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Cognitive impairment and elevated peripheral cytokines in breast cancer patients receiving chemotherapy

Comprometimento cognitivo e citocinas periféricas elevadas em pacientes com câncer de mama recebendo quimioterapia

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Abstract

Background Anthracyclines-based regimen (5-fluorouracil, doxorubicin, and cyclophosphamide (FAC); cyclophosphamide, epirubicin, and 5-fluorouracil [CEF]) and non-anthracycline based regimens (cyclophosphamide, methotrexate, and 5-fluorouracil [CMF]) are widely used as neoadjuvant chemotherapy for breast cancer patients.

Objective The present study was conducted to observe the effects of FAC, CEF, and CMF regimen on cognition and circulatory proinflammatory cytokines (interleukin 6 [IL-6] and interleukin 1β [IL-1β]) for the duration of three cycles of chemotherapy in breast cancer patients.

Methods Eighty newly diagnosed HER-2 negative breast cancer patients were enrolled and divided into 3 groups as FAC- ($n = 27$), CEF- ($n = 26$), and CMF- ($n = 27$) receiving patients. Serum IL-6 and IL-1β levels were measured by using enzyme-linked immunosorbent assay (ELISA), and cognition was assessed using the Mini-Mental State examination (MMSE) questionnaire.

Results Anthracycline-based regimen was found to increase the levels of IL-6, IL-1β, and decreased MMSE scores compared with CMF regimen ($p < 0.05$).

Conclusion Anthracycline-based regimen caused comparatively higher peripheral inflammation, which could be the reason for more decline in cognition in anthracycline-receiving patients than non-anthracycline group.

Keywords
- Cognitive Dysfunction
- Mental Status and Dementia
- Tests
- Breast Neoplasms
- Neoadjuvant Therapy
- Cytokines

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Resumo

Antecedentes Regime baseado em antraciclinas (5-fluorouracil, doxorrubicina e ciclofosfamida [FAC]; ciclofosfamida, epirrubicina e 5-fluorouracil [CEF]) e regimes não baseados em antraciclina (ciclofosfamida, metotrexato e 5-fluorouracil (CMF]) são amplamente utilizados como quimioterapia neoadjuvante para pacientes com câncer de mama.

Objetivo O presente estudo foi realizado para observar os efeitos do regime FAC, CEF e CMF na cognição e citocinas pró-inflamatórias circulatórias (interleucina 6 [IL-6] e interleucina 1β [IL-1β]) durante três ciclos de quimioterapia em pacientes com câncer de mama.

Métodos Oitenta pacientes recém-diagnosticadas com câncer de mama HER-2 negativo foram recrutadas e divididas em 3 grupos de pacientes que receberam FAC ($n = 27$), CEF ($n = 26$) ou CMF ($n = 27$). Os níveis séricos de IL-6 e IL-1β foram medidos por *enzyme-linked immunosorbent assay* (ELISA) e a cognição foi avaliada por meio do questionário *Mini-Mental State Examination* (MMSE).

Resultados O regime baseado em antraciclinas aumentou os níveis de IL-6, IL-1β e diminuiu os escores do MMSE em comparação com o regime CMF ($p < 0,05$).

Conclusão O regime baseado em antraciclinas causou inflamação periférica comparativamente mais alta, o que pode ser a razão para maior declínio na cognição em pacientes que receberam antraciclinas do que no grupo que não recebeu antraciclina.

Palavras-chave

- Disfunção Cognitiva
- Testes de Estado Mental e Demência
- Neoplasias da Mama
- Terapia Neoadjuvante
- Citocinas

