

CASE REPORT

Diagnostic performance of peripheral leukocyte telomere G-tail length for detecting breast cancer

Yumiko Koi^{1,2} | Yasuhiro Tsutani²  | Yukie Nishiyama³ | Miyuki Kanda⁴  |
Yoshitomo Shiroma³ | Yuki Yamamoto³  | Shinsuke Sasada²  | Tomoyuki Akita⁵  |
Norio Masumoto²  | Takayuki Kadoya²  | Ryou-u Takahashi³  | Junko Tanaka⁵  |
Morihiro Okada²  | Hidetoshi Tahara^{3,4,6} 

¹Surgical Oncology, Division of Radiation Biology and Medicine, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

²Department of Surgical Oncology, Research Center for Radiation Casualty Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

³Department of Cellular and Molecular Biology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

⁴Collaborative Laboratory of Liquid Biopsy, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

⁵Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan

⁶The Research Center for Drug Development and Biomarker Discovery, Hiroshima University, Hiroshima, Japan

Correspondence

Hidetoshi Tahara, Department of Cellular and Molecular Biology, Institute of Biomedical and Health Sciences, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8553, Japan.
Email: toshi@hiroshima-u.ac.jp

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Abstract

The telomere G-tail (G-tail) plays an essential role in maintaining chromosome stability. In this study, we assessed the leukocyte G-tail length of breast cancer (BC) patients and cancer-free individuals and evaluated the association between the G-tail length and the presence of BC. A significant shortening of the median G-tail length was observed in BC patients compared with cancer-free individuals and was found in the early phase of BC. Our study indicated that the leukocyte G-tail length might be a potential biomarker for BC detection.

KEYWORDS

biomarker, breast cancer, leukocyte, telomere, telomere G-tail

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1 | INTRODUCTION

Telomeres are simple repetitive DNA sequences at the ends of linear chromosomes that play an important role in chromosome integrity.¹ At the terminal 3'-end of the telomere, the G-rich repeat extends beyond the complementary C-rich repeat to form a single strand, known as the telomere 3'-overhang or telomere G-tail (G-tail).² The G-tail is a key component of the protective T-loop formation that hides the terminal end of the telomere to prevent it from being recognized as damaged DNA.^{3,4} A shortened G-tail is unable to maintain the T-loop formation, indicating that the G-tail is essential for chromosome stability.

Breast cancer (BC) is one of the most frequently diagnosed cancers worldwide.⁵ Telomere shortening in BC tissue has been reported in comparison with the adjacent benign breast tissue.^{6,7} Additionally, a relationship between the shortening of telomere length in leukocytes and BC risk or prognosis has been reported.^{8,9} Consequently, telomere length has attracted attention as a biomarker for the detection of BC and as a treatment target for BC. However, G-tail length in BC patients has not been assessed. In this study, we evaluated the association between G-tail length and the presence of BC by comparing the G-tail length in BC patients with that in cancer-free individuals.

2 | MATERIALS AND METHODS

2.1 | Ethics

This study was approved by the Institutional Research and Ethics Committee at Hiroshima University Hospital, Hiroshima, Japan. Written informed consent was obtained from all participants prior to study enrollment.

2.2 | Study participants

The study cohort comprised 153 participants, consisting of BC patients (BC group; n = 70) and cancer-free individuals (control group; n = 83). We recruited BC patients in clinical stages 0-III who underwent surgery between November 2016 and December 2017 at the Hiroshima University Hospital. The control group included age-matched female individuals who were revealed to be BC-free based on screening by mammography, ultrasonography, or an interview. Participants who had any cancer history or active inflammatory disease were considered ineligible for this study.

2.3 | Assessment of BC by immunohistochemistry

Estrogen receptor (ER) or progesterone receptor (PR) positivity was defined as when 1% or more of the cells were stained by immunohistochemistry (IHC). Tumors were defined as human epidermal growth factor receptor 2 (HER2)-positive when the IHC score was 2+ or 3+ and was accompanied by *HER2* amplification, as identified by FISH (*HER2* / centromere enumerator probe 17 (CEP17) ratio greater than 2.0).

