Original Article

Comparative Cytomorphological Changes in Breast FNAC and Cervical Smears in Women with Vitamin B12 Deficiency

Ashwini Rege-Gundawar[1], Roopal Rathi[2]

[1]Associate Professor, Department of Pathology, LNCT Medical College, Kanadiya, Indore, Madhya Pradesh, India, [2]Assistant Professor, Department of Pathology, LNCT Medical College, Kanadiya, Indore, Madhya Pradesh, India

Abstract

Background: Vitamin B12 deficiency is a typical nutritional disease that is known to influence rapidly replicating epithelial tissues, causing cytomorphological changes that mimic either premalignant or malignant alterations. There is little data on comparing such changes across various epithelial locations. The study was conducted to estimate and compare changes in the cytomorphology of breast fine-needle aspiration cytology (FNAC) and cervical smears in women with vitamin B12 deficiency. **Material and Methods:** A cross-sectional analysis of 186 women aged 2060 years undergoing both breast FNAC and cervical cytology revealed that the study was conducted in a hospital. Serum vitamin B12 levels were estimated, and participants were classified into vitamin B12-deficient (n = 93; <200 pg/mL) and vitamin B12-sufficient groups (n = 93). Blind cytopathologists determined whether cytologic smears met predetermined nuclear and cytoplasmic criteria. The statistical analysis was conducted using the relevant tests, and $p < 0.05$ was considered significant. **Results:** Women having vitamin B12 deficiency had a much greater prevalence of cytomorphological aberrations in both breast FNAC and cervical smears than controls. In breast FNAC, nuclear enhancement (62.4%), nuclear disvalidity (49.5%), hyperchromasia (44.1%), and nuclear-cytoplasmic proportions (41.9%) were extremely predominant in the deficient group ($p < 0.001$). Women deficient in vitamin B12 had an increased nuclear enlargement (65.6%), hyperchromasia (58.1%), nuclear membrane abnormality (51.6%), and cytoplasmic maturation defects (46.2%) ($p <0.001$). In the deficient group, the frequency of nuclear atypia was similar in both the breast and cervical cytology. **Conclusion:** Vitamin B 12 deficiency is associated with marked and consistent cytomorphological changes in cervical and breast epithelial cells, with many resembling epithelial atypia. The parameter of these changes and their recognition with respect to vitamin B12 status are essential to avoid misinterpretation of cytological parameters and unnecessary clinical intervention.

Keywords: Voice disorders; GRBAS; CSID; hypofunctional dysphonia; hyperfunctional dysphonia; perceptual voice assessment.

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Introduction

Vitamin B12 deficiency is quite common and clinically significant as a nutritional issue in environments with restricted access to animal-source foods. The level of biochemical deficiency within Indian populations has been reported to be very high in large observational samples, supporting the notion that the phenomenon is not a rare occurrence in everyday practice.[1] In addition to hematologic manifestations, the clinical spectrum is heterogeneous, and a delay in recognition may lead to otherwise preventable morbidity; therefore, timely recognition is important in the day-to-day management of patients.[2]

At the cellular level, vitamin B12 deficiency inhibits DNA synthesis by disrupting one-carbon metabolism, leading to the typical megaloblastic phenotype with nuclear-cytoplasmic asynchrony.[2,3] Although the classically known effects occur in bone marrow and peripheral blood, morphologic changes can also be observed in epithelial tissues. Cytologic literature from earlier times reported that exfoliated epithelial cell cultures in megaloblastic conditions may exhibit an enlarged nucleus and other features suggestive of the nucleus, features that may make interpretation difficult when screening or lesion evaluation is needed in cytology applications.[4]

This diagnostic overlap is particularly relevant for cervical cytology, where biologic and nutritional cofactors have been explored in relation to abnormal smears and pre-neoplastic lesions. Case–control data have shown lower serum vitamin B12 levels among women with abnormal cervical cytology compared with cytologically normal controls.[5] In addition, studies evaluating graded cervical abnormalities have reported an association between lower vitamin B12 status and higher-grade lesions.[6]

Similarly, in breast cytopathology, fine-needle aspiration cytology (FNAC) is widely used as part of the triple assessment of breast masses; however, interpretive “grey zones” exist

Address for correspondence: Dr. Ashwini Rege-Gundawar, Associate Professor, Department of Pathology, LNCT Medical College, Kanadiya, Indore, Madhya Pradesh, India
E-mail: gundawar.ashwini@gmail.com

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because benign proliferative changes and sampling limitations can overlap morphologically with malignant processes.[7] In this context, any systemic condition capable of inducing nuclear atypia can act as a confounder.

