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O IMPACTO DA VITAMINA D E DA INTERLEUCINA-6 COMO FATORES DE RISCO ASSOCIADOS À DEPRESSÃO EM ADOLESCENTS

THE IMPACT OF VITAMIN D AND INTERLEUKIN-6 AS RISK FACTORS ASSOCIATED WITH DEPRESSION ADOLESCENTS

تأثير فيتامين د والإنترلوكين 6 كعوامل خطر مرتبطة بالاكتئاب لدى المراهقين

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RESUMO

Introdução: Os adolescentes enfrentam um risco elevado de depressão devido a flutuações hormonais e ao impacto nos biomarcadores fisiológicos e imunológicos, que estão associados a um risco elevado de sintomas de depressão durante a puberdade. **Objetivos**: Investigar os papéis da interleucina-6 (IL-6) e da vitamina D (Vt.D) como fatores influentes no aumento do risco de depressão entre adolescentes. **Métodos**: Estudo transversal com 130 adolescentes com idades entre 12 e 18 anos, entre novembro de 2023 e fevereiro de 2024. Este estudo examinou os níveis séricos de IL-6 e Vt.D. Além disso, o cálculo se baseia no Inventário de Depressão de Beck para medir a gravidade da depressão. **Resultados**: Os adolescentes apresentam um aumento significativo nos sintomas de depressão, com 59,2% apresentando sinais de depressão. Adolescentes deprimidos mostram um aumento significativo (p<0,001) nos níveis de IL-6 e diminuição nos níveis de Vt.D em comparação com indivíduos não deprimidos. Os escores de depressão foram significativamente correlacionados positivamente com os níveis de IL-6 e negativamente com os níveis de Vt.D, e a análise de regressão linear exibe um preditor positivo significativo de IL-6 (B: 4,430; IC 95%: 3,023 a 5,836). Vt.D é um preditor significativamente negativo (B: -0,145; IC 95%: -0,220 a -0,071) associado aos escores de depressão. A regressão logística mostrou que o risco de depressão é maior no sexo feminino (59,7%; OR: 2,33; p=0,002) em comparação ao sexo masculino (40,3%; OR: 0,939; p=0,803), adolescentes com sobrepeso e obesos (OR: 3,750, p=0,019; e OR: 2,333, p=0,022), do que seus pares com peso normal e abaixo do peso. Eles previram altamente a depressão em adolescentes com deficiência de Vt.D (OR: 24,4, p<0,001), e a gravidade da depressão aumentou sem a suplementação de Vt.D (OR: 1,769, p=004), mais do que aqueles que haviam tomado suplemento de Vt.D (OR: 0,571, p=0,207). **Discussão**: A vitamina D afeta a síntese de serotonina por um mecanismo biológico único. A serotonina está sempre envolvida na fisiopatologia da depressão. A vitamina D pode ter um efeito neuroprotetor no processo inflamatório do corpo. O suporte à imunomodulação leva a um aumento nas citocinas inflamatórias e vitaminas, o que suprime a síntese de citocinas inflamatórias, como a IL-6, pelos monócitos. **Conclusões**: A probabilidade de depressão grave na adolescência foi associada a níveis elevados de IL-6 e deficiência de vitamina D, servindo como preditores disponíveis.

Palavras-chave: *Deficiência de Vt.D, IL-6, Depressão em adolescentes e Escores de depressão.*

ABSTRACT

Background: Adolescents face an increased risk of depression due to hormonal fluctuations and the impact on physiological and immunological biomarkers, which are associated with a heightened risk of symptoms of depression during puberty. **Aims**: Investigate the roles of interleukin-6 (IL-6) and vitamin D (Vt.D) as influential factors in increasing the risk of depression among adolescents. **Methods**: Cross-sectional study of 130 adolescents aged 12 to 18, between November 2023 and February 2024. This study examined serum IL-6 and

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Vt.D levels. Additionally, the calculation relies on the Beck Depression Inventory to measure the severity of depression. **Results**: Adolescents have a significant increase in depression symptoms, with 59.2% showing signs of depression. Depressed adolescents show a significant increase (p<0.001) in IL-6 and decreased Vt. D levels compared to non-depressed individuals. Depression scores were significantly correlated positively with IL-6 levels and negatively with Vt. D levels and Linear regression analysis exhibit a significant positive predictor of IL-6 (B: 4.430; 95% CI: 3.023 to 5.836). Vt. D is a significantly negative predictor (B: -0.145; 95% CI: -0.220 to -0.071) associated with depression scores. Logistics regression showed the risk of depression is higher in females (59.7%; OR: 2.33; p=0.002) compared to males (40.3%; OR: 0.939; p=0.803), overweight and obese adolescents (OR: 3.750, p=0.019; and OR: 2.333, p=0.022), than normal weight and underweight peers. They highly predicted depression in adolescents with deficiency Vt. D (OR: 24.4, p<0.001), and depressed severity increased with no take Vt. D supplement (OR: 1.769, p=004), more than those who had taken Vt. D Supplement (OR: 0.571, p=0.207). **Discussion**: Vitamin D affects serotonin synthesis by a unique biological mechanism. Serotonin is always involved in the pathophysiology of depression. Vitamin D may have a neuroprotective effect on the inflammatory process in the body. Support immunomodulation leads to an increase in inflammatory cytokines and vitamins, which suppresses the synthesis of inflammatory cytokines such as IL-6 by monocytes. **Conclusions**: The likelihood of severe adolescent depression was found to be associated with elevated IL-6 levels and a deficiency in Vitamin D, serving as available predictors.

