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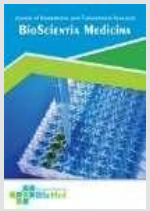

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Evaluating Progesterone Level Fluctuations as a Response Indicator to Chemotherapy in Triple Negative Breast Cancer

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A B S T R A C T

Background: Triple negative breast cancer (TNBC) represents an aggressive subtype with limited targeted therapies and often poorer prognosis. Chemotherapy remains a cornerstone of treatment, yet its systemic effects, including hormonal alterations, are not fully elucidated. The role of progesterone in TNBC progression and its modulation by chemotherapy is particularly complex and warrants investigation. This study aimed to evaluate changes in serum progesterone levels following chemotherapy in TNBC patients. **Methods:** A prospective cohort study was conducted at Dr. Moewardi General Hospital, Surakarta, involving 30 patients diagnosed with TNBC undergoing chemotherapy. Serum progesterone levels were quantified using the ELISA method before the first chemotherapy cycle and after the sixth cycle. Statistical analysis, primarily the Wilcoxon test, was used to compare pre- and post-chemotherapy levels. **Results:** The cohort had a mean age of 49.13 ± 8.98 years. Prior to chemotherapy, progesterone levels varied: 53.3% were below normal (<0.5 ng/mL), 23.3% were normal (0.5-5 ng/mL), and 23.3% were above normal (>5 ng/mL). Following six cycles of chemotherapy, a significant decrease in progesterone levels was observed ($p=0.020$). The proportion of patients with below-normal levels increased to 63.3%. Overall, 10 patients showed decreased levels, 18 remained stable, and 2 showed increased levels. No significant correlation was found between progesterone level changes and baseline patient characteristics like age, menarche, or menopausal status. **Conclusion:** Systemic chemotherapy significantly impacts progesterone levels in TNBC patients, leading to an overall decrease. Monitoring progesterone fluctuations during treatment may hold potential value for assessing therapeutic response or prognosis, warranting further investigation.

1. Introduction

Breast cancer stands as a formidable global health challenge, maintaining its position as the most frequently diagnosed malignancy among women on a global scale. The sheer magnitude of its impact is underscored by the fact that in 2020, an estimated 2.3 million women received a breast cancer diagnosis, and tragically, 685,000 women succumbed to the disease worldwide. This pervasive health issue transcends geographical boundaries, with Indonesia mirroring the global trend, where breast cancer holds the highest incidence, mortality, and prevalence rates among women. Within the diverse landscape of breast cancer, triple negative breast cancer (TNBC) emerges as a distinct and particularly recalcitrant subtype. TNBC is defined by the absence of the expression of three key receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). This unique biological profile characterizes approximately 10-20% of all breast cancer cases. TNBC is recognized for its inherent biological heterogeneity, which contributes to its

aggressive clinical behavior. This subtype is often associated with a propensity for early recurrence, an increased likelihood of metastasis to visceral organs and the brain, and an overall unfavorable prognosis when compared to other breast cancer subtypes. These aggressive features underscore the urgent need for a deeper understanding of TNBC biology and the development of more effective therapeutic strategies. Moreover, TNBC disproportionately affects specific demographics, with a higher prevalence observed in younger women (under 40 years of age), women of African descent, and individuals carrying germline BRCA1 mutations. This predilection for affecting younger women is particularly concerning, given the potential impact on their quality of life, fertility, and long-term well-being.[1-3]

The absence of the three key receptors (ER, PR, and HER2) in TNBC has significant therapeutic implications. Hormonal therapies, designed to target ER and PR, and HER2-targeted agents, are rendered ineffective in this subtype. Consequently, systemic chemotherapy remains the cornerstone of treatment for both early-stage and advanced TNBC. Chemotherapy plays a crucial role in the management of TNBC, with neoadjuvant and adjuvant approaches employed in various clinical settings. Neoadjuvant chemotherapy (NACT) is frequently utilized to downstage tumors prior to surgical intervention. This approach can potentially improve surgical outcomes and provide valuable in vivo assessment of treatment sensitivity, allowing clinicians to gauge the tumor's response to chemotherapy. Adjuvant chemotherapy, on the other hand, is administered after surgery with the aim of eradicating any remaining micrometastatic disease and reducing the risk of recurrence. While TNBC often demonstrates initial sensitivity to chemotherapy, achieving pathological complete response (pCR) rates that are even higher than those observed in hormone-receptor-positive cancers treated with NACT, the development of resistance remains a significant clinical challenge. This resistance frequently leads to high rates of relapse within the first 3-5 years following diagnosis, underscoring the need for strategies to overcome resistance and improve long-term outcomes. Therefore, a critical area of focus in TNBC research is understanding the intricate mechanisms that drive both response and resistance to chemotherapy. Elucidating these mechanisms is paramount for the development of novel therapeutic interventions and strategies to enhance treatment efficacy and ultimately improve outcomes for patients with TNBC.[4-6]

