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## Summary of Results

### 4. Outcome Measures

#### Primary Outcome

Vitamin D3 supplementation was associated with restoration of vitamin D3 status and favorable biological effects. Patients receiving Vitamin D3 demonstrated hematological stabilization, particularly improved hemoglobin levels, maintenance of magnesium homeostasis, preservation of lymphocyte counts, and regulation of CD8<sup>+</sup> T-cell activity.

In addition, Vitamin D3 supplementation was associated with suppression of VEGF levels, suggesting a potential inhibitory effect on tumor angiogenesis and modulation of the tumor microenvironment.

## Haemoglobin levels

The mean of Hb in (Group I) was ( $11.22 \pm 1.16$ ) before radiotherapy. After radiotherapy the mean increase slightly to ( $11.73 \pm 1.54$ ) but not reach the statistically significant ( $P=0.237$ ). The mean of Hb levels in (Group II) was ( $11.59 \pm 2.03$ ) before radiotherapy which decrease significantly to ( $10.68 \pm 1.25$ ) ( $p_0=0.003^*$ ). (Table 2)

Before radiotherapy, there was a statistically insignificant difference in Hb between groups ( $P = 0.400$ ). After radiotherapy, the vitamin D group showed significant increase in Hb (mean  $\pm$  SD:  $11.73 \pm 1.54$ ) compared to the non-vitamin D group ( $10.68 \pm 1.25$ ) ( $P = 0.012^*$ ).

**Table (2): Hb levels in the two studied groups**

| Hb (gdl)             | Group I<br>(Radiotherapy + Vitamin D3) (n=30) | Group II<br>(Radiotherapy Alone) (n=30) | p      |
|----------------------|-----------------------------------------------|-----------------------------------------|--------|
| <b>Before RT</b>     |                                               |                                         |        |
| Min. – Max.          | 9.60 – 15.70                                  | 2.0 – 14.50                             | 0.400  |
| Mean $\pm$ SD.       | $11.22 \pm 1.16$                              | $11.59 \pm 2.03$                        |        |
| Median (IQR)         | 11.10 (10.60 – 11.60)                         | 12.0 (11.10 – 12.50)                    |        |
| <b>After RT</b>      |                                               |                                         |        |
| Min. – Max.          | 9.20 – 16.70                                  | 9.20 – 12.70                            | 0.012* |
| Mean $\pm$ SD.       | $11.73 \pm 1.54$                              | $10.68 \pm 1.25$                        |        |
| Median (IQR)         | 11.25 (10.90 – 11.90)                         | 10.10 (9.60 – 12.10)                    |        |
| <b>p<sub>0</sub></b> | 0.237                                         | 0.003*                                  |        |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between **Before** and **After RT within the same group**

\*: Statistically significant at  $p \leq 0.05$

**Group I:** BCPs treated with radiotherapy in combination with Vit-D3 supplements

**Group II:** BCPs treated with radiotherapy alone

## Minerals levels (Ca, Mg)

### Ca levels

The mean Ca levels in (Group I) was ( $9.39 \pm 0.49$ ) before radiotherapy which showed insignificant difference with Ca levels ( $9.17 \pm 1.71$ ) after radiotherapy ( $p_0 = 0.991$ ). Ca levels in (Group II) were ( $7.73 \pm 3.46$ ) which increased significantly after radiotherapy to become ( $9.64 \pm 1.30$ ) ( $p_0 = 0.047^*$ ) (Table 3). No significant difference found between group I and group II in Ca levels before RT ( $P = 0.472$ ). The same is true after radiotherapy ( $P = 0.796$ ).

**Table (3): Ca levels in the two studied groups**

| Ca<br>(mg/dl)        | Group I<br>(Radiotherapy +<br>Vitamin D3) (n=30) | Group II<br>(Radiotherapy<br>Alone) (n=30) | p     |
|----------------------|--------------------------------------------------|--------------------------------------------|-------|
| <b>Before RT</b>     |                                                  |                                            |       |
| Min. – Max.          | 8.30 – 10.20                                     | 2.0 – 11.10                                | 0.472 |
| Mean $\pm$ SD.       | $9.39 \pm 0.49$                                  | $7.73 \pm 3.46$                            |       |
| Median (IQR)         | 9.45 (9.05 – 9.80)                               | 9.75 (3.0 – 10.10)                         |       |
| <b>After RT</b>      |                                                  |                                            |       |
| Min. – Max.          | 1.40 – 11.10                                     | 8.0 – 11.60                                | 0.796 |
| Mean $\pm$ SD.       | $9.17 \pm 1.71$                                  | $9.64 \pm 1.30$                            |       |
| Median (IQR)         | 9.35 (9.0 – 9.90)                                | 9.10 (8.50 – 11.0)                         |       |
| <b>p<sub>0</sub></b> | 0.991                                            | 0.047*                                     |       |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After **RT within the same group**