Keywords: *Deficiency Vt.D, IL-6, Adolescent depression, and Depress scores.*

الملخص:

الخلفية: يواجه المراهقون زيادة في خطر الإصابة بالاكتئاب بسبب التقلبات الهرمونية وتأثيرها على المؤشرات الحيوية الفسيولوجية والمناعية، والتي ترتبط بزيادة خطر ظهور أعراض الاكتئاب أثناء فترة البلوغ. **الهدف:** التحقق من دور الإنترلوكين 6 (IL-6) وفيتامين د (Vt.D) كعوامل مؤثرة في زيادة خطر الإصابة بالاكتئاب بين المراهقين. **الطرق:** دراسة مقطعية أجريت على 130 طالبًا مراهقًا تتراوح أعمارهم بين 12 و18 عامًا، في الفترة ما بين نوفمبر 2023 وفبراير 2024. فحصت هذه الدراسة مستويات IL-6 في الدم وفيتامين د. بالإضافة إلى ذلك، يعتمد الحساب على استبيان بيك للاكتئاب لقياس شدة الاكتئاب. **النتائج:** لدى المراهقين زيادة كبيرة في أعراض الاكتئاب، حيث ظهرت على 59.2% منهم علامات الاكتئاب. يظهر المراهقون المكتئبون زيادة ملحوظة (P<0.001) في مستوى IL-6 وانخفاض في مستويات Vt.D مقارنة بالأفراد غير المصابين بالاكتئاب. ارتبطت درجات الاكتئاب بشكل إيجابي مع مستويات IL-6 وسلبًا مع مستويات Vt.D ويظهر تحليل الانحدار الخطي مؤشرًا إيجابيًا مهمًا لـIL-6 (B: 4.430 ؛ 95٪ CI: 3.023إلى 5.836 Vt.D .(هو مؤشر سلبي إلى حد كبير(B: -0.145 ؛ 95٪ CI: -0.220إلى -0.071) يرتبط بدرجات الاكتئاب. أظهر الانحدار اللوجستي أن خطر الاكتئاب أعلى لدى الإناث (59.7%؛ نسبة الأرجحية: 2.33؛ قيمة احتمالية = 0.002) مقارنة بالذكور (40.3%؛ نسبة احتمالية: 0.939؛ قيمة احتمالية = 0.803)، والمراهقين الذين يعانون من زيادة الوزن والسمنة (نسبة الأرجحية: 3.750، قيمة احتمالية = 0.803). 0.019؛ ونسبة الأرجحية: 2.333، ع = 0.022)، من أقرانهم ذوي الوزن الطبيعي وناقصي الوزن. لقد تنبأوا بشكل كبير بالاكتئاب لدى المراهقين الذين يعانون من نقص فيتامين د (نسبة الأرجحية: 24.4، (p<0.001، وازدادت شدة الاكتئاب عند عدم تناول مكملات فيتامين د (نسبة الأرجحية: 1.769، قيمة الاحتمال = 004)، أكثر من أولئك الذين تناولوا فيتامين د. الملحق) D نسبة الأرجحية: 0.571، ع = 0.207). **المناقشة:** فيتامين (د) يؤثر على تخليق السيروتونين من خلال ميكانيكية بيولوجية فريدة من نوعها, وتشارك السيروتونين دائما في الفيزيولوجيا المرضية للاكتئاب. قد يكون لفيتامين (د) تأثير وقائي عصبي على العملية الالتهابية في الجسم. لدعم التعديل المناعي ، يؤدي إلى زيادة في السيتوكينات الالتهابية والفيتامينات التي كانت تمنع تخليق السيتوكينات الالتهابية مثل IL-6 بواسطة الخلايا الوحيدة

الاستنتاجات: وجد أن احتمال الإصابة بالاكتئاب الشديد لدى المراهقين يرتبط بارتفاع مستويات إنترلوكين 6 ونقص فيتامين د، وهو بمثابة تنبؤات متاحة.

الكلمات المفتاحية: نقص Vt.D، IL-6، اكتئاب المراهقين، مستويات الاكتئاب

1. INTRODUCTION

Adolescents are more susceptible to mental health problems during the formative and tumultuous years of adolescence due to changes in physiology, psychosocial development, and cognitive processes, depression is the fourth most common cause of illness and disability among adolescents, and mental health issues account for 16% of the worldwide burden of disease and damage among this population (Shorey *et al.,* 2022).

Depression is one of the most common mental illnesses in the world, affecting many people's quality of life (Harsanyi *et al*., 2023). Depression is characterized by the dysregulation of the hypothalamus-pituitary-adrenal (HPA) or hypothalamus-pituitary-gonadal (HPG) axes, as well as other factors such as brain serotonin depletion, the impact of inflammation, and the immune system response (Troubat *et al.;* 2021). It may result from low serotonin levels, which are influenced by low vitamin D levels (Kaviani *et al.,* 2020). Serotonin (5-HT) is a vital neurotransmitter in the central nervous system and a regulatory

hormone that regulates a wide variety of physiological functions.

Perhaps the most classically defined roles of 5-HT are central to mood, sleep, and anxiety management and peripheral to gastrointestinal motility modulation. Vitamin D, a steroid hormone, plays a vital role in maintaining bone and calcium homeostasis, as noted by Giustina *et al.* in 2020 and Di Molfetta *et al.* in 2024. It is significant for various physiological and pathological processes, including the modulation of the immune and inflammatory systems, neuroendocrine function, and cardiovascular health, as mentioned by Valer-Martinez *et al.* in 2023. Vitamin D is essential for the control of the serotonergic pathway as well as the generation of melatonin, showing the role of vitamin D not only in sleep but also in mood regulation (Huiberts *et al.,* 2021) and new research emphasizes its critical role in neurodevelopment (Todisco *et al.,* 2020). According to Mohammed and Al-Jawadi (2020), it has been recently observed that vitamin D may be related to cases of female infertility; however, studies are still very scarce in clarifying its role. Interleukin 6 (IL-6) is a cytokine produced from T cells, macrophages, and adipocytes, which can function as either an anti- or pro-inflammatory cytokine and be released into the bloodstream in response to an immunologic stimulation.