Against this background, the present study was designed to compare cytomorphological patterns observed in breast FNAC and cervical smears from women with vitamin B12 deficiency, to clarify the spectrum of deficiency-associated cytologic alterations, and reduce the likelihood of overcalling atypia at these two commonly sampled epithelial sites.

Materials and Methods

Study Design and Setting: A hospital-based cross-sectional analytical study was conducted over 12 months in the Department of Pathology at a tertiary care teaching hospital. The study was designed to evaluate and compare cytomorphological alterations observed in breast FNAC and cervical cytology among women with vitamin B12 deficiency.

Study Population: This study involved women aged 20 to 60 years presenting to the gynecology and surgical outpatient department and observed cases with FNAC of palpable breast lesions, as well as cervical cytology as part of their routine examination. The participants were recruited on a first-come, first-served basis.

Sample Size and Grouping: The study included 186 women selected through convenience sampling. The participants have been classified into 2 groups based on the serum vitamin B12 levels:

Vitamin B12–deficient group (n = 60): Serum vitamin B12 level <200 pg/mL

B12–sufficient control group (n = 60): Serum vitamin B12 level ≥200 pg/mL

The sample size was determined based on feasibility and prior cytomorphological studies assessing nutritional deficiencies.

Inclusion Criteria

- Women aged between 20 and 60 years
- Patients undergoing both breast FNAC and cervical cytology
- Availability of serum vitamin B12 estimation
- Willingness to participate in the study

Exclusion Criteria

- Pregnant or lactating women
- Known cases of breast or cervical malignancy

Patients who are already chemo-radiated or hormonally treated.

History of hematological disorders, long-standing liver disease, or renal failure.

Current vitamin B 12/folic acid supplementation.

Clinical and Lab Examination: A comprehensive clinical history was obtained, including age, presenting complaints, dietary habits, and medical history. Serum vitamin B12 levels were estimated from venous blood samples under aseptic conditions using the chemiluminescent immunoassay method.

Cytological Procedures

Breast FNAC: Breast FNAC was performed using a 22-gauge needle attached to a 10-mL syringe under standard aseptic precautions. Aspirated material was smeared onto clean glass slides. Air-dried smears were stained with May-Grünwald-Giemsa, while alcohol-fixed smears were stained with Papanicolaou stain.

Cervical Cytology: Cervical smears were obtained using an Ayre's spatula and endocervical brush. Smears were fixed immediately in 95% ethyl alcohol, then stained using the Papanicolaou method as per standard protocols.

Cytomorphological Assessment: The cytological smears of all participants were assessed by two experienced cytopathologists who were blinded to the participants' vitamin B12 status. The parameters evaluated were the following:

Breast FNAC P: Nuclear enlargement, nuclear abnormality, hyperchromasia, higher nuclear-cytoplasmic ratio, cytoplasmic vacuolization, and changes in the background.

Cervical Smears: Nuclear enlargement, nuclear hyperchromasia, nuclear membrane abnormality, binucleation or multinucleation, cytoplasmic maturation anomaly, and mimicked epithelial atypia.

Differences in interpretation were solved through a common review.

Statistical Analysis: Microsoft Excel was used to input data and analyze them using conventional statistical software. Categorical variables were reported as frequencies and percentages, whereas continuous variables were reported as means and standard deviations. The Chi-square test was used to compare the vitamin B12-deficient and sufficient groups. The p-value was deemed significant if it was below 0.05.

Results

In the study, 186 women participated, with half deficient in vitamin B12 and the other half not. Out of the total sample, 93 (50.0%) individuals were found to be vitamin B12 deficient, and 93 (50.0) were found to have normal serum vitamin B12 concentrations [Table 1].

The study sample was aged 20-60 years. The most significant age group in both groups was the 4150-year age group, comprising 31.2 percent of the vitamin B12-deficient women and 30.1 percent of the vitamin B12-sufficient women. There was no significant difference in age between the two groups [Table 2].

Cytomorphological evaluation of breast FNAC smears revealed a significantly higher frequency of nuclear abnormalities among women with vitamin B12 deficiency. Nuclear enlargement was the most common finding, observed in 62.4% of deficient cases compared with 22.6% of controls ($p < 0.001$). Other nuclear alterations, including nuclear irregularity (49.5% vs. 18.3%), hyperchromasia (44.1% vs. 15.1%), and increased nuclear–cytoplasmic ratio (41.9% vs. 14.0%), were also significantly more frequent in the vitamin B12–deficient group ($p < 0.001$ for all). Cytoplasmic vacuolization was identified in 36.6% of deficient cases, whereas it was present in only 11.8% of vitamin B12–sufficient women ($p < 0.001$). Background inflammatory changes did not differ significantly between the two groups [Table 3].