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Mg levels

The mean of Mg levels in (Group I) was ( $1.96 \pm 0.52$ ) before radiotherapy which increased significantly to ( $2.37 \pm 0.63$ ) after radiotherapy. In (Group II) Mg levels were ( $2.99 \pm 2.72$ ) before radiotherapy which decreased significantly to ( $1.71 \pm 0.40$ ) after radiotherapy ( $p_0 < 0.001^*$ ) (Table 4.4. and figure 4.4). Before treatment, the difference between groups was not significant ( $P = 0.126$ ), but after radiotherapy the vitamin D group had significantly higher Mg compared to the non-vitamin D group ( $P < 0.001$ ) (table 4).

**Table (4): Mg levels in the two studied groups**

| Mg<br>(mg/dl)        | Group I<br>(Radiotherapy + Vitamin D3) (n=30) | Group II<br>(Radiotherapy Alone) (n=30) | p       |
|----------------------|-----------------------------------------------|-----------------------------------------|---------|
| <b>Before RT</b>     |                                               |                                         |         |
| Min. – Max.          | 1.20 – 3.60                                   | 1.0 – 13.30                             | 0.126   |
| Mean $\pm$ SD.       | $1.96 \pm 0.52$                               | $2.99 \pm 2.72$                         |         |
| Median (IQR)         | 1.90 (1.70 – 2.0)                             | 2.0 (1.70 – 3.0)                        |         |
| <b>After RT</b>      |                                               |                                         |         |
| Min. – Max.          | 1.30 – 3.60                                   | 1.20 – 2.80                             | <0.001* |
| Mean $\pm$ SD.       | $2.37 \pm 0.63$                               | $1.71 \pm 0.40$                         |         |
| Median (IQR)         | 2.20 (1.80 – 2.80)                            | 1.60 (1.40 – 1.90)                      |         |
| <b>p<sub>0</sub></b> | 0.021*                                        | 0.001*                                  |         |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Vitamin B12 levels

Mean  $\pm$  SD of Vit B12 in (Group I) was ( $800.4 \pm 422.7$ ) before radiotherapy which increase insignificantly to ( $908.4 \pm 455.5$ ) after radiotherapy ( $p_0 = 0.309$ ). In (Group II) Vit-B12 was ( $752.5 \pm 644.4$ ) which increased significantly to ( $1398.9 \pm 530.0$ ) ( $p_0 < 0.001^*$ ) (table 5). Before radiotherapy, there was a significant difference between the two groups ( $P = 0.014$ ), The same is true after radiotherapy ( $P < 0.001$ ).

**Table (5): Vitamin B12 levels in the two studied groups**

| <b>Vitamin B12<br/>(pg/ml)</b> | <b>Group I<br/>(Radiotherapy +<br/>Vitamin D3) (n=30)</b> | <b>Group II<br/>(Radiotherapy<br/>Alone) (n=30)</b> | <b>P</b> |
|--------------------------------|-----------------------------------------------------------|-----------------------------------------------------|----------|
| <b>Before RT</b>               |                                                           |                                                     |          |
| Min. – Max.                    | 351.0 – 2000.0                                            | 255.0 – 2000.0                                      | 0.014*   |
| Mean $\pm$ SD.                 | $800.4 \pm 422.7$                                         | $752.5 \pm 644.4$                                   |          |
| Median (IQR)                   | 751.0(522.0 – 828.7)                                      | 477.0(371.0 – 656.0)                                |          |
| <b>After RT</b>                |                                                           |                                                     |          |
| Min. – Max.                    | 516.0 – 2000.0                                            | 624.0 – 2000.0                                      | <0.001*  |
| Mean $\pm$ SD.                 | $908.4 \pm 455.5$                                         | $1398.9 \pm 530.0$                                  |          |
| Median (IQR)                   | 744.5(650.0 – 822.0)                                      | 1462.5(850.0 – 1925.0)                              |          |
| <b>P<sub>0</sub></b>           | 0.309                                                     | <0.001*                                             |          |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After RT within the same group