In depression, cytokine responses to infections and stressors are increased. Responses, when combined with other risk factors, result in extended inflammatory processes, dysregulation of many axes, stress, pain, mood changes, anxiety, and depression (Harsanyi *et al.,* 2023). Depression and neurosis are diseases that currently affect most young people and affect their social behavior. To achieve this goal. This study examined the relationship between Vt.D and IL-6 as contributing factors to increased risk of depression in adolescents.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Study design:

This cross-sectional study was performed based on ethical clearance in line with the Declaration of Helsinki and verbal agreement from the study subjects' parents, which comprised one hundred and thirty (130) adolescents (males and females) with ages ranging from (12-18) years. Samples were collected from different areas in the center and districts of Al-Najaf al-Ashraf City, Iraq. It was conducted from the first of November 2023 to the end of January, 2024. Based on the study's experimental design and the formal data for every participant, The participants were healthy. All samples suffering from incurable diseases, cancer patients, tumors, endocrine disorders, mental and physical disabilities, and deaf and mute patients were excluded. The adolescents answered a questionnaire that included the Beck Depression Inventory to determine the degree of depression for each adolescent. Five ml of venous blood samples were obtained for hematological and biochemical screening tests.

2.2. Methods:

The collected blood was divided into two parts. Approximately 2 ml and put in an EDTA tube (ethylene diamine tetra acetic acid). In contrast, the other part (3 ml) was put in gel tubes for serum separation, centrifuged at 3000 rpm for 5 minutes to separate the serum, and transferred to new Eppendorf tubes and stored at -20 °C until the measurement IL-6 (No.E0090Hu) and Vt. D (No.E1546Hu levels by ELISA according to the kit supplied by (the Bioassay Technology Laboratory Company).

2.3. Statistical analysis:

All data were statistically analyzed by IBM SPSS 26 (SPSS Inc., Chicago, IL). Frequencies and percentages with the use of the chi-square test reported nominal data. The normal distribution used the Kolmogorov-Smirnov test. Numerical data were expressed as mean ± standard deviation (SD), an independent t-test between two groups, and an ANOVA with Tukey`s post hoc test for comparison among groups. Median (IQR) interquartile range for continuous variables with non-normal distribution and Kruskal-Wallis test for among groups. Pearson's or Spearman Rho coefficient correlation tests between serum levels of IL-6 and Serotonin with depressive scores. Linear and logistic nominal regression analyses were performed to predict the risk of depression. The significant level was considered as p-value <0.05.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Distribution of All Studied Categories in Adolescent Students.

The statistical analysis in Table 1 indicated that 130 adolescent students were enrolled aged (12-18), with (mean ± SD) 14.85±2.19. The results showed a significant increase (p=0.035) in number and percentage 77(59.2%) students had

depression symptom median (IQR) of scores about 16(13_21.5), and 53(40.8%) adolescents with non-depressed (scores 5(2.5_7), who considered as controls group, a statistically significant in depressed scores (p<0.001). As well the Beck depression scale the results indicated that depressive adolescents who had simple type scores were 35(26.9%), with a mean (of 12.57±1.72), mild was 25(19.2%) and mean about (18.44±1.89), and 17(13.1%) severe depression mean was (27.12±2.29), from these results, it was confirmed that there is a significant difference (p=0.042).

The study included 64(49.2%) males and 66 (50.8%) females of total adolescent students; Significant increases were observed in depressed females, 46 (59.7%) more than males 31 (40.3%) p=0.014. A significant (p<0.001) increase in mean BMI of depressive adolescents (23.3±4.3 kg/m2) when compared with (20.21±4.39 kg/m2). While, BMI categories showed depressive adolescents with underweight (20.8%), less than non-depressive (41.5%), but groups with normal weight (50.6%), overweight and obesity about (19.5%), and (9.1%), depressed highest than non-depressive adolescents about (45.3%), (7.5%), and (5.7%), respectively, with significant differences p=0.039. Depressed adolescents have a significant (p<0.001) increase in levels of IL-6 (pg/mL), (Mean:1.85, median (IQR): 1.8(1.3-2.24), and decreased Vt. D (ng/mL) (Mean: 22.32; median (IQR): 18.8(13.1_28.9) as compared with non-depressed (Mean: 1.17; median (IQR): 1.22(0.77-1.43) and 50.54; 52.22(42.2_62.4), respectively. According to Vt. D Status, depressive adolescents had deficiency Vt. D 49(63.6%) and Insufficiency Vt. D 15 (19.5%) more than non-depressed students 2 (3.8%), and 5(9.4%), but who with Sufficiency Vt. D about 13(16.9%) depressive, lowest 46 (86.8%) in non-depressive groups, these results showed a significant difference (p<0.001).

3.1.2. Vitamin D Association with Depressive Status

The findings in Table 2 suggested that Depressive adolescents who 49 (63.6%) Deficiency Vt. D (14.14±4.28) has mean scores of (20.29±5.77), a significant difference (p < 0.001) more than 15 (19.5%) who insufficiency (28.17±1.63) with mean scores (13.53±3.58) and 13 (16.9%) Sufficiency Vt. D (46.38±9.23) with mean scores of (12.69±1.25) as compared with vitamin D status 2 (3.8%), 5 (9.4%), and 46 (86.8%) in non-depressive adolescents, respectively.

3.1.3. IL-6 Association with Vt. D status

Serum levels of IL-6 in depressive adolescents with Deficiency Vt. D has a significant increase mean of about (2.08±0.76) than adolescents with Insufficiency Vt. D (1.48±0.35), p=0.003, and Sufficiency Vt. D (1.39±0.43), p=0.001 to compare with (1.23±0.65), (1.41±0.49), and (1.14±0.46) in non-depressive groups respectively (p=482). Figure 1.