The role of progesterone and its receptor (PR) in breast development and carcinogenesis is complex and multifaceted. In ER-positive breast cancer, PR status serves as a key predictive biomarker for endocrine therapy response. However, the significance of PR in TNBC, which is defined by PR-negativity based on standard immunohistochemistry (IHC) thresholds (typically <1%), is less clear and has been a subject of some controversy. It is important to note that standard IHC assays primarily assess nuclear PR (nPR). However, alternative progesterone signaling pathways exist, including those mediated by membrane progesterone receptors (mPRs), such as mPRα. Preclinical studies have yielded conflicting results regarding the role of progesterone in TNBC. Some studies have suggested that progesterone, acting via mPRα, may exert a suppressive effect on TNBC growth and metastasis. In contrast, other studies have implicated alternative progesterone signaling pathways, potentially involving receptors like PGRMC1 or GPR126, in promoting TNBC progression. These contradictory findings highlight the complexity of progesterone signaling in TNBC and the need for further research to clarify its precise role. Furthermore, it is crucial to consider that systemic chemotherapy itself can induce profound physiological changes in the body. These changes can include effects on the endocrine system and ovarian function, which may lead to alterations in circulating hormone levels, including progesterone. The specific impact of standard chemotherapy regimens on progesterone levels in TNBC patients and the potential clinical significance of these changes remain largely

unexplored areas. Given the aggressive nature of TNBC, the heavy reliance on chemotherapy for its treatment, and the ambiguous role of progesterone signaling beyond the classical nPR pathway, there is a compelling rationale for investigating the interplay between chemotherapy and systemic progesterone levels. Changes in hormone levels during treatment could potentially serve as indicators of treatment efficacy or toxicity, such as chemotherapy-induced ovarian failure. Moreover, these hormonal fluctuations may also modulate the tumor microenvironment or cancer cell biology in unforeseen ways, highlighting the need for a comprehensive understanding of these complex interactions.[7-10] This study was designed to address this gap in knowledge by quantifying the pharmacological effect of systemic chemotherapy on circulating progesterone levels in women undergoing treatment for Triple Negative Breast Cancer. The findings of this research will provide valuable insights into the endocrine consequences of cytotoxic therapy in this receptor-negative subtype, potentially paving the way for improved monitoring and management of TNBC patients.

2. Methods

This study was designed as a prospective observational cohort study. This methodological approach allowed for the observation of changes in progesterone levels over time in patients undergoing a standardized treatment protocol, without direct experimental manipulation of treatment regimens.

The study was carried out within the Surgical Oncology subdivision of Dr. Moewardi General Hospital, located in Surakarta, Indonesia. Dr. Moewardi General Hospital is a tertiary care referral center, providing specialized medical services and serving as a major healthcare provider in the region. The study protocol was meticulously developed and implemented in strict adherence to the ethical guidelines and principles governing clinical research. Prior to the commencement of any study-related procedures, the research protocol underwent rigorous review and received formal approval from the Institutional Review Board (IRB) or Ethics Committee of Dr. Moewardi General Hospital. This ethical review process ensured that the study design, patient recruitment, data collection, and handling of patient information complied with relevant ethical standards and regulations, safeguarding the rights and well-being of the study participants.

The study period spanned approximately one year, commencing in July 2024. This timeframe encompassed the recruitment of eligible patients, the collection of baseline data, the follow-up of patients during their chemotherapy treatment, and the subsequent collection of post-chemotherapy data. The one-year duration allowed for the accrual of a sufficient number of participants to meet the study's objectives and to capture the changes in progesterone levels following the completion of the planned chemotherapy cycles.

