 (51.7)	0.23	0	ref.	
Isolated-Nonlonely	92 (27.8)	0.29	0.007	−0.26 to 0.27	0.958
Nonisolated-Lonely	44 (13.3)	0.15	−0.462	−0.82 to −0.10	0.011
Isolated-Lonely	24 (7.3)	0.30	−0.185	−0.63 to 0.26	0.418
Working-age (<65YO) (n = 457)					
Nonisolated-Nonlonely	234 (51.2)	0.27	0	ref.	
Isolated-Nonlonely	98 (21.4)	0.28	−0.188	−0.43 to 0.05	0.127
Nonisolated-Lonely	74 (16.2)	0.23	−0.495	−0.76 to −0.23	<0.001
Isolated-Lonely	51 (11.2)	0.30	−0.138	−0.50 to 0.23	0.460
Older adults (65YO +) (n = 167)					
Nonisolated-Nonlonely	70 (41.9)	0.38	0	ref.	
Isolated-Nonlonely	67 (40.1)	0.45	0.265	−0.06 to 0.59	0.104
Nonisolated-Lonely	15 (9.0)	0.58	0.401	−0.18 to 0.98	0.172
Isolated-Lonely	15 (9.0)	0.48	0.182	−0.33 to 0.69	0.482

Abbreviations; CRP, C-reactive protein.
CRP were transformed to log scale for statistical analysis.
Isolated was defined as having 0, 1 or 2 social roles, and nonisolated was defined as having 3 or more social roles.
Model adjusted for age, gender (only model for all, working-age and older people participants), household socioeconomic status (household income and educational attainment of head of family), current lifestyle (frequency of exercise, drinking alcohol and smoking), medical history, BMI and depressive symptoms.
The analysis was repeated across imputed datasets and aggregated using the Rubin's rule.
17 and 7 participants were excluded due to missing CRP data and high CRP values (>10 mg/L), respectively.

hypotheses, we adjusted for current lifestyle and depressive symptoms, which could not explain all the associations. However, in addition to the potential residual confounding, previous studies reported that people under chronic stress including loneliness showed insensitivity of NLR to cortisol concentration (Cohen et al., 2012; Cole, 2008), indicating that neuroendocrine dysfunction may partially explain a pathway to elevated NLR. Women have been found to be resistant to stress because of sex hormones (McEwen, 2010), which also strengthened our findings that the association was observed mainly among men and older people. Another possible reason is that these groups are not very sociable

compared to other groups (Brook and Schmidt, 2020). The aforementioned mechanisms have been discussed for social isolation and loneliness independently, but our results implied that when both social isolation and loneliness exist simultaneously, these mechanisms may be further enhanced.

Counterintuitively, we found Nonisolated-Lonely was associated with *lower* CRP level among women and the working-age population. There have been several studies reporting similar direction of associations. A study of healthy, community-dwelling adults aged 30–54 years in the US showed that the frequency of negative marital interaction was associated with lower CRP levels (Bajaj et al., 2016), and another reported women aged over 50 years in the UK who felt lonely showed reduction in ferritin levels, a biomarker of chronic inflammation (Vingeliene et al., 2019). In addition, perceived support was associated with higher inflammation levels indexed by CRP and IL-6 among adults in Taiwan and the US, although reverse causation was likely (Glei et al., 2012). A Japanese study showed that men aged 40–69 years with higher perceived stress had lower CRP levels (Shimanoe et al., 2018). Thus, in a cross-sectional study, it is possible that those who were exposed to stress, such as an unsatisfactory well-connected social environment, may show lower CRP level.