INTRODUCTION

Neoadjuvant chemotherapy is an integral modality for treating breast cancer (BC). It improves the effectiveness of surgical treatment, suppresses systemic subclinical metastases to a certain degree, and improves the survival rate of patients.[1] Regardless of these positive effects, up to 70% of cancer-survivor patients experience cognitive deficit during or following chemotherapy treatment, which affects their quality of life[2]. Cognitive complaints are reported in more than 50% of BC patients after taking chemotherapy; however, clinical reports highlights that 15 to 25% of BC patients have objective cognitive decline.[3] Cognitive decline and psychological factors should be taken into consideration while the patient is on chemotherapy as it could significantly impact their quality of life.[4] Surprisingly, very scarce information is available regarding the differential cognitive effects of individual chemotherapeutic agent. Preclinical studies conducted by researchers demonstrated both indirect and direct neurological effects of doxorubicin, cisplatin, cyclophosphamide, and 5-fluorouracil,[5] which are commonly used to treat BC. Ironically, it is difficult to analyze which one is more toxic to the central nervous system (CNS). Clinically, several cross-sectional and longitudinal studies suggest that chemotherapy treatment during BC may cause impaired memory, attention deficit, lowering of speed of processing, difficulty in word finding, and other basic cognitive functions[6]. Limited studies have been done on cancer patients to determine the effects of different regimens on cognitive changes during chemotherapy.[7] Recent research that has evaluated cognitive complaints in cohorts of BC patients indicated that 46 to 60% of the survivors had cognitive complaints after 5 to 7 years of diagnosis.[8] However, another study conducted on BC patients indicated partial recovery from cognitive decline after 6 months of chemotherapy treatment but recovery observed was not similar to that of baseline cognitive status.[9] A clinical study conducted on BC survivors documented that the activation of peripheral proinflammatory cytokines by chemotherapy may be responsible for neurotoxic brain injury.[10] A mechanism proposed that there might be a significant association between peripherally and centrally expressed cytokines, by virtue of which there could be an exaggeration of inflammation processes in the brain.[11] A wide variety of studies indicate that circulating proinflammatory cytokines may contribute to cancer -associated behavioral changes like stress, fatigue, anxiety, and depression, leading to the appearance of cognitive decline.[12] Clinical studies were reported peripheral IL-1β and IL-6 levels were found to be induced in mild cognitive impairment and Alzheimer's disease patients.[13] The currently available literature on the correlation between chemotherapy, inflammatory cytokines, and cognitive difficulties supports the hypothesis that raised levels of inflammatory cytokines could be the underlying cause for the worsening of cognition in cancer patients. Consequently, it will be worthwhile to explore the effect of chemotherapy regimen on the levels of inflammatory cytokines in patients.

Anthracycline (ANTHR)-based (e.g., doxorubicin, epirubicin) chemotherapy regimens are frequently used to treat BC.[14] They are generally combined with cyclophosphamide and 5-fluorouracil to enhance cytotoxicity.[15] A clinical study on BC patients exhibited significant cognitive impairment and changes in brain treated with ANTHR-containing regimens.[16]

Thus, it is essential to compare the effects of ANTHR and non-ANTHR regimens on cognition in cancer patients receiving them. The involvement of peripheral-inflammation in the progression of behavioral impairment was also assessed to explore the role of the peripheral inflammatory mechanism during cognitive decline in BC patients who received ANTHR and those receiving a non-ANTHR regimen.

METHODS

Ethics approval and consent to participate

The study was conducted in agreement with the declaration of Helsinki and approved by the Institutional Review Board (IRB) of the Rajiv-Gandhi Cancer Institute and Research Centre, New Delhi, India (approval no-RGCIRC/IRB/64/2014) on August 21st, 2014. The Clinical-Trial Registry of India (CTRI) registration number for the present study is *CTRI/2016/02/006,677*. Patients were enrolled in the study after signing an informed consent form.

Study design and settings

The present study was designed as prospective longitudinal cohort study in which newly diagnosed HER 2-negative BC patients were enrolled. In total, 99 BC patients were recruited into the study. Out of these, 19 patients were ineligible, which included 14 patients who had not completed their 3 cycles of chemotherapy, 3 patients with organ-dysfunction (cardiac and renal) and 2 who had missed their scheduled cycles of chemotherapy. After excluding these 19 patients, 80 patients were included in the study and divided into 3 groups on the basis of the regimens they were receiving. Twenty-seven patients were in the group 1 (FAC), 27 in group 2 (CEF), and 26 in group 3 (CMF).

Group 1: FAC (doxorubicin 50 mg/m^2, 5-fluorouracil 500 mg/m^2, cyclophosphamide 500 mg/m^2) one time after an interval of 21 days for 3 cycles.