The study's target population consisted of all patients diagnosed with Triple Negative Breast Cancer (TNBC) who were scheduled to receive systemic chemotherapy as part of their treatment regimen. This inclusion criterion ensured that the study focused specifically on the population of interest, allowing for a detailed investigation of the impact of chemotherapy on progesterone levels within this specific breast cancer subtype. The accessible population comprised all TNBC patients who were under the care of the Surgical Oncology subdivision at Dr. Moewardi General Hospital during the study period. This represented the group of patients who were realistically available for recruitment into the study.

A purposive sampling technique was employed for the recruitment of study participants. Purposive sampling is a non-probability sampling method where researchers deliberately select participants based on specific criteria relevant to the research question. In this case, purposive sampling was utilized to ensure that only patients meeting the predefined inclusion criteria and not meeting any exclusion criteria were included in the study, thereby increasing the homogeneity of the sample and the internal validity of the findings.

Patients were deemed eligible for inclusion in the study if they fulfilled all of the following criteria; Histologically confirmed diagnosis of breast cancer: This criterion ensured that all participants had a definitive diagnosis of breast cancer, established through pathological examination of tissue samples; Immunohistochemistry (IHC) results confirming negative status for Estrogen Receptor (ER-), Progesterone Receptor (PR-), and Human Epidermal Growth Factor Receptor 2 (HER2-): This is the defining characteristic of Triple Negative Breast Cancer. The absence of these three receptors is crucial for classifying the tumor as TNBC and for determining the appropriate treatment strategies. Standard IHC definitions were applied, adhering to prevailing guidelines: less than 1% nuclear staining for ER/PR, and either an IHC score of 0 or 1+, or an IHC score of 2+ with a negative result from in situ hybridization (ISH) for HER2. These criteria are essential for accurate classification and are in line with established clinical practice; Scheduled to undergo standard systemic chemotherapy as part of their treatment plan: This criterion ensured that all participants were receiving the treatment of interest, allowing for a direct assessment of chemotherapy's effect on progesterone levels; Ability and willingness to provide written informed consent to participate in the study and undergo serial blood sampling: Ethical considerations mandate that all participants voluntarily agree to participate in the research after being fully informed about the study's purpose, procedures, potential risks and benefits, and their rights as research subjects. Written informed consent was obtained from each patient prior to their enrollment in the study. This also ensured compliance with the study protocol, particularly regarding the collection of blood samples at specified time points.

Patients were excluded from the final analysis if they met any of the following criteria; Did not adhere to the planned chemotherapy schedule: This criterion aimed to maintain the consistency of the treatment received by participants. Patients who deviated significantly from the intended chemotherapy regimen may have experienced different effects on their hormonal levels, potentially confounding the study results; Failed to complete the intended six cycles of chemotherapy for any reason (toxicity, disease progression): Completion of the planned chemotherapy cycles was crucial for assessing the full impact of the treatment on progesterone levels. Patients who discontinued chemotherapy prematurely may not have experienced the complete effects of the treatment, making their data unsuitable for inclusion in the final analysis; Had blood samples that were deemed incompatible or unsuitable for progesterone analysis (hemolysis, insufficient volume): The integrity and quality of blood samples are essential for accurate laboratory analysis. Hemolyzed samples (where red blood cells have been broken down) or samples with insufficient volume may yield unreliable results, necessitating their exclusion from the study; Died during the course of chemotherapy before the final blood sample could be obtained: The study design required paired blood samples (pre- and post-chemotherapy) from each participant. Patients who died before the post-chemotherapy sample could be collected could not contribute to the paired analysis, leading to their exclusion.