The cervical cytology examination showed the same pattern of cytomorphological changes. Female patients with vitamin B12 deficiency had a much greater prevalence of nuclear enlargement

(65.6% vs. 25.8%), atomic hyperchromasia (58.1% vs. 20.4%), and nuclear membrane irregularity (51.6% vs. 17.2%) than controls ($p < 0.001$ in all). Deficiency Binucleation or multinucleation was observed in 39.8% of the cases that were deficient, compared with the prevalence in healthy women with adequate vitamin B12 (15.1%). The deficient group also had more cytoplasmic maturation defects (46.2% vs. 19.4%). Elements that parodied epithelial atypia were found in almost a third of women with vitamin B12 Deficiency, but were not common in the controls [Table 4].

Among patients with vitamin B12 deficiency, nuclear atypia prevalence was similar in breast FNAC and cervical smears. Nuclear atypia was detected in 62.4 per cent of breast FNAC samples and 65.6per cent of cervical samples, which suggested that the two epithelial locations were equally susceptible to vitamin B12-related cytomorphological changes [Table 5].

A direct comparison of specific cytomorphological parameters between breast FNAC and cervical smears in vitamin B12-deficient women did not indicate statistically significant differences. Nuclear enlargement, hyperchromasia, nuclear membrane abnormality, and cytoplasmic changes in both cytological samples were equally frequent, indicating that the pattern of epithelial reaction to vitamin B12 deficiency across tissue locations was similar [Table 6].

Table 1: Distribution of Study Participants Based on Vitamin B12 Status (n = 186)

Vitamin B12 status	Serum vitamin B12 level (pg/mL)	Number of participants	Percentage (%)
Deficient	<200	93	50.0
Sufficient	≥200	93	50.0
Total	—	186	100.0

Table 2: Age Distribution of Study Participants in Vitamin B12–Deficient and Sufficient Groups

Age group (years)	Vitamin B12 deficient n (%)	Vitamin B12 sufficient n (%)	Total n (%)
20–30	18 (19.4)	20 (21.5)	38 (20.4)
31–40	27 (29.0)	25 (26.9)	52 (28.0)
41–50	29 (31.2)	28 (30.1)	57 (30.6)
51–60	19 (20.4)	20 (21.5)	39 (21.0)
Total	93 (100)	93 (100)	186 (100)

Table 3: Cytomorphological Features Observed in Breast FNAC in Relation to Vitamin B12 Status

Cytomorphological feature	Vitamin B12 deficient n (%)	Vitamin B12 sufficient n (%)	p-value
Nuclear enlargement	58 (62.4)	21 (22.6)	<0.001
Nuclear irregularity	46 (49.5)	17 (18.3)	<0.001
Hyperchromasia	41 (44.1)	14 (15.1)	<0.001
Increased N:C ratio	39 (41.9)	13 (14.0)	<0.001
Cytoplasmic vacuolization	34 (36.6)	11 (11.8)	<0.001
Background inflammation	29 (31.2)	23 (24.7)	0.31

Table 4: Cytomorphological Features Observed in Cervical Smears in Relation to Vitamin B12 Status

Cytomorphological feature	Vitamin B12 deficient n (%)	Vitamin B12 sufficient n (%)	p-value
Nuclear enlargement	61 (65.6)	24 (25.8)	<0.01
Nuclear hyperchromasia	54 (58.1)	19 (20.4)	<0.01
Nuclear membrane irregularity	48 (51.6)	16 (17.2)	<0.01
Binucleation/multinucleation	37 (39.8)	14 (15.1)	<0.01
Cytoplasmic maturation defects	43 (46.2)	18 (19.4)	<0.01
Features mimicking epithelial atypia	28 (30.1)	9 (9.7)	<0.01

Table 5: Comparative Frequency of Nuclear Atypia in Breast FNAC and Cervical Smears among Vitamin B12–Deficient Women (n = 93)

Site of cytology	Presence of nuclear atypia n (%)	Absence of nuclear atypia n (%)
Breast FNAC	58 (62.4)	35 (37.6)
Cervical smear	61 (65.6)	32 (34.4)

Table 6: Comparison of Overall Cytomorphological Alterations Between Breast FNAC and Cervical Smears in Vitamin B12–Deficient Group

Parameter	Breast FNAC n (%)	Cervical smear n (%)	p-value
Nuclear enlargement	58 (62.4)	61 (65.6)	0.64
Hyperchromasia	41 (44.1)	54 (58.1)	0.06
Nuclear membrane irregularity	46 (49.5)	48 (51.6)	0.77
Cytoplasmic abnormalities	34 (36.6)	43 (46.2)	0.18