Group 2: CEF (epirubicin 60 mg/m^2, cyclophosphamide 75 mg/m^2, 5-fluorouracil 500 mg/m^2) one time after an interval of 21 days for 3 cycles.

Group 3: CMF (5-fluorouracil 600 mg/m^2, cyclophosphamide 100 mg/m^2, methotrexate 40 mg/m^2) one time after an interval of 21 days for 3 cycles.

Patients

The inclusion criteria for patients were: 1) patients within the 18–55 years age range; 2) newly diagnosed HER 2-negative BC patients; 3) BC patients receiving FAC, CMF, and CEF as chemotherapeutic regimen; 3) patients ready to provide a signed informed consent; 4) patients well versed in either English or Hindi language. The exclusion criteria were 1) patients having history of head injury or head trauma; 2) patients with any CNS disease or any medical condition that may impact cognitive function (CF) (e.g., multiple-sclerosis); 3) evidence of disorder like dementia, epilepsy, or severe learning disability in the past or present; 4) patients having substance abuse or dependence or having psychotic-spectrum disorder; 5) history of brain surgery or irradiation; 6) patients having history of chemotherapy treatment in the past; 7) patients suffering from any inflammatory and/or autoimmune inflammatory disorder (e.g., rheumatoid-arthritis, systemic lupus erythematosus, vasculitis) or conditions that may initiate the inflammatory processes (e.g., insulin-dependent diabetes; uncontrolled allergic condition, chronic obstructive pulmonary disease (COPD) or asthma); 8) patients having thyroid disorders; 9) patients who were undergoing hormonal therapy; 10) patients who were on chronic steroid medication; 11) patients who underwent stem-cell transplantation; 12) chronic kidney failure to values greater than normally found in a particular age group or recently raised level of serum-creatinine. In this study, patients older than 55 years of age were excluded due to known age-related cognitive changes after 55 years of age. Diseases or medical conditions were also excluded due to their known potential effect on inflammation and cognitive function.

Assessment of cognition

Cognitive-functions were evaluated using the Mini-Mental State Examination (MMSE).[17] Cognitive assessment was done at 4 time-points by using the MMSE scale at intervals of $\sim$ 21 days. The 1st time point (TP0) was at baseline, before the first dose of chemotherapy for all patients. The 2nd time point (TP1) was at $\sim$ 21 days after TP0, and this was also the day before the initiation of the 2nd cycle of chemotherapy. The 3rd time point (TP2) was at 42 days and 21 days after TP0 and TP1, respectively, which was the day before the start of the 3rd cycle of chemotherapy. The 4th time point (TP3) was $\sim$ 63, 42, and 21 days after TP0, TP1, and TP2, respectively, which was the day before the start of 4th cycle of chemotherapy. To simplify, the approximate duration between each time point of assessment was 21 days.

Estimation of IL-6 and IL-1β

A 5-ml blood sample was collected from patients at each time point (TP0, TP1, TP2, and TP3) before the administration of drugs during each cycle of chemotherapy. Samples were then centrifuged at 4,000 rpm for 15 minutes to separate serum. Supernatant serum was divided into aliquots and stored aseptically at -80°C until analysis. Serum IL-6 and IL-1β were analyzed for each 50 µL of serum sample using a highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (Krishgen-Biosystems, India) in duplicate.

Statistical analysis

Frequencies with respective proportions were represented in the scale and categorical variables as mean $\pm$ standard deviation (SD). The Kolmogorov-Smirnov test was used to test the normality of all scale parameters. Multivariate repeated-measures analysis of variance (ANOVA) was used for analysis of data for various IL-6, IL-1β, and MMSE parameters over time for BC patients by adjusting age and other parameters. The Bonferroni correction was applied for within the group comparisons. Percentage change had been calculated by the formula (% change = initial value-final value $\times$ 100/initial value). The initial values used for the calculation of % change were the mean values of each variable at TP0, and the final value was the mean values of each variable at TP3. The IBM SPSS Statistics for Windows, version 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses in the present study.