Baseline demographic and clinical data were collected for all enrolled participants. This comprehensive data collection allowed for a thorough characterization of the study population and the exploration of potential factors that might influence progesterone levels. The following specific data points were recorded; Age: This is a fundamental demographic variable that can influence hormonal levels and cancer characteristics; Age at menarche: This reflects the onset of reproductive function and may have long-term implications for breast cancer risk and hormonal profiles; Menopausal status: This is a critical factor influencing progesterone levels, as menopause leads to significant hormonal changes; Parity (number of children): Reproductive history, including the number of pregnancies, can affect breast cancer risk and hormonal patterns; Age at first pregnancy: Similar to parity, the timing of first

pregnancy can have implications for breast cancer development and hormonal exposure; History of breastfeeding: Breastfeeding has been associated with protective effects against breast cancer and can influence long-term hormonal profiles; Use of hormonal contraception (including duration): Exogenous hormone use can significantly impact endogenous hormone levels and may influence the response to chemotherapy; Family history of cancer: A family history of cancer, particularly breast or ovarian cancer, can indicate a genetic predisposition and may be associated with specific tumor characteristics; Clinical stage of the disease at diagnosis: The stage of the cancer at diagnosis is a crucial prognostic factor and can influence treatment decisions.

The primary variable of interest in this study was the serum progesterone level. To assess the impact of chemotherapy on progesterone levels, venous blood samples were collected from each participant at two specific time points; Pre-chemotherapy (Baseline): A blood sample was drawn before the administration of the first cycle of chemotherapy. This baseline measurement established the initial progesterone level for each patient prior to any chemotherapy exposure; Post-chemotherapy: A second blood sample was collected three weeks after the completion of the sixth and final cycle of chemotherapy. This time point was chosen to allow for the assessment of the cumulative effect of chemotherapy on progesterone levels after the standard treatment course was completed. Blood samples were processed according to standard laboratory protocols to ensure the integrity and quality of the serum. Serum was separated from the blood cells and stored appropriately under controlled conditions until the time of analysis. Progesterone levels in the collected serum samples were quantitatively determined using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. ELISA is a widely used and reliable laboratory technique for measuring hormone concentrations in biological samples. The ELISA assays were performed following the manufacturer's instructions, ensuring consistency and accuracy in the measurement of progesterone levels. The progesterone levels were reported in nanograms per milliliter (ng/mL), the standard unit of measurement for this hormone. For the purpose of categorization and clinical interpretation, progesterone levels were further classified based on common laboratory reference ranges; Below normal: less than 0.5 ng/mL; Normal: 0.5 to 5 ng/mL; Above normal: greater than 5 ng/mL. These categories allowed for a clear understanding of the distribution of progesterone levels within the study population and facilitated the comparison of pre- and post-chemotherapy levels.

All collected data, including demographic information and progesterone levels, were entered into a computerized database. The Statistical Package for the Social Sciences (SPSS) software, version 25 (IBM Corp., Armonk, NY, USA), was utilized for the statistical analysis of the data. Descriptive statistics were employed to summarize the baseline characteristics of the study participants and to describe the distribution of progesterone levels. For continuous variables, such as age and progesterone levels, the mean and standard deviation (SD) were calculated. For categorical variables, such as menopausal status and family history of cancer, frequencies and percentages were used to represent the data. The primary statistical analysis focused on comparing the progesterone levels measured before the first chemotherapy cycle (pre-test) with those measured after the sixth cycle (post-test). Given the paired nature of the data (each patient had two measurements) and the potential for non-normal distribution of progesterone levels, the Wilcoxon signed-rank test was chosen as the appropriate statistical test. The Wilcoxon signed-rank test is a non-parametric test that is specifically designed for comparing paired samples when the data may not follow a normal distribution. It assesses whether there is a statistically significant difference between the two related samples. In addition to the primary analysis, correlation analyses were conducted to explore potential relationships between the change in progesterone levels and various baseline patient

characteristics. These analyses aimed to identify any factors that might be associated with the magnitude or direction of progesterone level changes following chemotherapy. The baseline characteristics included age, age at menarche, age at marriage, age at first pregnancy, menopausal status, number of children, history of breastfeeding, and family history of cancer. The specific correlation tests used would depend on the nature of the variables being compared (e.g., Pearson correlation for continuous variables, Spearman correlation for non-parametric data). For all statistical analyses, a p-value of less than 0.05 was pre-determined to be the threshold for statistical significance. This means that any result with a p-value less than 0.05 was considered unlikely to have occurred by chance, indicating a statistically significant finding.

