The Effects of Socioeconomic Position on Breast Cancer: Based on the DNA Methylation Profiles

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Abstract

Background: The association between socioeconomic position (SEP) and breast cancer outcomes is significantly influenced, and comprehending this connection relies on the analysis of DNA methylation (DNAm) profiles. Studies suggest that socioeconomically disadvantaged individuals, characterized by lower levels of education and household income, may exhibit specific DNAm patterns in breast cancer patients. These patterns have the potential to influence the genesis and progression of cancer. This study investigates the unique DNAm patterns exhibited by socioeconomically disadvantaged individuals, which may have an impact on the progression of cancer. Methods: The study examined the DNAm profiles of breast cancer patients and their socioeconomic backgrounds. The profiles were analyzed using Illumina Infinium MethylationEPIC v2.0 and scanned using an iScan machine (Illumina). The bioinformatics analysis was conducted in R/Bioconductor. Results: The study uncovered noteworthy DNAm differences in persons with lower socioeconomic status. Significant hyper- and hypomethylation occurred at crucial CpG sites, specifically hypomethylation in

cg18843803 and cg17751872 biomarkers. The overexpression of genes, such as TSHZ3 and ZNF714, in the low SEP groups suggests a direct biological pathway through which socioeconomic factors can influence cancer outcomes. These genes are implicated in increasing cell survival, invasiveness, and epithelial–mesenchymal transition, thereby contributing to more aggressive cancer phenotypes. The study also emphasized demographic inequities, with those of lower income being diagnosed at younger ages and having lower levels of education. Conclusion: This study highlights the biological significance of SEP on breast cancer outcomes. Through the identification of precise DNAm biomarkers, this analysis offers valuable insights into the impact of socioeconomic factors on cancer biology. It promotes the incorporation of socioeconomic factors into cancer treatment and prevention methods to tackle health disparities.

Keywords: Breast cancer, Bioinformatics analysis, Epigenomics biomarkers, DNA methylation (DNAm), Socioeconomic position (SEP).

1. Introduction

The association between socioeconomic position (SEP) and breast cancer outcomes is significantly influenced, and comprehending this connection relies on the analysis of DNA methylation (DNAm) profiles. Studies suggest that socioeconomically disadvantaged individuals, characterized by lower levels of education and household income, may exhibit specific DNAm patterns in breast cancer patients. These patterns have the potential to influence the genesis and progression of cancer.[1] According to the Central Bureau of Statistics of the Republic of Indonesia, individuals in Indonesia are considered poor if their household income is below 160 USD as per March 2023.[2] Researchers have discovered certain CpG sites that are linked to family income. These sites are found in genes that play a role in stress signaling and immunological responses. This suggests that SEP can have a biological impact on cancer by affecting DNAm.

In addition, there is a correlation between neighborhood socioeconomic deprivation and alterations in DNAm that stimulate inflammation and modify the immunological milieu. This, in turn, affects the course of breast cancer and the response to therapy. Research has demonstrated that people living in underprivileged neighborhoods undergo more pronounced changes in their DNAm patterns, including both hypo- and hypermethylation in genes that control tumor growth. These variations may result in a poorer prognosis and increased mortality rates. Utilizing DNAm biomarkers presents a promising approach to investigating the biological incorporation of SEP, serving as a personal measuring device for exposure and offering a more reliable measure to assess the influence of SEP on health outcomes.[3] Additionally, the timing, length, and nature of SEP exposure play critical roles, given early life and consistently low SEP are strongly linked to alterations in DNAm. This underscores the intricate connection between SEP and DNAm throughout a person's lifetime.[4] In summary, these findings highlight the significance of taking SEP into account in breast cancer research. DNAm profiles can provide valuable information on how socioeconomic factors impact cancer biology and patient outcomes.

2. MATERIAL AND METHODS

Patients

A cross-sectional study was conducted utilizing blood samples obtained from 48 female patients diagnosed with breast cancer at Cipto Mangunkusumo National Hospital and Dharmais National Cancer Center Hospital, both in Jakarta. The criteria included female patients 18 years old and above, with invasive breast carcinoma and having thorough medical records and histological data. Patients with comorbidities were excluded.

DNA extraction and DNA isolate quality control

DNA samples were obtained from the blood by separating the buffy coat. The DNA was subsequently isolated using the Genomic DNA Mini Kit (GeneAid). The purity of the DNA isolate was evaluated using Nanodrop by quantifying the absorbance ratio of 260/280. The Qubit® 3.0 Fluorometer and the Qubit dsDNA BR Assay Kit from Thermo Fisher Scientific were used to measure the concentration of double-stranded DNA.