3.1.4. Correlations and Regression Analysis of Vt. D and IL-6 levels with depression scores

Statistics results reported Depression scores were reverse significant correlated (p<0.0001) with levels of Vt. D (r= -0.786), Figure 2. At the same time, were significantly positively correlated (p<0.0001) with levels of IL-6 (r=0.751), Figure (3). IL-6 was significantly negatively correlated (p<0.0001) with levels of Vt. D (r= -0.570). Figure 4. The results showed in Table 3 that age (year) was not a significant predictor of depression scores (B: 0.231; p= 0.234; 95% CI: -0.153 to 0.616), and BMI (B: -0.172; 0.079; 95% CI: -0.365 to 0.021). IL-6 exhibits a highly significant positive predictor (B: 4.430; p<0.001; 95% CI: 3.023 to 5.836). At the same time, Vt. D showed highly significantly negative predictors. (B: -0.145; p<0.001; 95% CI: -0.220 to -0.071).

3.1.5. Logistic Regression analysis to predict the risk factors associated with depression.

The nominal regression analysis in Table 4 shows that the risk of depression associated with females (OR: 2.33; p=0.002) is higher than that of males (OR: 0.939; p=0.803). Those overweight adolescents and obese were predicted to be more depressive (OR: 3.750, p=0.019; and OR: 2.333, p=0.022) than normal weight and underweight (OR:1.625, p=0.061; and OR: 0.727, p=0.332), respectively. The risk of depression was elevated in adolescents with no Vt. D Supplement (OR: 1.769, p=004), more than those who had taken Vt. D Supplement (OR: 0.571, p=0.207). There was Highly predicted depression in adolescents with deficiency Vt. D (OR: 24.4, p<0.001), as compared with insufficiency (OR: 3.00, p=0.033), but no effects in and sufficiency adolescent and (OR:0.283, p<0.001). adolescent (OR: 486, p=0.015).

3.2. Discussion

This study aimed to investigate the relationship between IL-6 and Vitamin D, which

may exacerbate the risk of depression in adolescents. The study enrolled 130 adolescents with a mean age of 14.85 ± 2.19. It was found that 77 (59.2%) of the adolescents showed symptoms of depression. Among those who experienced depression, mild depression was more prevalent (26.9%) than moderate (19.2%) or severe (13.1%) depression. Also, Depression rates are higher in females than males. Many studies support the findings of the current study, indicating a higher prevalence and severity of depression among female adolescents. These findings agree with several studies, which showed the prevalence of major depressive occurrences among adolescents aged 12-17 years and increased rate from 8.1 - 15.8% in the United States, and discovered a prevalence of depression by 34.0% of Chinese adolescents aged 11-16 year, (Daly, 2022, Sun *et al*.; 2021).

A recent study by Shorey showed adolescents are more susceptible to psychological problems during the formative and turbulent adolescent years due to changes in their bodies, minds, and social interactions. Adolescents worldwide between the ages of 10 and 19 are at risk of developing clinical depression at 34%, a rate higher than the estimated for people between the ages of 18 and 25 (Shorey *et al*., 2022) adolescence and early adulthood are key periods for growth and development (Norris *et al*.; 2022) Previously an Iraqi study in a systematic review of Ahmed (2022) reported that furthermost of children have depressive symptoms, particularly the children above the age of nine who are more affected, as well as depression is more common among males than females, but the overall prevalence rate of depression in Iraq is 3.7% according to estimates of WHO in 2021 (Ahmed, 2022).

According to depression status, our outcomes confirmed with a recent Iraqi study revealed the level of depression among adolescent girls. There were four different types of depression: mild mood disturbance (16%), moderate depression (13.4%), severe depression (3.1%), and borderline clinical depression (24.3%) (Alkabban & Alkhazrajy, 2023). Another study observed depression in adolescents between the ages of 13 and 18 and was categorized into three groups in a different study: 10.1% had high depression (severe), 48.4% had low depression (mild), and 41.5% had no depression. The detection rate of high depression was significantly higher in girls (14.5%) than in boys (9.6%), with an OR of 1.60 (95% CI, 1.059–2.406), $p < 0.05$ (Zhang *et al*., 2023). Furthermore, depressed adolescents have higher BMI compared with those non-depression, there were (20.8%) of depressed adolescents were underweight, (50.6%) were normal weight, (19.5%) overweight (19.5%), and (9.1%) obese, these results corresponded with the findings of researchers (Alkabban & Alkhazrajy, 2023), who explore the level of depression among adolescent girls, and its association with BMI found that 14% of the participants were underweight, 59% were of normal weight, 21% were overweight, and 6% were obese.

The results showed vitamin D lowest in depressed Adolescents than non- depression, and those who take vitamin D as a dietary supplement had fewer depression symptoms about 22(16.9%) had depressive symptoms 8(10.4%), and 14(26.4%) non-depressives, as compared with a total student 108(83.1%) who don't take Vt. D Supplement about 69(89.6%) with depressive, and 39(73.6%) who non-depressive. The results of the study are also shown in Figure (3) and Table 3 Vt. D showed highly significantly negative predictors for Depression scores (B: -0.145; $p<0.001$; 95% CI: -0.220 to -0.071). Depression may result from low serotonin levels, which are influenced by low vitamin D levels (23). Our findings showed that adolescents with no Vt. D Supplement (OR: 1.769, p=004) were more than those who had taken Vt. D Supplement (OR: 0.571, p=0.207). There was Highly predicted depression in adolescents with deficiency Vt. D (OR: 24.4, $p<0.001$), as compared with insufficiency (OR: 3.00, p=0.033), but no effects in and sufficiency adolescent and (OR:0.283, $p<0.001$). adolescent (OR: 486, p=0.015).