3. Results

Table 1 presents the baseline demographic and clinical characteristics of the 30 patients with triple negative breast cancer (TNBC) who participated in the study. These baseline characteristics are important for understanding the study cohort's composition and for assessing potential factors that might influence the study results; Total Participants: The study included a total of 30 patients. This sample size is important to consider when evaluating the generalizability of the findings; Age: The mean age of the patients was 49.13 years, with a standard deviation of 8.98 years. This indicates that the patients' ages ranged around 49 years, with some variability. The standard deviation helps quantify how spread out the ages are; Reproductive History: The mean age at menarche (the onset of menstruation) was 13.47 years, with a standard deviation of 1.07 years. This suggests that the patients experienced menarche at a typical age, with relatively little variation within the group. A large majority (90%) of the patients were married at or before the age of 20 years. This is a notable characteristic, potentially reflecting cultural or regional patterns. The mean age at first pregnancy was 19.72 years, with a standard deviation of 1.41 years. Similar to the age at marriage, this indicates a relatively young age at the start of childbearing for this cohort. Nearly half (46.7%) of the patients were postmenopausal. This is an important factor, as menopausal status significantly influences hormone levels, including progesterone. The most common parity was 1-2 children, reported by 66.7% of the patients. This provides information about the patients' childbearing history; Exposures & History: A substantial proportion (83.3%) of the patients reported a history of breastfeeding. Breastfeeding is a factor that has been studied in relation to breast cancer risk. A significant majority (75%) of the patients had used hormonal birth control at some point. The use of exogenous hormones can influence endogenous hormone levels and may be relevant to the study. A considerable percentage (73.3%) of the patients reported a family history of cancer. This suggests a potential genetic predisposition to cancer in this cohort; Clinical Presentation: The majority of patients (63.3%) presented with locally advanced breast cancer (LABC). This indicates that a large portion of the cohort had tumors that had already spread regionally at the time of diagnosis.

Table 2 illustrates the distribution of serum progesterone levels in 30 Triple Negative Breast Cancer (TNBC) patients, both before the initiation of chemotherapy and after the completion of six chemotherapy cycles. It also presents the statistical significance of the overall change in progesterone levels; Progesterone Level Category: The table categorizes progesterone levels into three groups: Below Normal (< 0.5 ng/mL), Normal (0.5 - 5 ng/mL), and Above Normal (> 5 ng/mL). These categories provide a clinically relevant framework for understanding the progesterone levels in these patients; Before Chemotherapy: Initially, before chemotherapy, the largest proportion of patients (53.3%, n=16) had progesterone levels that fell Below Normal. Normal and Above Normal progesterone levels were observed in equal proportions (23.3%, n=7 for each category); After 6 Cycles Chemotherapy: Following six cycles of chemotherapy, there was a shift

in the distribution. The proportion of patients with Below Normal progesterone levels increased to 63.3% (n=19). The proportion of patients with Normal progesterone levels also increased, to 33.3% (n=10). Conversely, the proportion of patients with Above Normal progesterone levels decreased substantially to 3.3% (n=1); Overall Change: The table indicates an overall significant decrease in progesterone levels following chemotherapy, with a p-value of 0.020. This statistically significant p-value suggests that the observed decrease in progesterone levels is unlikely to be due to chance.

Table 3 presents a detailed breakdown of how individual patients' progesterone levels changed between the pre-chemotherapy (initial) measurement and the post-chemotherapy measurement. It categorizes patients based on their initial progesterone level (Low, Normal, or High) and then shows how many patients in each of those initial categories ended up in each of the three categories after chemotherapy; Initial Low Progesterone: Of the 16 patients who started with low progesterone levels (<0.5 ng/mL), the majority (14 patients, or 87.5%) remained in the low progesterone category after chemotherapy. A small number (2 patients, or 12.5%) initially with low progesterone saw their levels increase to the normal range following chemotherapy; Initial Normal Progesterone: Among the 7 patients who began with normal progesterone levels (0.5-5 ng/mL), a little over half (4 patients, or 57.1%) experienced a decrease in progesterone levels, falling into the low range after chemotherapy. The remaining patients (3 patients, or 42.9%) who started with normal progesterone levels maintained progesterone levels within the normal range after chemotherapy; Initial High Progesterone: In the group of 7 patients who initially had high progesterone levels (>5 ng/mL), most (5 patients, or 71.4%) showed a decrease to the normal range after chemotherapy. One patient (14.3%) with initially high progesterone experienced a more substantial decrease, with levels falling into the low range post-chemotherapy. One patient (14.3%) initially with high progesterone continued to have high progesterone levels after chemotherapy; Post-Chemotherapy Distribution: Looking at the total patient distribution post-chemotherapy, out of the 30 patients, 19 (63.3%) had low progesterone levels, 10 (33.3%) had normal levels, and 1 (3.3%) had high levels.