RESULTS

Demographic of patients

The demographic data at baseline were obtained by taking an interview of the patient and from their medical records. Eighty newly diagnosed HER 2-negative BC patients were

enrolled in the present study. The median age was 43 years (range, 18–55). Twenty-seven patients were progesterone receptor-negative (PR-) or estrogen-receptor-negative (ER-), and 53 patients were found progesterone-receptor-positive (PR+) or estrogen-receptor-positive (ER+). Forty BC patients were postmenopausal, and another 40 patients were premenopausal women. The patients were at different clinical stages of BC before undergoing chemotherapy. The details are 14, 27, 27, and 12 patients were at clinical stages I, II, III, and IV at the time of enrolment, respectively. Chemotherapy regimens CMF, FAC and CEF were received by 27,27 and 26 patients, respectively (►**Table 1**).

The effect of each cycle of chemotherapy on cognition and inflammation of all patients

Effect of cycles of chemotherapy on IL-1β and IL-6 levels of overall patients

The mean values of IL-1β and IL-6 levels between TP0 and TP2, TP0 and TP3, TP0 and TP1, TP1 and TP3, and TP2 and TP3

Table 1 Patient characteristics

		FAC n (%)	CMF n (%)	FEC n (%)	All n (%)
Median age (range), years		43 (18–55)			43 (18–55)
Age range	18–25	1 (4)	0 (0)	1 (4)	2 (2.5)
	26–35	8 (30)	9 (33)	7 (27)	24 (30)
	36–45	9 (33)	9 (33)	11 (42)	29 (36.25)
	46–55	9 (33)	9 (33)	7 (27)	26 (32.50)
Patients		27 (33.75%)	27 (33.75%)	26 (32.50%)	80 (100)
Hormone receptor status	ER+ and PR+	17 (63)	18 (67)	18 (69)	53(66)
	ER- and PR-	10 (37)	9 (33)	8 (31)	27 (33)
Menopause	Pre	13 (48)	13 (48)	14 (54)	40 (50)
	Post	14 (52)	14 (52)	12 (46)	40 (50)
Laterality of disease	Right	15 (56)	14 (52)	11(42)	40 (50)
	Left	12 (44)	13 (48)	15(58)	40 (50)
Socio-economic status[¥]	Upper class	4 (15)	5 (19)	6 (23)	12 (15)
	Upper middle	8 (30)	6 (22)	4 (15)	18 (23)
	Lower middle	8(30)	9(33)	9 (35)	30 (38)
	Upper lower	3 (10)	4 (15)	3 (12)	10 (12)
	Lower	4 (15)	3 (11)	4 (15)	10 (12)
TNM status[@] *T status*	T1	5 (18.5)	5 (18.5)	4 (15)	14 (17)
	T2	9 (33)	8 (30)	10 (38)	27 (34)
	T3	8 (30)	9 (33)	8 (32)	27 (34)
	T4	5 (18.5)	5 (18.5)	4 (15)	12 (15)
N status	N0	6 (22)	5 (18.5)	5 (19)	16 (20)
	N1	7 (26)	7 (26)	7 (27)	21 (26)
	N2	8 (30)	9 (33)	8 (31)	25 (31)
	N3	6 (22)	6 (22)	6 (23)	18 (23)
M status	M0	23 (85)	23 (85)	22 (85)	68 (85)
	M1	4 (15)	4(15)	4 (15)	12 (15)
Stage	I	5 (18.5)	5 (18.5)	4 (15)	14 (17)
	II	9 (33)	9 (33)	9 (35)	27 (34)
	III	9 (33)	9 (33)	9 (35)	27 (34)
	IV	4 (15)	4 (15)	4 (15)	12 (15)

Abbreviations: CMF, cyclophosphamide methotrexate 5-fluorouracil; FAC 5, fluorouracil adriamycin cyclophosphamide; FEC, 5-fluorouracil epirubicin cyclophosphamide; $p < 0.05$, statistically significant; P, value among the groups; [a]$p < 0.001$ versus baseline; [b]$p = 0.001$ versus 1st cycle; [c]$p < 0.0001$ versus 2nd cycle; [d]$p = 0.028$ versus baseline; [e]$p = 0.003$ versus baseline; [f]$p = 0.056$ versus 1st cycle; [g]$p = 0.004$ versus baseline; [h]$p = 0.028$ versus 2nd cycle; [i]$p = 0.003$ versus 1st cycle; [j]$p = 0.008$ versus 2nd cycle; [k]$p = 0.083$ versus baseline; [l]$p = 0.005$ versus baseline; [m]$p = 0.009$ versus 1st cycle; [n]$p < 0.010$ versus 2nd cycle; [o]$p = 0.011$ versus baseline; [p]$p = 0.027$ versus baseline; [q]$p = 0.004$ versus baseline; [r]$p = 0.025$ versus 1st cycle; (-): increase from baseline; (+): decrease from baseline.