Vitamin D affects serotonin synthesis by a unique biological mechanism, and Serotonin is always involved in the pathophysiology of depression. A previous study explained depressive disorder is widely thought to be caused by insufficient vitamin D levels. They are thought to be related because the human brain has vitamin D receptors (VDRs), which have the potential to produce psychiatric disorders through the following mechanisms: Certain regions of the human brain, including the thalamus, cerebellum, amygdala, and hippocampus, produce enzymes called 1α-hydroxylases that can convert 25(OH)D into 1,25(OH)2D3, Vitamin D and its receptors (VDR) in the human brain are linked to CYP 24A1 and CYP 27B1 enzymes, suggesting that vitamin D plays a crucial role in neuroprotection, Vitamin D may have a neuroprotective effect on the inflammatory process in the body. To support

immunomodulation, there has been an increase in inflammatory cytokines and vitamin D, (Ghaseminejad-Raeini *et al*., 2023). The findings support the concept that vitamin D administration helps alleviate unpleasant moods. Supplementation is most likely to aid patients suffering from major depressive disorder and those who are vitamin D deficient (Cheng *et al*., 2020; Krivošíková *et al*., 2022).

The results indicate an inverse relationship between vitamin D and interleukin-6 Figure (4) There are previous studies that support this validity, and Vitamin D suppresses the synthesis of inflammatory cytokines, particularly interleukin 6 (IL-6), by monocytes, which triggers the systemic inflammatory response syndrome, (Aranow *et al*., 2011).

Recent epidemiological data has demonstrated a strong correlation between vitamin D insufficiency and a higher risk of developing infectious diseases and inflammatory autoimmune disorders or an exacerbation of these conditions (Ao *et al*., 2021). Vitamin D and its metabolites limit the adaptive immune response, which, if left unchecked, promotes the inflammatory response that can result in acute respiratory distress syndrome and death. In contrast, they support the innate immune response, which offers the first line of defense against bacterial and viral infections (Bikle, 2022). Vitamin D helps reduce autoimmunity caused by T cells, specifically Th1. Vitamin D receptor agonists block the development of dendritic cells, inflammatory and pro-pathogenic T cells (Th1 and Th17), promoting a shift to the Th2 pathway and better immunological tolerance (Aranow *et al*., 2011).

Adolescent depression had a higher significant increase in IL-6 levels compared with non-depression. Some studies revealed that Interleukin-6 (IL-6), an inflammatory cytokine, has been implicated in an increased risk of depression in adults, but nothing is known about this relationship in adolescents. Our findings are confirmed with a recent study (Elgellaie *et al*., 2023), which suggested that plasma IL-6 was higher in the major depressive disorder (MDD) group versus control, but with a sex interaction for IL-6, with this group difference only among females, and correlated with depressive severity, anxiety, hostility, stress, and BMI, these confirmed of findings obtained in current study The risk of depression associated with females (OR: 2.33; p=0.002), is higher than males (OR: 0.939; p=0.803). Those overweight adolescents and obese were predicted to be more depressive (OR: 3.750, p=0.019; and OR: 2.333, p=0.022) than normal weight and underweight (OR:1.625, p=0.061; and OR: 0.727, p=0.332), respectively.

Interleukin 6 (IL-6) is a cytokine produced from T cells, macrophages, and adipocytes that can function as either an anti- or pro-inflammatory cytokine and be released into the bloodstream in response to an immunologic stimulant. In depression, cytokine responses to infections and stressors are increased. Responses, when combined with other risk factors, resulting in extended inflammatory processes, dysregulation of many axes, stress, pain, mood changes, anxiety, and depression (Harsanyi *et al*., 2023), these outcomes confirmed by our results IL-6 exhibits a highly significant positive predictor (B: 4.430; p<0.001; 95% CI: 3.023 to 5.836). Inflammatory markers may play a key role in the diverse profile of youth depression. The recent proposal by Toenders and colleagues (2022) that depression in youth may be characterized by multiple distinct immunophenotypes that correlate with particular depression symptom profiles. Dysregulation of inflammatory cascades, thus, needs to be taken into account as a plausible cause of neurobiological dysfunction in depression in children and adolescents (Bhatt *et al*., 2023). Recently, Anett and colleagues (2024) examined the association between IL-6, TNF-α, and neurobiological function. Correlations between those factors were seen; however, the direction of the correlations varied.

4. CONCLUSION

Decreased vitamin D and elevated IL-6 levels may indicate a relationship between immune dysregulation and vitamin D metabolism. The likelihood of severe adolescent depression was found to be associated with IL-6, especially with vitamin D deficiency, serving as available predictors of the severity of depression.

5. DECLARATIONS

5.1. Study Limitations

The relatively small sample size of 130 adolescents may limit the generalizability. Exclusion criteria omitted participants with certain health conditions, potentially introducing bias. The cross-sectional design prevents establishing causal relationships. Reliance on self-report measures and ELISA kits, which may introduce measurement error. Potential confounding

variables were not accounted for in the statistical analysis. Lack of control for external factors like seasonal vitamin D variations**.**

5.2. Acknowledgements

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5.3. Funding source

The authors funded this research.

5.4. Competing Interests

The authors declare that they have no conflicts of interest regarding the publication of this article.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

This cross-sectional study comprised one hundred and thirty (130) adolescents (males and females) with ages ranging from (12-18) years, performed based on ethical clearance in line with the Declaration of Helsinki and verbally approved by the Ethics Committee of the Faculty of Sciences, Kufa University, with approval number of (28568) and date of (11/07/2023).

After explaining the risk of depression in adolescents and its connection with hormonal changes, nutrition, and lifestyle, a special questionnaire was designed based on The Beck Depression Inventory (BDI). The BDI is a 21-item assessment tool used to identify depressive symptoms and typical attitudes (Beck *et al*., 1961). The study subjects' parents provided written informed consent, and formal data for each participant was coded and recorded. This included information such as name, age, gender, family history of hereditary diseases, use of vitamin D as a nutritional supplement, weight, and height.