TABLE 1 Clinicopathologic characteristics of study participants

	BC (n = 70)	Control (n = 83)	P value
Median age (range), years	56 (30-84)	55 (21-80)	.14
BC stage			
0	13 (18.6%)	-	-
I	31 (44.3%)	-	-
II	21 (30.0%)	-	-
III	5 (7.1%)	-	-
Estrogen receptor (n = 57)			
Positive	49 (86.0%)	-	-
Negative	8 (14.0%)	-	-
Progesterone receptor (n = 57)			
Positive	43 (75.4%)	-	-
Negative	14 (24.6%)	-	-
HER2 (n = 57)			
Positive	10 (17.5%)	-	-
Negative	47 (82.5%)	-	-
Leukocytes (range),/ μL (BC n = 34, control n = 43)	5290 (3390-8520)	5900 (3600-10700)	.19
Granulocytes (range),/μL	3175 (1920-6480)	3400 (1900-7500)	.41
Lymphocytes (range),/μL	1680 (990-3840)	2000 (600-4700)	.09
Monocytes (range),/ μL	260 (150-540)	200 (100-400)	.09

Abbreviations: -, not applicable; BC, breast cancer; HER2, human epidermal growth factor receptor 2.

2.4 | Measurement of leukocyte G-tail length and total telomere length

Peripheral whole blood samples were collected from each participant. A telomere hybridization protection assay (HPA) and G-tail HPA were undertaken on the leukocytes to determine the total telomere length and G-tail length, as previously described.^{10,11} The total telomere length and G-tail length were determined using 0.2 μg denatured genomic DNA or 1 μg nondenatured genomic DNA, respectively. The genomic DNA of RKO cells was used as a control to correct for interassay variability. The average coefficient of variance for the G-tail and total telomere length was 4.0% and 4.4%, respectively.

2.5 | Statistical analysis

JMP 12 software (SAS Institute) was used for all statistical analyses. Comparisons between the 2 groups were estimated using