Table 4 summarizes the general trend of how progesterone levels changed in the 30 patients from before chemotherapy to after the completion of chemotherapy. It simplifies the more detailed individual changes shown in Table 3 by grouping them into three main categories; Decrease: This category includes patients whose progesterone levels moved to a lower category. According to the table, 10 patients (33.3% of the total cohort) experienced a decrease in their progesterone levels; Stable / Minimal Change: This category represents patients whose progesterone levels remained relatively stable. This could include patients who stayed within the "Low," "Normal," or "High" categories throughout the study. 18 patients (60.0% of the total cohort) fell into this "stable" category; Increase: This category consists of patients whose progesterone levels moved to a higher category. In this study, 2 patients (6.7% of the total cohort) experienced an increase in their progesterone levels.

4. Discussion

The most salient observation of this study is the statistically significant decrease in overall progesterone levels following the administration of six cycles of chemotherapy. This finding carries substantial weight, as it robustly demonstrates that standard chemotherapy regimens, as administered in this specific clinical setting, exert a tangible effect on the systemic hormonal environment. Notably, this effect extends to progesterone, a hormone of considerable importance in reproductive physiology and potentially in breast cancer biology, and this occurs even within TNBC, a cancer subtype defined by the absence of the classical progesterone receptor.

Table 1. Baseline characteristics of triple negative breast cancer patients (N=30).

Characteristic	Value
Total participants	30
Age (years)	
Mean ± SD	49.13 ± 8.98
Reproductive history	
Age at menarche (years)	
Mean ± SD	13.47 ± 1.07
Age at Marriage	
≤ 20 years	27 (90.0%)
Age at first pregnancy (years)	
Mean ± SD	19.72 ± 1.41
Menopausal status	
Postmenopausal	14 (46.7%)
Parity (Number of Children)	
1-2 children	20 (66.7%)
History of breastfeeding	
Yes	25 (83.3%)
Exposures & history	
Use of hormonal birth control	
Yes	22 (75.0%)
Family history of cancer	
Yes	22 (73.3%)
Clinical presentation	
Disease stage	
Locally advanced (LABC)	19 (63.3%)

Table 2. Serum progesterone levels before and after six cycles of chemotherapy in triple negative breast cancer patients (N=30).

Progesterone level category	Level range (ng/mL)	Before chemotherapy (N=30)	After 6 cycles of chemotherapy (N=30)
Below normal	< 0.5	16 (53.3%)	19 (63.3%)
Normal	0.5 - 5	7 (23.3%)	10 (33.3%)
Above normal	> 5	7 (23.3%)	1 (3.3%)
Overall change			**Significant Decrease**
(p-value)			p = 0.020

Table 3. Patient distribution by pre- and post-chemotherapy progesterone levels.

Initial progesterone level (Pre-Chemo)	Count (Pre-Chemo)	Post-chemo level: Low (<0.5 ng/mL)	Post-chemo level: Normal (0.5-5 ng/mL)	Post-chemo level: High (>5 ng/mL)
Low (<0.5 ng/mL)	**16**	**14** (87.5% of initial Low)	**2** (12.5% of initial Low)	**0** (0.0% of initial Low)
Normal (0.5-5 ng/mL)	**7**	**4** (57.1% of initial Normal)	**3** (42.9% of initial Normal)	**0** (0.0% of initial Normal)
High (>5 ng/mL)	**7**	**1** (14.3% of initial High)	**5** (71.4% of initial High)	**1** (14.3% of initial High)
Total patients (Post-Chemo)	**N=30**	**19** (63.3% of total)	10 (33.3% of total)	1 (3.3% of total)

Table 4. Overall direction of progesterone change across all patients.