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Table 1. *Distribution of All Studied Categories in Adolescent Students.*

Variables	Categories	Depressive	Non-Depressive	p-value
Age (year)		14.94±2.2	14.72±2.2	0.579
Studied samples		77 (59.2%)	53 (40.8%)	X^2=4.431 0.035*
Depression Scores		17.69±5.97 16(13_21.5)#	4.98±2.74 5(2.5_7)#	<0.001**
Types of Depression	**Simple**	35 (26.9%)	-	X^2=6.338 0.042*
	Mild	25 (19.2%)	-	
	Severe	17 (13.1%)	-	
Sex	**Male**	31(40.3%)	33 (62.3%)	X^2= 6.081 0.014*
	Female	46 (59.7%)	20 (37.7%)	
BMI (kg/m^2)	**Mean ± SD**	23.3±4.3	20.21±4.39	<0.001**
	Underweight **Normal weight** **Overweight** **Obesity**	16 (20.8%) 39 (50.6%) 15 (19.5%) 7 (9.1%)	22 (41.5%) 24 (45.3%) 4 (7.5%) 3 (5.7%)	X^2=8.341 0.039*
IL-6(pg/ml)		1.85±0.72 1.8(1.3-2.24)#	1.17±0.47 1.22(0.77-1.43)#	<0.001**
Vt.D (ng/mL)		22.32±13.21 18.8(13.1_28.9)#	50.54±14.85 52.22(42.2_62.4)#	<0.001**
Vt. D Supplement	**Yes**	8 (10.4%)	14 (26.4%)	X^2=5.734 0.017*
	No	69 (89.6%)	39 (73.6%)	
Vt. D Status	**Deficiency**	49 (63.6%)	2 (3.8%)	X^2= 4.54 <0.001**
	Insufficiency	15 (19.5%)	5 (9.4%)	
	Sufficiency	13 (16.9%)	46 (86.8%)	

Significant differences at p-value. **<0.01, *<0.5. X^2: Chi-Square test. #: Median (IQR). BMI: (body mass index)

Table 2. *Depression Scores Association with Vt. D status*

Vt. D status	Depression Scores		p-value
	Depressive	Non-Depressive	
Deficiency Vt. D	49 (63.6%) 20.29±5.77	2 (3.8%) 8.5±0.71	**<0.001****
Insufficiency Vt. D	15 (19.5%) 13.53±3.58	5 (9.4%) 7±1.22	
Sufficiency Vt. D	13 (16.9%) 12.69±1.25	46 (86.8%) 4.61±2.71	

*Significant differences at p-value **<0.01, p*<0.05. data of Depression Scores expressed as Mean ± SD*

Table (3). *Linear regression analysis is used to predict risk factors associated with depression scores.*

Predicts parameters	B	SE	p-value	95.0% CI
(Constant)	13.265	4.108	0.002	5.076 to 21.455
Age (year)	0.231	0.193	0.234	-0.153 to 0.616
BMI (kg/m^2)	-0.172	0.097	0.079	-0.365 to 0.021
IL-6 (pg/mL)	4.430	0.706	<0.001	3.023 to 5.836
Vt.D (ng/mL)	-0.145	0.037	<0.001	-0.220 to -0.071

*Significant differences at p-value *<0.05, p **<0.01. B: Unstandardized Coefficients (effect size). SE: standard error. CI: Confidence Interval. Dependent Variable: depression* Scores.

Table 4. *Nominal Regression analysis to predict the categories of risk factors associated with depressive status.*

Predicts [a]		B	p-value	OR	95% CI
Sex	**Male**	-0.063	0.803	0.939	0.575 to 1.534
	Female	0.833	0.002	2.300*	1.361 to 3.888
BMI Categories	**Underweight**	-0.318	0.332	0.727	0.382 to 1.385
	Normal weight	0.486	0.061	1.625	0.977 to 2.702
	Overweight	1.322	0.019	3.750*	1.245 to 11.299
	Obesity	0.847	0.220	2.333	0.603 to 9.023
Vt.D Supplement	**Yes**	-0.560	0.207	0.571	0.240 to 1.362
	No	0.571*	0.004	1.769	1.195 to 2.620
Vt. D_ Status	**Deficiency**	3.199	0.0001	24.50**	5.96 to 100.74
	Insufficiency	1.099	0.033	3.00 *	1.09 to 8.254
	Sufficiency	-1.264	0.0001	0.283**	0.153 to 0.523

*Significant differences at p-value *<0.05, p**<0.01. a: The category references are non-depressive. 95% Confidence Interval for OR*

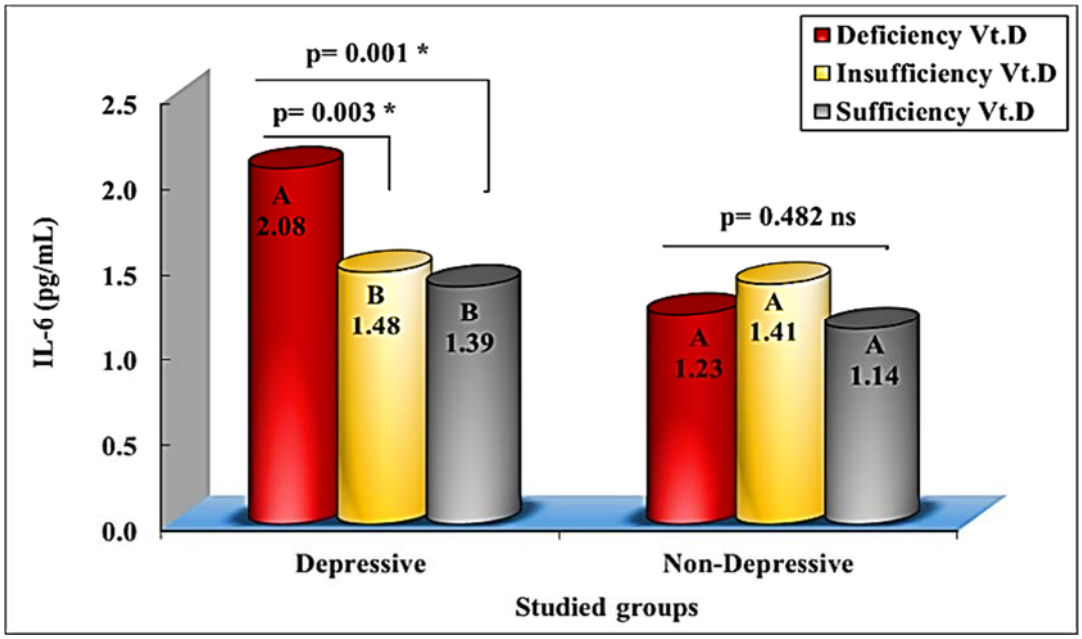

Figure 1: *Serum IL-6 levels in depressive adolescents according to Vt. D status*
Deafferent letters Significant differences at p-value<0.05. ns: non-significant.