Table 2 The effect of different cycles of chemotherapy on IL-6 and IL-1β levels, and MMSE score of all breast cancer patients ($n = 80$)

		TP0 mean ± SD	TP1 mean ± SD	TP2 mean ± SD	TP3 mean ± SD	% change TP0 to TP3
Interleukins	IL-6 (pg/ml)	23.58 ± 14.01	33.42 ± 13.45[a]	40.36 ± 14.23[a,b]	52.96 ± 14.45[a,c,d]	-124.59
	IL-1β (pg/ml)	23.22 ± 8.02	30.47 ± 8.78[a]	39.91 ± 10.85[a,c]	52.07 ± 12.55[a,c,d]	-124.24
MMSE Score		27.47 ± 1.17	27.09 ± 1.33[f]	26.79 ± 1.27[e]	26.32 ± 1.34[a,f, g]	4.18

Note: Adjusted for covariate Age; $p < 0.05$ statistically significant.
Negative value (-) = increase from baseline, positive value (+) = decrease from baseline; [a]$p < 0.0001$ versus Baseline; [b]$p = 0.0018$ versus 1st cycle; [c]$p < 0.0001$ versus 1st cycle; [d]$p < 0.001$ versus 2nd cycle; [e]$p = 0.0006$ versus baseline; [f]$p = 0.0004$ versus 1st cycle; [g]$p = 0.024$ versus 2nd cycle.

were found to be significantly increased ($p < 0.0001$). Interleukin 6 and IL-1β levels were found increased at TP3, as shown by percentage change data, which were 124.59 and 124.24, respectively (►**Table 2**).

The effect of cycles of chemotherapy on the MMSE scores of overall patients

The mean values of MMSE score between TP0 and TP3, TP0 and TP2, TP1 and TP3, and TP2 and TP3 were found to be significantly decreased ($p < 0.0001$, $p = 0.0006$, $p = 0.0004$ and $p = 0.024$, respectively). Percentage change data indicated decreased MMSE score by 4.18 percent at TP3 (►**Table 2**).

The effect of FAC, CMF, and CEF chemotherapy between the cycles on levels of proinflammatory cytokines and cognition

The effect of cycles of FAC, CMF, and CEF Regimen on MMSE Score

The mean difference in MMSE score between TP0 and TP1 was found significantly decreased only in FAC-receiving patients ($p < 0.083$). However, the mean difference in MMSE score between TP0 and TP2 was more statistically significant in FAC-receiving patients than in those treated with CEF ($p < 0.005$, 0.027 respectively). Further, the mean difference of MMSE score for TP0 and TP3 was found significantly decreased in patients receiving FAC (highest; $p < 0.0001$) as compared who were treated with CEF (intermediate; $p = 0.004$) and CMF (lowest; $p = 0.011$). However, the mean difference between TP1 and TP3 was more significantly decreased in FAC- than in CEF-receiving patients ($p = 0.009$, $p = 0.025$ respectively). Mini-Mental State Examination score was found significantly decreased between TP2 and TP3 only in FAC-receiving patients ($p = 0.010$). The percentage change in the MMSE score of patients was found reduced in patients receiving FAC regimen (highest) 6.05% as compared with those receiving CEF (intermediate) 3.80% and CMF (lowest) 2.68% (►**Table 3**).