Microarray preparations

Two hundred nanograms (ng) of DNA isolates were treated with bisulfite using the Zymo EZ-96 DNA-methylation kit (Zymo Research, Orange, CA, USA) before being prepared for microarray analysis with Infinium MethylationEPIC v2.0 (Illumina). The investigation was carried out using a scanner outfitted with the Illumina iScan platform. The scan generates images in the .idat file format that consists of two distinct intensity colors – red and green.

Microarray data analysis

The Limma tool, written in R/Bioconductor, was employed to identify differentially methylated CpG (DMC), which refers to specific sites where changes in methylation levels are seen. The DMC was identified as a CpG probe with an adjusted p-value of less than 0.05.[5] Hypermethylation DMC was defined as the log2 fold change being larger than 1, and hypomethylation DMC was defined as the log2 fold change being less than -1.[6]

3. RESULTS

Patient populations

Table 1 displays the descriptive attributes of breast cancer patients categorized by household income. It compares patients with household incomes below 160 USD (n = 19) to those with incomes above 160 USD (n = 29). To determine if there were notable disparities between the two income groups across several categorical factors (such as age at diagnosis, subtype, grade, family history, and education), we conducted a chi-square test for independence. The statistical analysis indicates notable disparities among the economic categories regarding age at diagnosis (p-value = 0.0006) and education level (p-value = 0.0015). The findings indicate that individuals from lower-income families were diagnosed at earlier stages of life and possessed lower levels of education than individuals from higher-income households. There were no notable distinctions among the groups for breast cancer subtype, grade, or family history.

Table 1. Descriptive characteristics of breast cancer patients

	Household income (below 160 USD; n(%)) n = 19	Household income (above 160 USD; n(%)) n = 29
Age at diagnosis		
Below 45	8 (42.1%)	0 (0%)
45–55	6 (31.5%)	11 (37.9%)
55–65	4 (21.1%)	7 (24.2%)
Above 65	1 (5.3%)	11 (37.9%)
Subtype		
Luminal A	4 (21.1%)	8 (27.4%)
Luminal B	5 (26.3%)	7 (24.2%)
HER2+	5 (26.3%)	7 (24.2%)
TNBC	5 (26.3%)	7 (24.2%)
Grade		
I	1 (5.3%)	3 (10.3%)
II	4 (21.1%)	7 (24.2%)
III	9 (47.5%)	13 (44.8%)
IV	4 (21.1%)	6 (20.7%)
Family history		
Yes	13 (68.5%)	13 (44.8%)
No	6 (31.5%)	16 (55.2%)
Education		
Below high school	11 (57.9%)	12 (41.4%)
High School	6 (31.5%)	1 (3.4%)
College	2 (10.6%)	16 (55.2%)

Breast tumor DNA methylation with socioeconomic status

When examining the relationship between DNAm and household income (categorized as income < 160 and income > 160), six CpG probes (specifically DMC) were found to have a statistically significant association (with an adjusted p-value of less than 0.05), as shown in Table 2. The results demonstrated a significant correlation between methylation status and family income in a breast cancer setting. The cg18843803 and cg17751872 biomarkers indicated demethylation in the promoter areas of the TSHZ3 and ZNF71 genes, respectively (Figure 1). These genes may contribute to the biological processes that connect family income to breast cancer by means of epigenetic changes.

Table 2. DMC biomarker with significant association between household income and breast cancer DNA methylation

Methylation Status	CpG Locus	Methylation positions	CpG regions	GeneID	Adj P-Value	Log2FC
Hypermethylation	cg03468837	Chr2: 127621085	Shelf	-	5.2e-03	1.017
Hypomethylation	cg18843803	Chr19: 31308500	OpenSea; Promoter	TSHZ3	3e-02	-1.8422
	cg17751872	Chr19: 21082176	Shore; Promoter	ZNF714	8.4e-04	-1.1480
	cg04758026	Chr2: 151303077	OpenSea	-	1.7e-03	-1.009
	cg03834478	Chr11: 69509210	OpenSea	-	3.8e-02	-1.2865
	cg19458741	Chr5: 31268848	OpenSea	RP11-152K4.2	1.5e-02	-1.0261