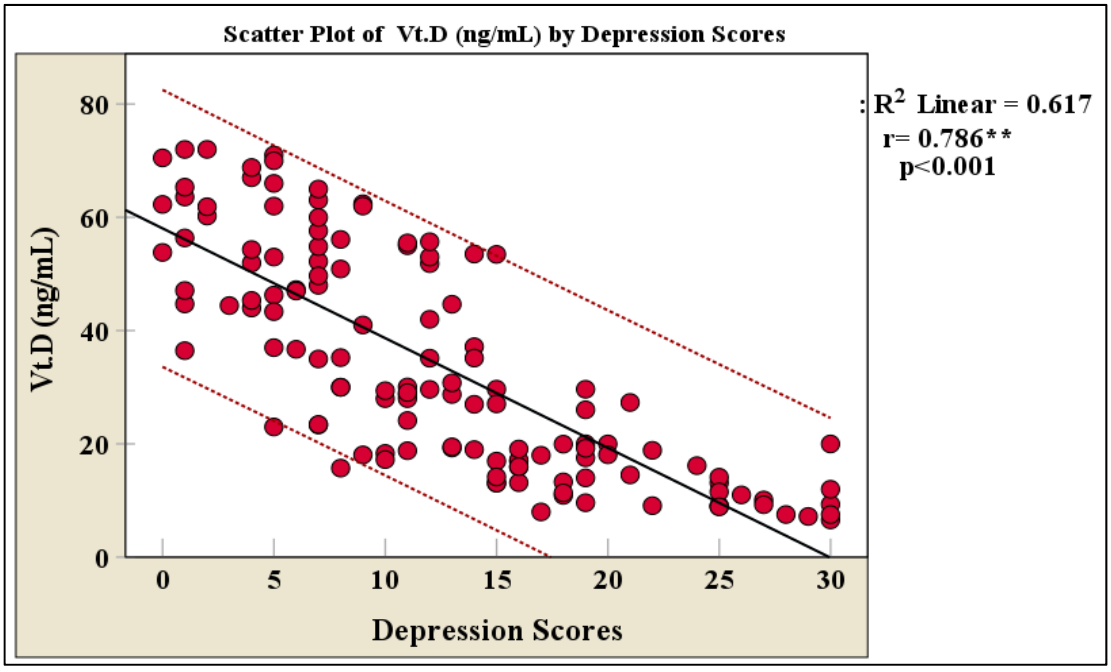

Figure 2. Pearson Correlation of Vt. D with depression scores
The separated red line is a 95% confidence interval

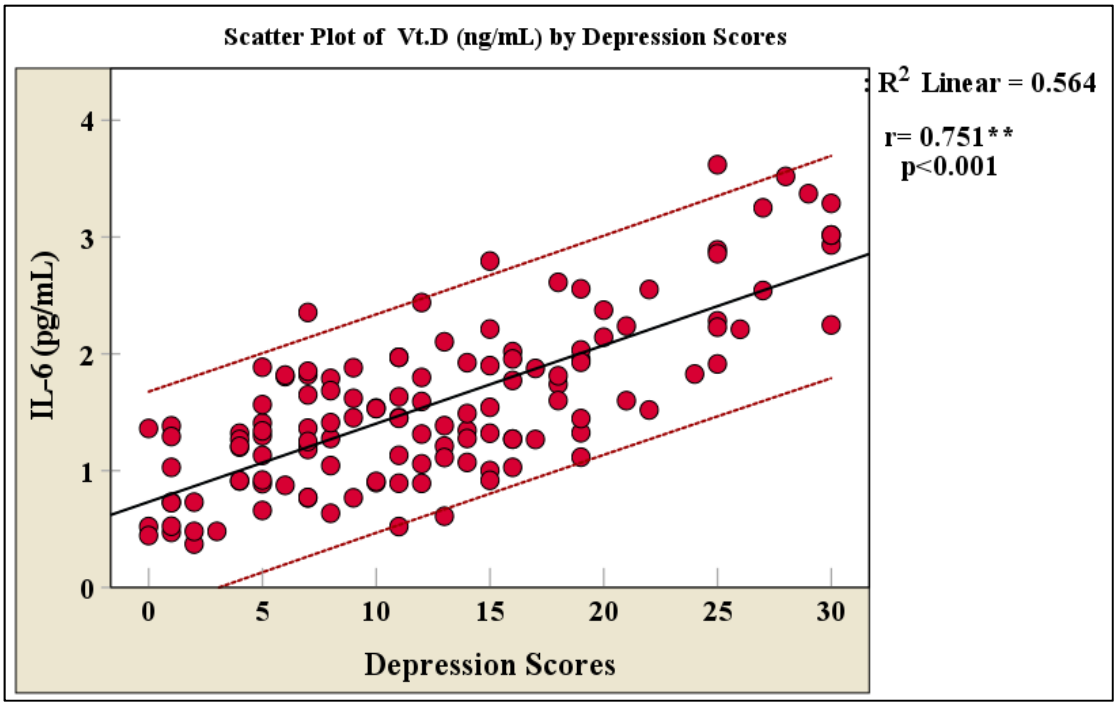

Figure 3. *Pearson Correlation of IL-6 with depression scores.*
The separated red line is a 95% confidence interval.