Effect of FAC, CMF and CEF on IL-6

Patients who were treated with CMF have significantly increased IL-6 levels at TP3 only. The difference in IL-6 levels between the time points TP0 and TP2, TP0 and TP3, TP1 and TP3, and TP2 and TP3 for both FAC and CEF regimens was found equally significant ($p < 0.0001$). However, IL-6 levels between the time points TP0 and TP1 were found more

Table 3 The effects of different cycles of chemotherapy on IL-6 and IL-1β levels, and MMSE score of patients receiving FAC ($n = 27$), CMF ($n = 27$) and FEC ($n = 26$) as chemotherapy regimen

	Regimen	TP0 Mean ± SD	TP1 Mean ± SD	TP2 Mean ± SD	TP3 Mean ± SD	% change TP0 to TP3
IL-6 (pg/ml)	FAC	22.26 ± 14.84	36.65 ± 12.30[a]	48.48 ± 14.6[a,b]	69.21 ± 16.21[a,b,c]	-210.91
	CMF	25.95 ± 14.05	29.64 ± 16.40	32.35 ± 16.03	35.08 ± 16.66[d]	-35.18
	FEC	22.17 ± 12.92	33.99 ± 11.61[e]	40.27 ± 12.15[a,f]	54.60 ± 11.33[a,b,c]	-146.27
IL-1β (pg/ml)	FAC	24.52 ± 7.83	36.48 ± 8.53[a]	50.43 ± 12.31[a,b]	71.51 ± 12.71[a,b,c]	-191.63
	CMF	23.39 ± 10.75	26.23 ± 10.36	30.79 ± 11.43[g]	35.60 ± 10.43[a,h]	-52.20
	FEC	21.76 ± 7.17	28.72 ± 10.42[a]	38.53 ± 9.78[a,i]	49.12 ± 15.57[a,b,j]	-125.73
MMSE score	FAC	27.59 ± 1.01	27.03 ± 1.25[k]	26.74 ± 1.12[l]	25.92 ± 1.20[b,m,n]	6.05
	CMF	27.51 ± 1.15	27.18 ± 1.27	27.03 ± 1.37	26.77 ± 1.36°	2.68
	FEC	27.33 ± 1.33	27.07 ± 1.49	26.62 ± 1.33[p]	26.29 ± 1.46[q,r]	3.80

Abbreviations: CMF, cyclophosphamide methotrexate 5-fluorouracil; FAC 5, fluorouracil adriamycin cyclophosphamide; FEC, 5-fluorouracil epirubicin cyclophosphamide; $p < 0.05$, statistically significant; P, value among the groups; [a]$p < 0.001$ versus baseline; [b]$p = 0.001$ versus 1st cycle; [c]$p < 0.0001$ versus 2nd cycle; [d]$p = 0.028$ versus baseline; [e]$p = 0.003$ versus baseline; [f]$p = 0.056$ versus 1st cycle; [g]$p = 0.004$ versus baseline; [h]$p = 0.028$ versus 2nd cycle; [i]$p = 0.003$ versus 1st cycle; [j]$p = 0.008$ versus 2nd cycle; [k]$p = 0.083$ versus baseline; [l]$p = 0.005$ versus baseline; [m]$p = 0.009$ versus 1st cycle; [n]$p < 0.010$ versus 2nd cycle; [o]$p = 0.011$ versus baseline; [p]$p = 0.027$ versus baseline; [q]$p = 0.004$ versus baseline; [r]$p = 0.025$ versus 1st cycle; (-): increase from baseline; (+): decrease from baseline.

significantly increased in FAC-receiving patients than in those treated with CEF ($p < 0.001$, $p = 0.003$ respectively). Similarly, between the time points TP1 versus TP2, levels of IL-6 were found more significantly increased in FAC-receiving patients than in those treated with CEF ($p = 0.001$, $p = 0.056$ respectively). However, IL-6 levels for CMF receiving patients were found significantly increased only between TP0 versus TP3 (lowest, $p = 0.028$). The percentage change in the IL-6 levels of patients was found increased in FAC (highest) 210.91% compared with CEF (intermediate) 146.27% and CMF patients (lowest) 35.18%.