Direction of change	Number of patients	Percentage of total cohort (N=30)	Notes
Decrease	10	33.3%	Patients whose levels moved to a lower category
Stable / Minimal Change	18	60.0%	Patients remaining within their initial category (Low→Low, Normal→Normal, High→High).
Increase	2	6.7%	Patients whose levels moved to a higher category (Low to Normal)
Total	**30**	**100.0%**	

This observation prompts a deeper consideration of the mechanisms through which chemotherapy might induce such a reduction in progesterone. Chemotherapeutic agents, while primarily targeting rapidly dividing cancer cells, are not entirely specific in their action. They can also affect other rapidly proliferating cells within the body, including those of the ovaries, the primary site of progesterone production in premenopausal women. The potential for chemotherapy-induced ovarian suppression or damage is a well-recognized phenomenon, and this study's results align with this understanding. However, it is crucial to acknowledge that the study design, while effectively demonstrating the change in progesterone levels, does not definitively pinpoint the precise mechanisms responsible for this change. Further investigations incorporating more detailed endocrine assessments would be necessary to fully elucidate the relative contributions of ovarian suppression, adrenal gland effects, and other potential factors. The statistical significance of the decrease ($p = 0.020$) lends considerable confidence to the finding. In statistical terms, a p-value of 0.020 indicates that there is only a 2% probability of observing the obtained results (i.e., the decrease in progesterone levels) if there were truly no effect of chemotherapy on progesterone. This low probability strongly suggests that the observed decrease is a genuine consequence of the chemotherapy treatment and not simply a random occurrence.[11,12]

An important contextual element to consider is the baseline distribution of progesterone levels within the study cohort, that is, the progesterone levels measured before the initiation of chemotherapy. The study revealed a considerable degree of variability in these baseline levels. Notably, over half of the patients already exhibited low progesterone levels (<0.5 ng/mL) even before commencing treatment. This observation raises several intriguing questions and warrants careful consideration of the factors that might contribute to this baseline heterogeneity. Several potential explanations can be offered for the observed variability in pre-chemotherapy progesterone levels. One prominent factor is menopausal status. As the study appropriately acknowledges, nearly half of the patient cohort had already experienced menopause prior to the study. Menopause is characterized by significant hormonal shifts, including a natural decline in ovarian progesterone production. Therefore, the lower progesterone levels observed in a substantial proportion of patients could, at least in part, be a reflection of this physiological transition. However, it is crucial to emphasize that the study population also included premenopausal women, in whom other factors might be influencing progesterone levels. Beyond menopausal status, it is important to recognize that even within a relatively homogeneous population, there exists a degree of natural inter-individual variation in hormone levels. Factors such as age, body mass index, lifestyle factors, and underlying health conditions can all contribute to differences in circulating progesterone concentrations. Furthermore, the presence of breast cancer itself, even before treatment, might exert some influence on systemic hormone balance. While the precise nature and extent of this influence remain to be fully elucidated, it is plausible that the tumor or the body's response to it could affect hormone production or metabolism. The study's finding of substantial baseline variability underscores the importance of measuring hormone levels before initiating any intervention. Establishing a baseline for each individual allows for a more accurate assessment of subsequent changes and helps to account for pre-existing differences that might otherwise confound the interpretation of results.[13,14]

The study's primary finding of a chemotherapy-induced reduction in progesterone levels warrants a more detailed examination. While the statistical significance of the overall decrease is clear, it is important to consider the nuances of individual patient responses and the magnitude of the observed changes. As noted earlier, chemotherapeutic agents can exert cytotoxic effects on ovarian cells, leading to a decrease in progesterone production. This effect is particularly relevant in premenopausal women, where

the ovaries are the primary source of progesterone. However, even in postmenopausal women, where ovarian progesterone production is minimal, chemotherapy might still influence progesterone levels through other mechanisms. The adrenal glands also produce small amounts of progesterone, and it is conceivable that chemotherapy could affect adrenal steroidogenesis, although this is less well-understood. It is important to acknowledge that the impact of chemotherapy on ovarian function can vary considerably among individuals. Factors such as the specific chemotherapy regimen used, the cumulative dose of chemotherapy, the patient's age, and their baseline ovarian reserve can all influence the extent of ovarian damage and the resulting decrease in progesterone levels. Some women might experience only a temporary decline in ovarian function, with hormone levels recovering after the completion of chemotherapy. Others might develop more persistent or even permanent ovarian failure, leading to long-term hormonal changes. In the context of this study, while the overall trend was a decrease in progesterone, there was likely a range of individual responses. Some patients might have experienced a substantial drop in progesterone levels, while others might have shown only a modest change. Understanding this variability is crucial for interpreting the clinical implications of the findings.[15,16]