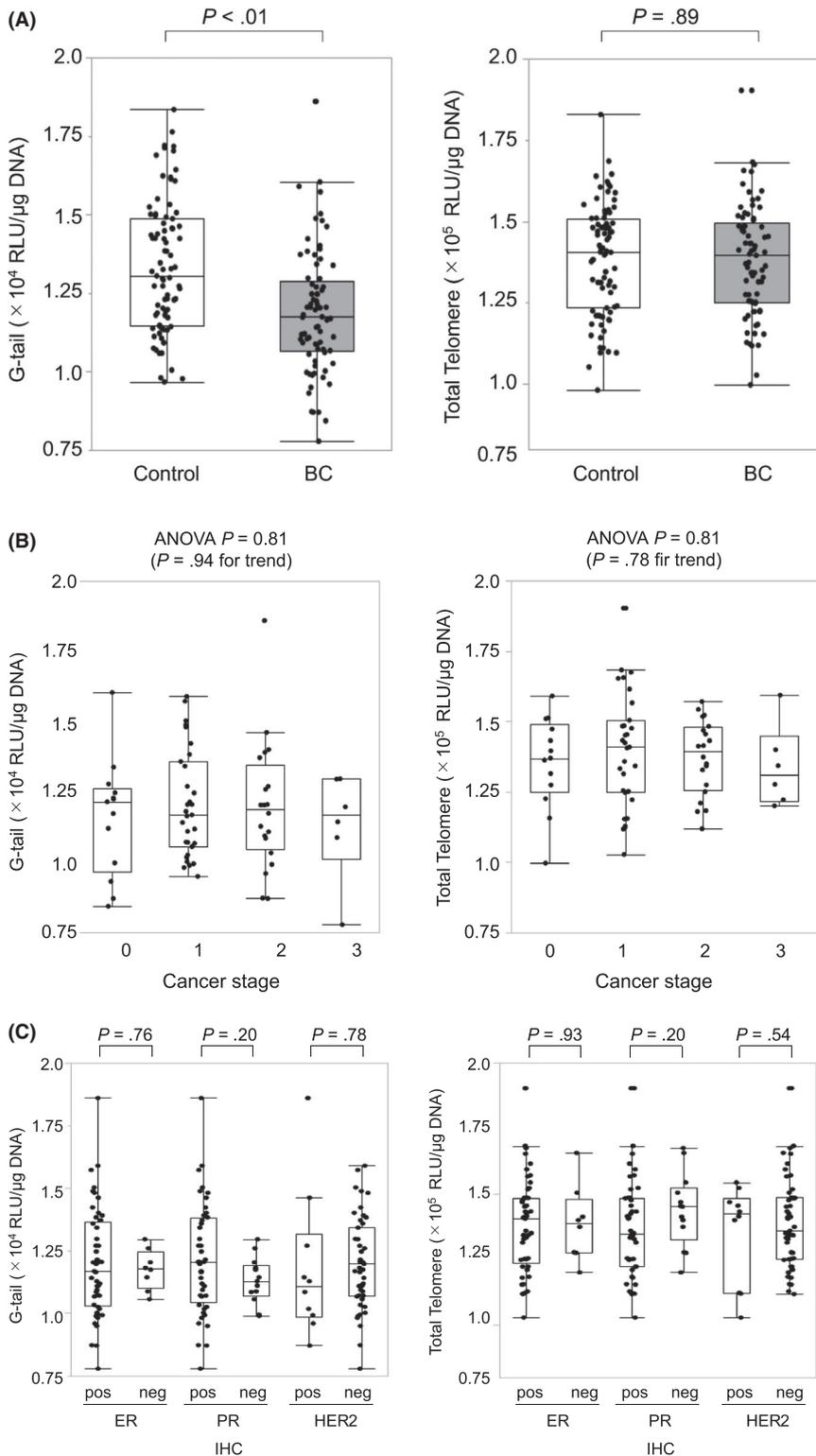


FIGURE 1 Comparison of the G-tail length (left panels) and total telomere length (right panels) in leukocytes between (A) breast cancer (BC) and control groups, (B) among BC patients by BC stage ($n = 70$), and (C) among patients with invasive BC by immunohistochemical (IHC) analysis of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) ($n = 57$). neg, negative; pos, positive; RLU, relative light unit

Student's unpaired *t* test. Comparison among cancer stages was estimated using ANOVA following the Jonckheere-Terpstra test for trend. Spearman's rank correlation analysis was used to calculate associations between the G-tail length or telomere length and the presence of BC. The significance threshold was set at $P < .05$.

3 | RESULTS

The median age for the BC and control group was 56 (range, 30-84) and 55 (range, 21-80) years, respectively ($P = .14$). Thirteen patients (18.6%) had stage 0 cancer. Immunohistochemical analysis revealed 49 (86.0%), 43 (75.4%), and 10 patients (17.5%) with ER-, PR- and

HER2-positive cancer. No significant difference in complete blood count was observed between the BC (n = 34) and control group (n = 43) (Table 1).

The median luminescence signals of the leukocyte total telomere length in the BC and control group were $137\,738.2 \pm 17\,072.95$ and $138\,459.0 \pm 16\,949.77$ relative light units (RLU)/ μg DNA, respectively ($P = .89$, Figure 1A). Interestingly, the median signals of the leukocyte G-tail length were significantly lower in the BC group than in the control group ($11\,886.7 \pm 2008.74$ vs $13\,336.0 \pm 2072.31$ RLU/ μg DNA, $P < .01$). Furthermore, no significant difference between leukocyte G-tail length and the BC stage was identified ($P = .81$), suggesting a significantly shorter leukocyte G-tail length was observed in patients diagnosed with stage 0 (Figure 1B). The IHC staining did not reveal any significant difference for either leukocyte G-tail or total telomere length, with or without stain positivity (Figure 1C). Additionally, the leukocyte G-tail length was positively correlated with the leukocyte total telomere length in the control group ($\rho, 0.29$; $P < .01$); however, there was no correlation between the leukocyte G-tail length and leukocyte total telomere length in the BC group ($\rho, -0.09$; $P = .48$; Figure 2A). Furthermore, the leukocyte G-tail length was negatively correlated with age in the control group ($\rho, -0.29$;

$P < .01$), whereas there was no correlation between the leukocyte G-tail length and age in the BC group ($\rho, -0.09$; $P = .48$; Figure 2B).

