Vitamin D plays an essential role in bone metabolism and immunity. Hence, it might affect the development and extent of periodontal disease. This study aimed to assess Vitamin D status in periodontal disease. A total of 150 are participants in the current study, including 50 patients with head and neck cancer who received radiotherapy post-six months (HNC post-RT), 50 chronic periodontitis (CP) patients without HNC, and 50 periodontally healthy (control) enrolled. Clinical Attachment Loss (CAL), Probing Pocket Depth (PPD), Plaque Index (PI), and Gingival Bleeding Index (GBI) were recorded. An electrochemiluminescence immunoassay (eCLIA) was constructed to quantify serum Vitamin D levels. Chronic periodontitis with HNC post-RT patients presented a non-significant proportion of Vitamin D levels compared to chronic periodontitis without HNC(p>0.05) and patients with CP+HNC post-RT and CP without HNC exhibited significant Vitamin D levels compared to control (p<0.05). A significant negative correlation was found between serum Vitamin D levels and CAL, PPD, PI, and GBI in the periodontal disease groups. Moreover, a significant positive correlation was observed between serum Vitamin D levels and hyposalivation. Hyposalivation was increased in patients (CP+HNC post-RT; 0.15 [0.11-0.23] ml/min, *P*=0.001) and (CP without HNC; 0.30 [0.25-0.41] ml/min, *P*=0.001), compared to healthy controls; 0.35 [0.28-0.54] ml/min, *P*=0.001). In this case-control study, Vitamin D deficiency is significantly associated with chronic periodontitis groups. The assessment of vitamin D levels in patients presenting with chronic periodontitis seems advisable, as vitamin D deficiency might be involved in the onset and progression of chronic periodontitis.