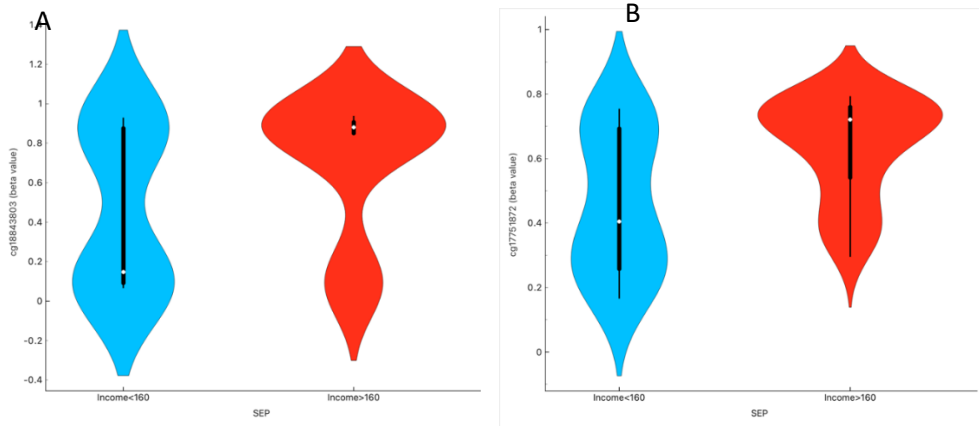

Figure 1. Violin plot of the top 2 DMC biomarkers related to SEP: A. cg18843803, B. cg17751872

We attempted to analyze and contrast the CpG biomarkers among the different subtypes, namely, Luminal A, Luminal B, HER2+, and TNBC, as depicted in Figure 2. According to the statistical analysis, there were no notable variations in the distribution of beta values for the cg18843803 biomarkers among the HER2, LMNA, LMNB, and TNBC subtypes. The beta values for each subtype exhibited comparable measures of central tendency and dispersion. The ANOVA analysis revealed a statistically significant disparity in the means of cg17757872 among the four subtypes (p-value < 0.05). Additionally, post hoc analysis using Tukey's HSD test revealed statistically significant differences between numerous pairs of subtypes. Specifically, there were significant differences between HER2 and LMNA (p = 0.011), HER2 and LMNB (p = 0.001), and LMNB and TNBC (p = 0.001). However, no significant differences were seen between the remaining pairings of subtypes.

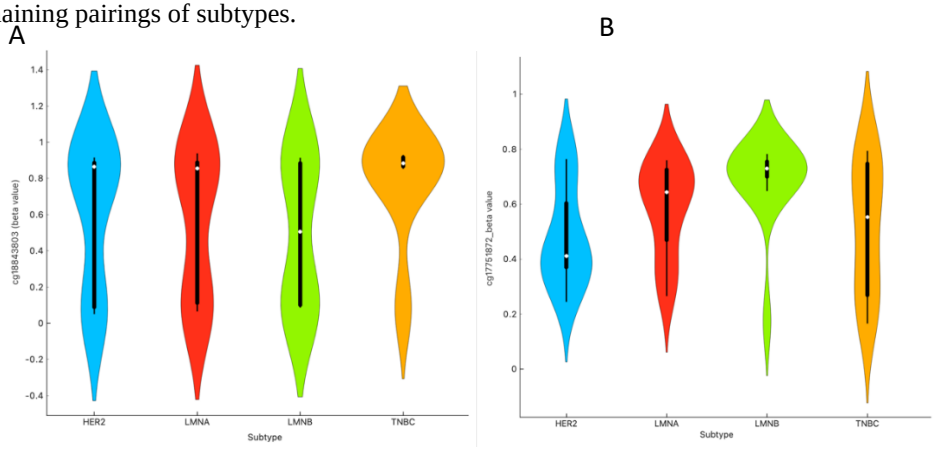

Figure 2. Violin plot of the top 2 DMC biomarkers related to breast cancer subtypes: A. cg18843803, B. cg17751872

4. DISCUSSION

This study explored the complex connection between SEP and DNAm patterns in breast cancer patients, highlighting the biological consequences of socioeconomic inequalities. The examination of CpGs that have undergone DMCs found notable connections between family income and DNAm, particularly in the genes TSHZ3 and ZNF714. The genes involved in stress signaling and immunological responses highlight the potential molecular mechanisms by which SEP affects the development of breast cancer.