4 | DISCUSSION

Our results showed a significant shortage of leukocyte G-tail length in BC patients compared with cancer-free individuals irrespective of cancer status, such as stage or IHC positivity, suggesting that leukocyte G-tail length could be a potential biomarker for BC diagnosis.

Previous studies have reported shortened leukocyte G-tail length were associated with age-related disease.^{12,13} As for cancers, G-tail length shortening in tissue samples has been found to be associated with carcinogenesis, including hepatocellular carcinoma and endometrial cancer.^{14,15} However, studies evaluating leukocyte G-tail length in cancers are sparse. In our study, leukocyte G-tail length was significantly shortened in the BC group compared with cancer-free individuals and the shortening was found in patients the stage 0 BC. It is known that chromosomal aneuploidy is present in the early stage of BC.^{16,17} Additionally, a previous study reported that the G-tail length shortens due to chromosomal instability.¹⁸ Although

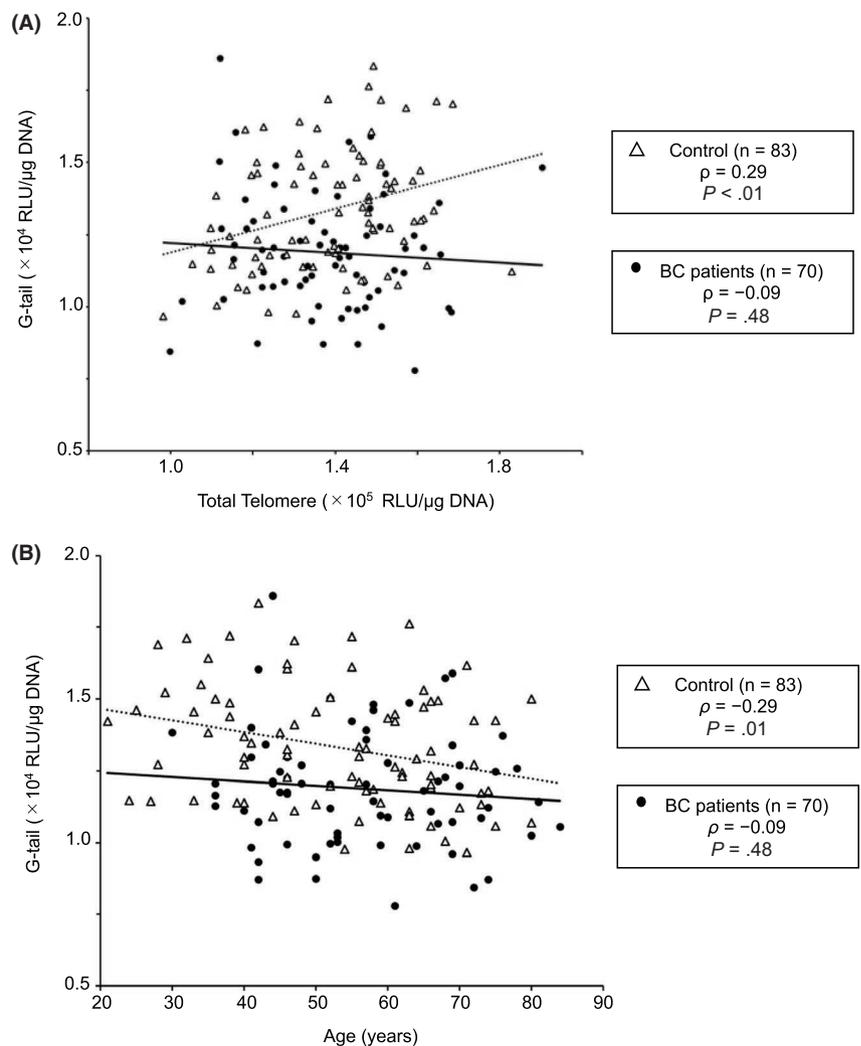


FIGURE 2 Scatter plots of breast cancer (BC) and control groups indicating the relationship between G-tail length and total telomere length in leukocytes (A) and that between leukocyte G-tail length and age (B). RLU, relative light unit