While the study highlights the significant overall decrease in progesterone levels, it is equally important to acknowledge the observation that a substantial proportion of patients (60%) exhibited relatively stable progesterone levels throughout the course of chemotherapy. This finding might seem contradictory to the overall trend of decreasing progesterone, but it is essential to interpret it carefully. The classification of "stable" in this context likely encompasses a range of scenarios. It might include patients whose progesterone levels remained consistently low both before and after chemotherapy, as well as those whose levels remained consistently within the normal range. It is also possible that some patients experienced minor fluctuations in progesterone levels that did not cross the predefined thresholds for "low," "normal," or "high," and were therefore categorized as "stable." The observation of stability in a significant proportion of patients underscores the complexity of the hormonal response to chemotherapy. It suggests that while chemotherapy can indeed induce a decrease in progesterone levels in some individuals, it does not do so uniformly in all patients. There are likely individual factors that influence the degree to which chemotherapy affects progesterone production or metabolism. It is crucial to avoid oversimplifying the interpretation of the "stable" category. It does not necessarily imply a complete absence of any effect of chemotherapy on progesterone. Rather, it suggests that in these patients, the net effect of chemotherapy on progesterone levels, whatever the underlying mechanisms, did not result in a significant shift across the predefined categories.[17,18]

The study also reported the intriguing observation that a small minority of patients (approximately 6.7%) experienced an increase in their progesterone levels following chemotherapy. This finding is notable because it deviates from the overall trend of decreasing progesterone and warrants careful consideration. The presence of outliers in any dataset is an important phenomenon that can provide valuable insights into the underlying biological processes. In this case, the two patients who exhibited increased progesterone levels might represent unique clinical or biological circumstances that influenced their hormonal response to chemotherapy. Several potential explanations can be offered for this paradoxical increase in progesterone levels. One possibility is that these patients might have had underlying conditions or physiological variations that affected their hormone production or metabolism. For instance, certain ovarian or adrenal abnormalities could lead to increased progesterone production. Alternatively, there might have been individual differences in the way these patients metabolized or eliminated the chemotherapeutic agents, resulting in differential effects on their endocrine systems. It is also important to consider the possibility of measurement error or

other technical factors. While the study employed a standardized ELISA assay for progesterone measurement, there is always a potential for variability in laboratory testing. However, given the overall consistency of the findings in the rest of the cohort, it is less likely that measurement error would be the sole explanation for the observed increase in progesterone levels in these two patients. The small number of patients exhibiting increased progesterone levels highlights the importance of cautious interpretation of this finding. It is difficult to draw definitive conclusions based on such a small sample size. Further investigations involving a larger cohort and more detailed clinical and endocrine evaluations would be necessary to fully understand the mechanisms underlying this paradoxical increase in progesterone in a subset of patients.[19,20]

5. Conclusion

This study provides compelling evidence that systemic chemotherapy significantly influences progesterone levels in women undergoing treatment for Triple Negative Breast Cancer. The key finding is the statistically significant decrease in overall progesterone levels following six cycles of chemotherapy, highlighting the systemic impact of cytotoxic treatment on hormonal function. The study also reveals the heterogeneity of progesterone levels in TNBC patients, both before and after chemotherapy. Baseline progesterone levels varied considerably, with a substantial proportion of patients exhibiting low levels even prior to treatment, underscoring the importance of considering individual hormonal profiles. While the overall trend was a decrease in progesterone levels post-chemotherapy, a subset of patients showed stable or even increased levels, suggesting complex individual responses to treatment. These findings have potential implications for clinical practice and future research. Monitoring progesterone fluctuations during chemotherapy could offer valuable insights into therapeutic response or potential side effects, such as chemotherapy-induced ovarian dysfunction. Further studies are warranted to elucidate the precise mechanisms underlying chemotherapy-induced hormonal changes and to explore the prognostic significance of progesterone level variations in TNBC patients.

6. References

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