Effect of cycles of FAC, CMF and FEC regimen on IL-1β levels

Patients who were treated with CMF have significantly increased IL-1β levels at TP2 and TP3 only. The difference in IL-1β levels between the time points TP0 and TP2, TP0 and TP3 and TP1 and TP3 for both FAC and CEF regimens was found equally significant ($p < 0.0001$). However, IL-1β levels between the time points TP0 and TP1 were found more significantly increased in FAC-receiving patients than in those treated with CEF ($p < 0.0001$, $p = 0.001$ respectively). Similarly, between the time points TP1 and TP2, the levels of IL-1β were found more significantly increased in FAC-receiving patients than in those treated with FEC ($p < 0.0001$, $p = 0.003$ respectively). In the same way, FAC-receiving patients were found to have more significantly increased IL-1β levels than those treated with CEF between the time points TP2 and TP3 ($p < 0.0001$, $p = 0.008$ respectively). However, the IL-1β levels for CMF-receiving patients were found significantly increased between TP0 and TP3 and TP0 and TP2 ($p < 0.0001$, $p = 0.004$ respectively). The percentage change in the IL-1β levels of patients was found increased in those treated with FAC regimen (highest) 191.63% when compared with CEF- (intermediate) 125.73% and CMF-receiving patients (lowest) 52.20% (►**Table 3**).

DISCUSSION

The cognition was assessed in the end of each cycle using the MMSE questionnaire, which demonstrated that the scores were decreased significantly after each chemotherapy cycle in all the three groups. The present findings are supported by studies conducted on BC and lymphoma patients, in whom derangement of cognitive function and speed performance were significant during and postchemotherapy.[18,19] In a previous study, it was found that 75% of cancer participants had cancer-related cognitive complaints.[4] All three regimens include cyclophosphamide and 5-fluorouracil as common drugs, and it was observed previously in preclinical studies on mice that these drugs have adverse impact on cognition.[20] Interestingly, all three regimens contain drugs like cyclophosphamide and 5-fu, which readily cross the blood brain barrier (BBB).[21] The preclinical findings are further supported by outcomes of an observational study and clinical trial conducted on BC patients in whom decreased cognitive function was observed in patients receiving ANTHR-based regimen and methotrexate-based chemotherapy regimen.[22] This decreased cognitive performance after each cycle of chemotherapy observed in the present study could be an indicator of prospective cognitive impairment. Further, MMSE score was found more decreased in the FAC-receiving group when compared with the CMF-receiving group, which can be supported by a clinical trial on BC patients in which FAC regimen-receiving patients were having more decreased cognitive function when compared with patients receiving CMF regimen.[22] It was also further confirmed by an observational study on BC patients in which an ANTHR-based regimen had shown more alteration on cognitive function than non-ANTHR-based chemotherapy.[23]

In the present study, ANTHR regimen- (FAC, CEF) receiving patients had more increased serum IL-1β and IL-6 levels than CMF regimen-receiving patients. In previous research, it was noted that both pro and antiinflammatory cytokines concentrations increased in CMF-treated mice.[24] In a preclinical study, it was indicated that doxorubicin and cyclophosphamide (AC) administration has increased the levels of proinflammatory cytokines (TNF-α and IL-6).[25] Concurrently, a clinical study conducted on patients with Hodgkin lymphoma indicated that patients receiving ANTHR-based regimen had increased IL-1β levels.[26] There is rising evidence that the proinflammatory cytokine IL-1β may play a significant role in the behavioral symptoms linked with ANTHR-based regimen. In a preclinical study, the serum levels of IL-1β were found increased in doxorubicin-treated mice in comparison to their untreated counterparts.[27] Several studies on BC survivors treated with ANTHR-containing regimen were found in which cognitive impairment and brain injury could be due to cytokine mediated neuro-inflammation.[28,29] Additionally, ANTHRs were found to induce cytokine as compared with non-ANTHRs[22] drugs.

In contrast to ANTHRs, methotrexate is an antimetabolite that inhibits the synthesis of purine and pyrimidine precursors in cancer cells and possesses antiinflammatory properties.[30] A clinical study on patients with juvenile rheumatoid arthritis confirmed the antiinflammatory properties of methotrexate as it was decreasing IL-6 levels. Methotrexate specifically inhibits monocytes and macrophage growth, which can produce cytokines.[31] In the present study, the CMF-receiving group were having comparatively lower levels of serum IL-6 and IL-1β, which could be attributed to the antiinflammatory potential of methotrexate.