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## VEGF levels

Mean  $\pm$  SD of VEGF levels in (Group I) was ( $100.4 \pm 12.88$ ) before radiotherapy which decreased significantly to ( $93.20 \pm 13.98$ ) after radiotherapy ( $p_0 = 0.045$ ). In (Group II) VEGF was ( $96.10 \pm 13.02$ ) before radiotherapy which increased significantly to ( $108.5 \pm 29.75$ ) ( $p_0 = 0.035^*$ ). (Table 6)

No baseline difference between the two groups in VEGF levels. ( $P = 0.203$ ), but after radiotherapy, the control group II (without vitamin D) had significantly higher VEGF levels than the vitamin D group ( $P = 0.015$ ).

**Table (6): VEGF levels in the two studied groups**

| VEGF<br>(pg/ml)      | Group<br>(Radiotherapy +<br>Vitamin D3) (n=30) | Group<br>(Radiotherapy<br>Alone) (n=30) | p      |
|----------------------|------------------------------------------------|-----------------------------------------|--------|
| <b>Before RT</b>     |                                                |                                         |        |
| Min. – Max.          | 75.0 – 135.0                                   | 70.0 – 121.0                            | 0.203  |
| Mean $\pm$ SD.       | $100.4 \pm 12.88$                              | $96.10 \pm 13.02$                       |        |
| Median (IQR)         | 101.0 (92.0 – 109.0)                           | 94.0 (88.0 – 104.0)                     |        |
| <b>After RT</b>      |                                                |                                         |        |
| Min. – Max.          | 52.0 – 112.0                                   | 75.0 – 195.0                            | 0.015* |
| Mean $\pm$ SD.       | $93.20 \pm 13.98$                              | $108.5 \pm 29.75$                       |        |
| Median (IQR)         | 96.0 (83.0 – 105.0)                            | 100.5 (95.0 – 112.0)                    |        |
| <b>p<sub>0</sub></b> | 0.045                                          | 0.035*                                  |        |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between before and after radiotherapy within the same group

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## NF-κB levels

Mean  $\pm$  SD of NF-κB in (Group I) before radiotherapy was ( $0.62 \pm 0.15$ ) which decreased insignificantly to ( $0.55 \pm 0.19$ ) after radiotherapy ( $p_0 = 0.128$ ). In (Group II) the mean  $\pm$  SD of NF-κB was ( $0.65 \pm 0.18$ ) which showed insignificant difference with after radiotherapy ( $p_0 = 0.064$ ). No significant changes or differences was found between the two studied groups in NF-κB levels either before or after radiotherapy ( $P > 0.05$ ). (Table 7)

**Table (7): NF-κB levels in the two studied groups**

| NF-κB<br>(ng/ml)     | Group I<br>(Radiotherapy +<br>Vitamin D3) (n=30) | Group II<br>(Radiotherapy<br>Alone) (n=30) | p     |
|----------------------|--------------------------------------------------|--------------------------------------------|-------|
| <b>Before RT</b>     |                                                  |                                            |       |
| Min. – Max.          | 0.33 – 0.91                                      | 0.31 – 0.93                                | 0.610 |
| Mean $\pm$ SD.       | $0.62 \pm 0.15$                                  | $0.65 \pm 0.18$                            |       |
| Median (IQR)         | 0.61 (0.52 – 0.71)                               | 0.58 (0.52 – 0.85)                         |       |
| <b>After RT</b>      |                                                  |                                            |       |
| Min. – Max.          | 0.31 – 0.91                                      | 0.11 – 0.93                                | 0.994 |
| Mean $\pm$ SD.       | $0.55 \pm 0.19$                                  | $0.54 \pm 0.21$                            |       |
| Median (IQR)         | 0.51 (0.43 – 0.62)                               | 0.52 (0.32 – 0.71)                         |       |
| <b>p<sub>0</sub></b> | 0.128                                            | 0.064                                      |       |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Immunological parameters