We could not deny the possibility of chance findings and the existence of confounding; however, median CRP levels of Nonisolated-Lonely women and working-age population were significantly lower than those of their counterparts. This may indicate that women and working-age adults of Nonisolated-Lonely may be in a distinct condition, that is, a Nonisolated-Lonely condition reflects a situation where people are connected physically or socially but not mentally (i.e., connected via superficial relationships). Since they cannot interact satisfactorily with people in the network, they may not be able to receive optimal level of stress in interaction even though they have many connections, which may be reflected in the lower CRP levels. The notion that the optimal level of stress is advantageous for survival is justified in the context of stress-response hormesis; stress is beneficial at a low level but harmful at high level (Gems and Partridge, 2008), which was demonstrated as the effect of stress in longevity in animal models (Gems and Partridge, 2008) and the relationship between cortisol and cognitive functions in human (Lupien et al., 2007). Alternatively, we can also speculate that stressed Nonisolated-Lonely women and working-age population may enjoy anti-inflammatory feedback effect of cortisol (Yeager et al., 2011) more strongly than other groups. Heterogeneity of effects by age is reasonable considering that inversed associations between stressful events related to social relationships and inflammation were observed in younger populations (Bajaj et al., 2016; Glei et al., 2012; Shimanoe et al., 2018), which accorded with our findings.

Another novelty of this study is that we implemented the study after the first wave of COVID-19 pandemic in Japan, which altered social structure dramatically. Thus, we could observe particular characteristics of newly recognized population of Nonisolated-Lonely. Importantly, our findings warranted the assessment of both social isolation and loneliness including the interplay between them since Nonisolated-Lonely population, i.e., socially connected but feeling lonely population, has been neglected but may have different inflammatory conditions. However, we need to acknowledge that we did not have the data on isolation and loneliness status before the pandemic, and could not separate the effects of stable individual differences from pandemic-induced changes in social contact and experienced connection. In this regard, when we assessed lifestyle changes due to COVID-19, we found that lifestyle changes were correlated with social isolation and loneliness status, supporting the possibility that the distinct social relationships during the COVID-19 pandemic may be specifically captured. There also might be reverse causation in that inflammation may elevate loneliness among men and older people, or inflammation may increase the desire in women and the working-age population to get along with others (Eisenberg et al., 2012; Smith et al., 2020). We need more studies, especially longitudinal studies using pre-existing data on social isolation and loneliness in pre-pandemic time, to replicate our findings.

The current study has several limitations. First, there was no information on the duration of social isolation and loneliness. Although we assumed their experiences reflected the impact of the COVID-19 pandemic, it was unclear whether they had experienced them long enough to change their physical condition. Also, we did not mention whether interaction was in person or virtually, and did not define the frequency in "regular basis" in the assessment of social isolation. However, the previous studies did not define those details as well. Second, we assessed social isolation and loneliness via a self-reported questionnaire, but as lonely people tend to rate their social interactions more negatively (Miller, 2011), there might be measurement bias. Third, although we adjusted for potential confounders including socioeconomic status and lifestyle such as exercise, drinking and smoking, medical history and BMI, we did not adjust for medical prescription, which may also affect inflammation. Fourth, although it was a population-based study, the participation rate was not high. Thus, sampling bias was likely. The power may also not be sufficient, in particular, gender- and age-stratified analysis. Further larger population-based study with a higher response rate is warranted.

5. Conclusion

In spite of the potential limitations, our study provided novel evidence on the interplay between social isolation and loneliness. Isolated-Lonely (i.e., socially isolated and feeling lonely) was associated with higher NLR among men while Nonisolated-Lonely (i.e., not socially isolated but feeling lonely) was associated with lower CRP among women and the working-age population (aged 18–64 years). Our findings emphasized the importance of assessment of both subjective and objective social relationships not only for future research, but also for the intervention perspective especially under the COVID-19 pandemic. Our results indicated that not only socially isolated people but also those who were socially connected with loneliness may need help. To effectively mitigate the impact of COVID-19 pandemic on health, particularly sociopsychological health aside from infection, future studies to identify the populations at risk and the complex interplay between subjective and objective social relationships are warranted.

Acknowledgement

We thank Euma Ishii, Yoshifumi Fukuya, Keitaro Miyamura, Yu Funakoshi, and medical students at TMDU, who participated in the data collection, medical staffs in Kuramochi Clinic Interpark, and all the participants in this study.

Declaration of Competing Interest

Authors declare no Conflict of Interests for this article.

Funding

This research was supported by the Japan Agency for Medical Research and Development (AMED) under Grant Number 2033648, Pfizer Health Research Foundation, Innovative Research Program on Suicide Countermeasures (IRPSC), and Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number 19H04879).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.03.007.

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