there have been no data representing the association of G-tail length between breast tissue and peripheral leukocytes, a previous study reported that telomere length in somatic tissues was shortened at a similar rate for different tissue types, including leukocytes.¹⁹ Therefore, it is conceivable that G-tail length might also shorten at similar rates in tissues and blood. These findings raise the possibility of leukocyte G-tail length as a reflector of chromosomal instability.

Furthermore, we identified that cancer-free individuals showed a positive correlation between the G-tail length and total telomere length; however, in the BC group, no significant correlation between these parameters was observed. Previous *in vitro* studies have reported that erosion of the G-tail, rather than total telomere length, triggers cellular senescence and consequently results in the loss of cellular viability.²⁰ In addition, no correlation between G-tail length and age was found in the BC group, whereas significant correlation between these parameters was observed in the control group, which is consistent with a previous study.¹³ These results suggest a new hypothesis that a shortened G-tail might be observed, with or without shortened total telomere length, in the early stage of cancer development, regardless of age.

The association between leukocyte telomere length and BC has been evaluated in previous studies but the results are still inconsistent.^{8,21-23} This could be attributed to heterogeneity between studies such as sample size, methodology for telomere length measurement, or participant characteristics.²⁴ In our study, we observed no significant difference in telomere length between the BC and control groups. We used leukocytes to evaluate telomere length with regard to diagnostic marker discovery using blood samples. It has been reported that granulocytes have shorter telomere length than lymphocytes, indicating that differential count of leukocytes could influence telomere length as well as G-tail length.²⁵ Generally, a complete blood count is not required for breast cancer screening, therefore, we could not obtain the complete blood count of all participants. As the obtained data showed a nonsignificant difference in number between the BC and control groups, it might not significantly affect our analysis. However, further studies are needed to evaluate telomere length among differential leukocyte counts to confirm our results.

Neither G-tail length nor total telomere length showed significant differences among the ER-, PR-, and HER2-positive samples in our study. Further studies with a larger sample size are warranted to elucidate the correlation of G-tail length among tumor subtypes. Moreover, considering that chromosomal instability is commonly seen in various cancers, it is possible that leukocyte G-tail length is also shortened in other cancers. Further studies are needed to evaluate leukocyte G-tail length in other cancers to confirm the biomarker potential of leukocyte G-tail length for cancer detection.

In conclusion, a significantly shorter leukocyte G-tail length in BC patients suggests that leukocyte G-tail length is a potential biomarker for BC diagnosis. Additionally, no correlation between leukocyte G-tail length and total telomere length in BC patients was observed, which indicates that the shortened G-tail length observed in the early phases of cancer might not be accompanied by a shortened total telomere length.

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CONFLICT OF INTEREST

Professor Hidetoshi Tahara is a founder, stock owner, and board director of MiRTEL Co. Ltd. A family member, Kanoko Tahara, is an employee of MiRTEL Co. Ltd. The other authors declare no conflict of interest.

ORCID

Yasuhiro Tsutani  <https://orcid.org/0000-0001-8836-1027>

Miyuki Kanda  <https://orcid.org/0000-0001-6410-9441>

Yuki Yamamoto  <https://orcid.org/0000-0001-7733-3741>

Shinsuke Sasada  <https://orcid.org/0000-0003-1623-869X>

Tomoyuki Akita  <https://orcid.org/0000-0002-1056-3611>

Norio Masumoto  <https://orcid.org/0000-0002-2274-0558>

Takayuki Kadoya  <https://orcid.org/0000-0003-1835-1266>

Ryou-u Takahashi  <https://orcid.org/0000-0002-9192-2929>

Junko Tanaka  <https://orcid.org/0000-0002-5669-4051>

Morihito Okada  <https://orcid.org/0000-0001-7068-6608>

Hidetoshi Tahara  <https://orcid.org/0000-0003-3054-4175>

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