Discussion

This work shows that women with biochemical deficiency of vitamin B12 had a significantly greater prevalence of cytomorphological abnormalities in both breast FNAC and cervical smears than did vitamin B12-adequate controls. The typical image was that of nuclear atypia- nuclear enlargement,

hyperchromasia, irregular nuclear shapes, and elevated N: C ratio with cytoplasmic alteration (vacuolization in breast FNAC, and maturation defects in cervical smears). Notably, the abundance of nuclear atypia in the deficient cohort was generally similar across the two epithelial locations, confirming that B12-associated nuclear alterations can occur in rapidly proliferating epithelia and thus serve as a global confounder in cytology reporting.

The biological explanation for such results is the central role of vitamin B12 in the metabolism of one-carbon compounds and in thymidylate production. B12 deficiency can lower methionine/S-adenosylmethionine availability and disrupt nucleotide balance, increasing dUMPmisincorporation and promoting genomic instability—mechanisms that can translate morphologically into enlarged, hyperchromatic, irregular nuclei and nuclear–cytoplasmic dyssynchrony.[8] In clinical hematology, megaloblastic states are classically attributed to defective DNA synthesis and maturational arrest. At the same time, these descriptions focus on hematopoietic precursors; the same principle can reasonably be extended to other renewing tissues and help interpret the epithelial nuclear atypia observed in our smears.[9,10]

With respect to cervical cytology, historical clinicopathologic observations have reported "megaloblastic" cervicovaginal cell abnormalities resembling those described in severe folate/vitamin B12 deficiency, and these changes are reversible in at least some contexts.[11]

More recent clinical work has explored vitamin B12/folate status in women with borderline cytologic abnormalities (e.g., ASCUS) and HPV-related outcomes, supporting a link between micronutrient status, epithelial cell-cycle control, and cervical disease trajectories. Although our study was not designed to evaluate HPV status or lesion progression, the significantly higher frequency of nuclear and maturation-related abnormalities in deficient women aligns with the broader literature indicating that micronutrient deficiencies can influence cervical epithelial morphology and potentially complicate smear interpretation.[12,13]

In breast FNAC, the interpretive impact of B12-associated atypia is clinically important because the "atypical" category in breast cytology is recognized as a diagnostic gray zone with variable outcomes; no single morphologic criterion consistently predicts malignancy, and indeterminate cytology is commonly managed using clinical–radiologic correlation and/or tissue sampling.[14] Contemporary reporting systems (e.g., the IAC Yokohama framework) emphasize risk stratification and explicitly aim to reduce overtreatment by clarifying indeterminate categories, including atypical/low-risk groups.[15] Large cytohistologic datasets also show that "atypia" within proliferative breast disease can be associated with a meaningful malignancy risk, reinforcing the need to consider potentially reversible causes of atypia—such as nutritional deficiency—before escalation when the clinical-radiologic context is not strongly suspicious.[16] Our observation that nuclear enlargement and related atypical features were markedly more frequent in B12-deficient women than in controls supports the practical recommendation that B12 status (or broader nutritional assessment, where appropriate) be reviewed when cytologic atypia is mild, diffuse, and discordant with imaging/clinical findings.

Altogether, the most important implication of our findings is the interpretive significance of vitamin B12 deficiency: it can cause cytomorphologic patterns that may be mistakenly interpreted as epithelial atypia in both breast and cervical samples, posing a risk of overcalling the presence of premalignant or malignant change without consideration of biochemical correlation. This does not imply that there is anything wrong with cytologic atypia; it is just that, in combination with nutritional deficiency, it is preferable to consider cytomorphology as a possible modifiable factor before making a management decision, in line with the full principles of a triple assessment of breast lesions and standard clinical algorithms for abnormal cervical cytology.

Conclusion

This is shown in the current research, which links vitamin B12 deficiency to considerable changes in cytomorphology in breast FNAC and cervical cytology, marked by nuclear enlargement, hyperchromasia, irregular nuclear contours, and cytoplasmic maturation defects. These adaptations were observed with similar frequency in both breast and cervical epithelial cells, suggesting an identical response of epithelial cells to vitamin B12 deficiency across tissues. Significantly, some of these changes resemble cytological atypia, highlighting the potential for diagnostic overinterpretation when the clinical and biochemical alignment is missing. The importance of vitamin B12 deficiency as a reversible etiology of epithelial cytological atypia to avoid misdiagnosis and unnecessary interventions, and of interventions as an important element of cytological examination integration with nutritional assessment.

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Conflicts of interest

There are no conflicts of interest.

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