### CD8+ (%)

In (Group I) mean  $\pm$  SD of **CD8+ % before radiotherapy was** ( $24.93 \pm 5.88$ ) which decreased significantly to ( $21.57 \pm 6.18$ ) after radiotherapy ( $p_0 < 0.001^*$ ). In (Group II) CD8+ before radiotherapy was ( $18.10 \pm 8.07$ ) which decreased significantly to ( $18.10 \pm 8.07$ ) after radiotherapy ( $p_0 = 0.003$ ). There was no significant difference between groups at baseline ( $P = 0.761$ ), but after radiotherapy, Group II showed significant lower CD8+% when compared to Group I. (Table 8).

**Table (8): CD8+ % in the two studied groups.**

| CD8+ (%)             | Group I<br>(Radiotherapy + Vitamin D3) (n=30) | Group II<br>(Radiotherapy Alone) (n=30) | p      |
|----------------------|-----------------------------------------------|-----------------------------------------|--------|
| <b>Before RT</b>     |                                               |                                         |        |
| Min. – Max.          | 12.0 – 36.0                                   | 11.0 – 51.0                             | 0.761  |
| Mean $\pm$ SD.       | $24.93 \pm 5.88$                              | $25.60 \pm 10.40$                       |        |
| Median (IQR)         | 25.0 (21.0 – 30.0)                            | 25.50 (17.0 – 34.0)                     |        |
| <b>After RT</b>      |                                               |                                         |        |
| Min. – Max.          | 10.0 – 34.0                                   | 7.0 – 37.0                              | 0.047* |
| Mean $\pm$ SD.       | $21.57 \pm 6.18$                              | $18.10 \pm 8.07$                        |        |
| Median (IQR)         | 22.0 (17.0 – 25.0)                            | 16.0 (12.0 – 22.0)                      |        |
| <b>p<sub>0</sub></b> | $<0.001^*$                                    | $0.003^*$                               |        |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Absolute CD8+ Count

In (Group I) mean  $\pm$  SD of absolute CD8+ before radiotherapy was ( $420.2 \pm 256.10$ ) which increased significantly to ( $533.4 \pm 275.8$ ) after radiotherapy ( $p_0 = 0.040$ ). In (Group II) absolute CD8+ before radiotherapy was ( $527.1 \pm 298.6$ ) which decreased insignificantly to ( $514.5 \pm 285.9$ ) after radiotherapy ( $p_0 = 0.837$ ). No significant differences either before or after radiotherapy between the two groups ( $P > 0.05$ ). (table 9)

**Table (9): Absolute CD8+ count in the two studied groups.**

| Absolute CD8+        | Group I<br>(Radiotherapy +<br>Vitamin D3) (n=30) | Group II<br>(Radiotherapy<br>Alone) (n=30) | p     |
|----------------------|--------------------------------------------------|--------------------------------------------|-------|
| <b>Before RT</b>     |                                                  |                                            |       |
| Min. – Max.          | 81.0 – 979.0                                     | 88.0 – 1018.0                              | 0.156 |
| Mean $\pm$ SD.       | $420.2 \pm 256.10$                               | $527.1 \pm 298.6$                          |       |
| Median (IQR)         | 356.5(235.0 – 525.0)                             | 566.5(221.0 – 751.0)                       |       |
| <b>After RT</b>      |                                                  |                                            |       |
| Min. – Max.          | 88.0 – 1018.0                                    | 118.0 – 1018.0                             | 0.813 |
| Mean $\pm$ SD.       | $533.4 \pm 275.8$                                | $514.5 \pm 285.9$                          |       |
| Median (IQR)         | 486.0(332.0 – 720.0)                             | 590.5(238.0 – 697.0)                       |       |
| <b>p<sub>0</sub></b> | 0.040*                                           | 0.837                                      |       |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Absolute lymphocytes count