TSHZ3, a T-box transcription factor, has a major effect on the course of breast cancer by increasing malignancy through many routes. Research conducted using the 21T cell lines, which simulate several stages of breast cancer advancement, has demonstrated that TSHZ3 is present in large quantities in invasive mammary carcinoma cells (21MT-1) but is only expressed to a small extent in non-invasive cells (21NT and 21PT). The overexpression of TSHZ3 isoforms in 21NT cells resulted in enhanced cell survival, increased ability to form colonies, and greater invasion capacity. Conversely, the suppression of TSHZ3 in 21MT-1 cells led to a smaller colony size and less invasive morphology, showing its involvement in promoting aggressive cancer characteristics.[7] The overexpression of TSHZ3 in malignant cells relative to normal tissues makes it a possible diagnostic marker. The overexpression of this gene is strongly linked to 78.4% sensitivity and 79.6% specificity in detecting breast cancer. However, it is not significantly associated with other breast cancer markers, such as estrogen receptors or cancer antigen 15-3.[8] In addition, TSHZ3, together with TBX2, is commonly upregulated in breast cancer, leading to the inhibition of senescence, stimulation of epithelial-mesenchymal transition (EMT), and enhancement of invasive cell movement.[9] In summary, the excessive expression of TSHZ3 plays a key role in the advancement of breast cancer by increasing the survival of cells, their ability to invade surrounding tissues, and the occurrence of epithelial-mesenchymal transition (EMT). This makes TSHZ3 a crucial element in the development of malignant breast cancer cells.

A direct study of ZNF714 overexpression in breast cancer has not been conducted under the given circumstances. However, knowledge can be gained by examining the functions of other zinc finger proteins (ZNFs) in the evolution of breast cancer. Overexpression of ZNF703 is linked to a more aggressive clinical phenotype, especially in the luminal B subtype, where it enhances cellular proliferation, progression through the cell cycle, and resistance to endocrine therapy.[10,11] Based on these results, it is reasonable to suggest that the overexpression of ZNF714 may have a comparable effect on the advancement of breast cancer by influencing important signaling networks and cellular processes.

Our results support earlier studies showing that individuals from poorer socioeconomic backgrounds have unique DNAm patterns that may worsen the course of cancer and affect the effectiveness of treatment. Specific CpG sites in genes crucial for tumor growth and immunological modulation were found to exhibit hypermethylation and hypomethylation. This indicates that socioeconomic variables not only impact the ability to obtain healthcare and make lifestyle choices but also have significant biological consequences that can influence the progression of diseases.

The study also emphasizes notable disparities in the age of diagnosis and educational achievement between low-and high-income groups, with those from lower-income backgrounds being diagnosed at a younger age and having lower levels of education. Demographic inequalities exacerbate the physiological impacts of SEP, which may result in poorer prognoses and increased mortality rates among marginalized people. Furthermore, our data indicate that specific DNAm biomarkers, such as cg18843803 and cg17751872, can be utilized as important instruments for comprehending epigenetic alterations linked to SEP. These indicators offer valuable information on how early-life and ongoing low SEP exposures contribute to changes in DNAm, which in turn impacts the development and progression of breast cancer.

In summary, this study emphasizes the need to incorporate socioeconomic aspects into cancer research and treatment efforts. By recognizing the biological foundations of SEP, we can create more focused treatments to reduce the negative impact of socioeconomic inequalities on breast cancer outcomes.

5. CONCLUSION

This study emphasizes the complex relationship between SEP and DNAm in breast cancer patients. The upregulation of genes such as TSHZ3 and maybe ZNF714 in low SEP groups indicates a direct biological mechanism by which socioeconomic variables can impact cancer outcomes. Ultimately, the study highlights the importance of tackling socioeconomic inequalities to enhance the outlook and chances of survival for individuals with breast cancer. It advocates for a comprehensive strategy that takes into account both biological and social factors that influence health outcomes.

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Authors' contributions

RIP: Formal analysis, write the manuscript; SIW, SSP, FF: Conceptualize and supervise the research; resources: SSP, IGNGW, NS

Ethics approval and consent to participate

The study received clearance from the Ethical Committee of the Faculty of Medicine, Universitas Indonesia, on 4 September 2023 (approval number: KET-1140/UN2.F1/ETIK/PPM.00.02/2023). The procedures were conducted in accordance with the relevant norms and regulations. Prior to the collection of the patients' samples, all individuals provided their informed consent for participation in this investigation. Clinical and socioeconomics data were collected using participant surveys and computerized medical records.

Competing interests

The authors declare that they have no competing interests.

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