Mechanistically, studies suggest that cytokines may penetrate the BBB through circumventricular regions in the brain via active transport, where they may release other inflammatory mediators, for example, prostaglandins, nitric oxide, chemokine, and cell-adhesion molecules by binding to the endothelial receptors which affects the integrity of the BBB by inducing structural damage to the brain.[32] Beside inflammation, normal levels of cytokines are essential for functioning of the CNS as they contribute in the modulation of glial cell, neuronal functioning, neuronal repair, and the metabolism of serotonin and dopamine, which are essential for normal cognitive functioning. Cytokine dysregulation has been linked to direct neurotoxicity leading to neurodegenerative disorders like Alzheimer disease and Parkinson disease.[33] The increased levels of IL-1β and IL-6 during

dementia and Alzheimer disease hint at the involvement of proinflammatory cytokines in neurodegeneration.[34]

The strength of the present study was that we have assessed cognition and inflammation in patients receiving three different regimens up to three cycles of chemotherapy with the design opted for the assessment of cognition and inflammation in BC patients and, consequently, the outcomes generated after the completion of the present study helped us in drawing the contrast between the effects of three different regimens for up to three cycles of chemotherapy. The study was further strengthened by measuring cytokines prior to and after each cycle of chemotherapy indicating, over the period, changes in the patients.

The limitation of the study was the absence of a concurrent BC-patient group not receiving chemotherapy. Thus, a correlation between cognitive impairment and cytokine dysregulation and the progression of cancer was not possible. A small sample size is the other limitation of the study. Assessment of other inflammatory mediators with their exact signaling pathways could have cleared the complexity of inflammatory responses after chemotherapy administration. The MMSE scale used for the chemotherapy-related cognitive impairment (CRCI) assessment of BC patients in the present study cannot be termed to meet state of art criteria. Objective cognition assessment tests (MMSE scale) are reasonable for the cognitive assessment; however, we recommend self-reported subjective questionnaire at different time periods and settings.[35] The problem of 'practice effect' for shorter interval repeated assessments may persist with the MMSE scale in a longitudinal study. Furthermore, the limitation of the MMSE scale is that it measures the episodic memory and executive function insufficiently, and, thus, a more robust scale is needed. A more reliable cognitive assessment can be done by using subjective cognitive assessment test (e.g., FACT-COG) in addition to the MMSE scale. International and Cognitive Cancer Task Force (ICCTF) recommendation to include healthy control and cancer control subjects for the assessment of cognition was not followed in designing the study.[36] Therefore, in the absence of a concurrent BC control group, it was difficult to find out a correlation between the alteration of cytokines level and cognitive derangement along with the progression of cancer. Thus, we suggest that future studies include both a healthy control and a non-chemotherapy-receiving BC-patient group as reference to help get a better inference of the outcome. Another limitation is that the minimum standardized test for harmonization of studies of cognition and cancer (COWA, HVLT-R, and Trail Making test) in accordance with the ICCTF were not followed in the present study due to non-availability of the HVLT-R scale in Hindi language and the problem of practice effect with the COWA and Trail Making scales. Due to time constraints and limited resources, these battery-of-cognition assessment tests were not included. Another limitation is that no evaluation was done at both baseline and after chemotherapy for common comorbidities in BC patients, such as insomnia, anxiety, depression, pain, and fatigue that could interfere with cognitive assessment.

In conclusion, these results should be considered preliminary and hypothesis-generating. Larger studies should be done to confirm the effect of different chemotherapy regimens on various inflammatory pathways and their association with the development and progression on cognitive decline. To analyze the direct effect of cytokines on the derangement of various functions of brain, we suggest the assessment of cytokines in the cerebrospinal fluid (CSF) as compared with the circulating cytokines, which might not show the true picture. Thus, it can be concluded that the FAC, CEF, and CMF regimens may induce proinflammatory cytokines, leading to rupture of the BBB with cognitive decline in the treated BC patients.

Authors' Contributions

MAK, DB, NA: had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; N. A., M. A. K.: study concept and design, drafting of the manuscript; M. A. K.: statistical analysis; All authors: approved the final manuscript as submitted and agreed to be accountable for all aspects of the work; acquisition, analysis, or interpretation of data; and critical revision of the manuscript for important intellectual content.

Conflict of Interest

The authors have no conflict of interests to declare.

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