In (Group I) mean  $\pm$  SD of absolute lymphocyte before radiotherapy was ( $1420.4 \pm 557.8$ ) which increased significantly to ( $1729.2 \pm 605.5$ ) after radiotherapy ( $p_0 = 0.021^*$ ). In (Group II) absolute lymphocytes count before radiotherapy was ( $2055.9 \pm 726.0$ ) which decreased significantly to ( $1268.6 \pm 608.6$ ) after radiotherapy ( $p_0 < 0.001^*$ ). Before treatment, significantly lower counts in the vitamin D group compared to control ( $p < 0.001$ ). After radiotherapy, there was a significant within-group increase in the vitamin D group ( $p_0 = 0.021$ ), and a decrease in the control group ( $P < 0.001$ ). (table10)

**Table (10): Absolute lymphocytes in the two studied groups**

| Abs. lymph           | Group I<br>(Radiotherapy + Vitamin D3) (n=30) | Group II<br>(Radiotherapy Alone) (n=30) | p       |
|----------------------|-----------------------------------------------|-----------------------------------------|---------|
| <b>Before RT</b>     |                                               |                                         |         |
| Min. – Max.          | 350.0 – 3060.0                                | 350.0 – 3180.0                          | <0.001* |
| Mean $\pm$ SD.       | 1420.4 $\pm$ 557.8                            | 2055.9 $\pm$ 726.0                      |         |
| Median (IQR)         | 1293.5(1200.0 – 1665.0)                       | 2210.0(1510.0 – 2470.0)                 |         |
| <b>After RT</b>      |                                               |                                         |         |
| Min. – Max.          | 769.0 – 2510.0                                | 412.0 – 2800.0                          | 0.005*  |
| Mean $\pm$ SD.       | 1729.2 $\pm$ 605.5                            | 1268.6 $\pm$ 608.6                      |         |
| Median (IQR)         | 1800.0(1234.0 – 2210.0)                       | 1293.0 (873.0 – 1820.0)                 |         |
| <b>p<sub>0</sub></b> | 0.021*                                        | <0.001*                                 |         |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone

## Secondary Outcome

Vitamin D3 supplementation was associated with restoration of vitamin D3 normal levels

### Vitamin D3 levels

Vit-D3 mean levels in (Group I) was ( $13.86 \pm 9.57$ ) before radiotherapy which increased significantly to ( $29.84 \pm 7.67$ ) after radiotherapy  $P_0 < 0.001^*$ . The mean levels in (Group II) was ( $12.03 \pm 8.79$ ) before radiotherapy which show further decrease after radiotherapy ( $7.83 \pm 2.47$ ) ( $p_0 = 0.044^*$ ) (Table 4.5 and figure 4.5). As expected, supplementation raised vitamin D significantly in Group I (vitamin D), while the control group decreased significantly ( $p_0 = 0.044$ ) (table11)

**Table (11): Vitamin D3 levels in the two studied groups**

| <b>Vitamin D<br/>(ng/ml)</b> | <b>Group I<br/>(Radiotherapy +<br/>Vitamin D3) (n=30)</b> | <b>Group II<br/>(Radiotherapy<br/>Alone) (n=30)</b> | <b>p</b> |
|------------------------------|-----------------------------------------------------------|-----------------------------------------------------|----------|
| <b>Before RT</b>             |                                                           |                                                     |          |
| Min. – Max.                  | 2.04 – 52.0                                               | 2.04 – 44.50                                        | 0.249    |
| Mean $\pm$ SD.               | $13.86 \pm 9.57$                                          | $12.03 \pm 8.79$                                    |          |
| Median (IQR)                 | 11.70 (8.0 – 17.24)                                       | 9.90 (6.70 – 13.10)                                 |          |
| <b>After RT</b>              |                                                           |                                                     |          |
| Min. – Max.                  | 15.90 – 43.50                                             | 1.40 – 11.30                                        | <0.001*  |
| Mean $\pm$ SD.               | $29.84 \pm 7.67$                                          | $7.83 \pm 2.47$                                     |          |
| Median (IQR)                 | 28.55(23.30 – 38.0)                                       | 8.25 (6.10 – 9.80)                                  |          |
| <b>p<sub>0</sub></b>         | <0.001*                                                   | 0.044*                                              |          |

IQR: Inter quartile range

SD: Standard deviation

p: p value for comparing between the two studied groups

p<sub>0</sub>: p value for comparing between Before and After RT **within the same group**

\*: Statistically significant at  $p \leq 0.05$

Group I: BCPs treated with radiotherapy in combination with Vit-D3 supplements

Group II: BCPs treated with radiotherapy alone