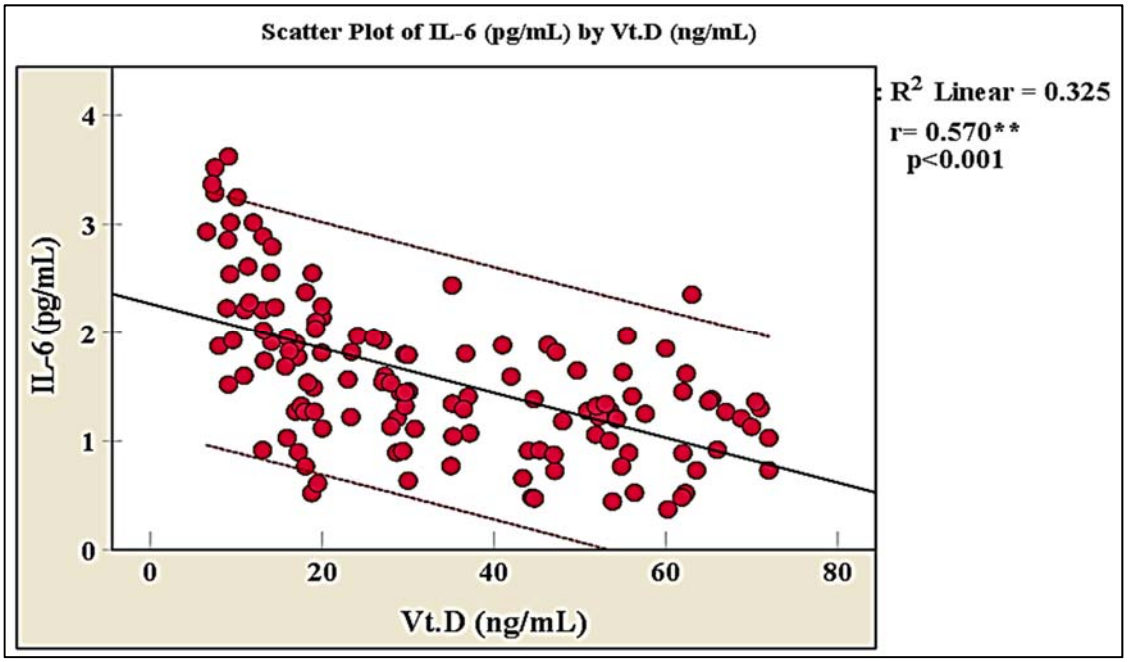

Figure 4. *Pearson Correlation of IL-6 with Vt. D*
The separated red line is a 95% confidence interval

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
0. I do not feel sad.
1. I feel sad
2. I am sad all the time and I can't snap out of it.
3. I am so sad and unhappy that I can't stand it.
2.
0. I am not particularly discouraged about the future.
1. I feel discouraged about the future.
2. I feel I have nothing to look forward to.
3. I feel the future is hopeless and that things cannot improve.
3.
0. I do not feel like a failure.
1. I feel I have failed more than the average person.
2. As I look back on my life, all I can see is a lot of failures.
3. I feel I am a complete failure as a person.
4.
0. I get as much satisfaction out of things as I used to.
1. I don't enjoy things the way I used to.
2. I don't get real satisfaction out of anything anymore.
3. I am dissatisfied or bored with everything.
5.
0. I don't feel particularly guilty
1. I feel guilty a good part of the time.
2. I feel quite guilty most of the time.
3. I feel guilty all of the time.
6.
0. I don't feel I am being punished.
1. I feel I may be punished.
2. I expect to be punished.
3. I feel I am being punished.
7.
0. I don't feel disappointed in myself.
1. I am disappointed in myself.
2. I am disgusted with myself.
3. I hate myself.
8.
0. I don't feel I am any worse than anybody else.
1. I am critical of myself for my weaknesses or mistakes.
2. I blame myself all the time for my faults.
3. I blame myself for everything bad that happens.
9.
0. I don't have any thoughts of killing myself.
1. I have thoughts of killing myself, but I would not carry them out.
2. I would like to kill myself.
3. I would kill myself if I had the chance.
10.
0. I don't cry any more than usual.
1. I cry more now than I used to.
2. I cry all the time now.
3. I used to be able to cry, but now I can't cry even though I want to.
11.
0. I am no more irritated by things than I ever was.
1. I am slightly more irritated now than usual.
2. I am quite annoyed or irritated a good deal of the time.

3. I feel irritated all the time.
12.
0. I have not lost interest in other people.
1. I am less interested in other people than I used to be.
2. I have lost most of my interest in other people.
3. I have lost all of my interest in other people.
13.
0. I make decisions about as well as I ever could.
1. I put off making decisions more than I used to.
2. I have greater difficulty in making decisions more than I used to.
3. I can't make decisions at all anymore.
14.
0. I don't feel that I look any worse than I used to.
1. I am worried that I am looking old or unattractive.
2. I feel there are permanent changes in my appearance that make me look unattractive
3. I believe that I look ugly.
15.
0. I can work about as well as before.
1. It takes an extra effort to get started at doing something.
2. I have to push myself very hard to do anything.
3. I can't do any work at all.
16.
0. I can sleep as well as usual.
1. I don't sleep as well as I used to.
2. I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3. I wake up several hours earlier than I used to and cannot get back to sleep.
17.
0. I don't get more tired than usual.
1. I get tired more easily than I used to.
2. I get tired from doing almost anything.
3. I am too tired to do anything.
18.
0. My appetite is no worse than usual.
1. My appetite is not as good as it used to be.
2. My appetite is much worse now.
3. I have no appetite at all anymore.
19.
0. I haven't lost much weight, if any, lately.
1. I have lost more than five pounds.
2. I have lost more than ten pounds.
3. I have lost more than fifteen pounds.
20.
0. I am no more worried about my health than usual.
1. I am worried about physical problems like aches, pains, upset stomach, or constipation.
2. I am very worried about physical problems and it's hard to think of much else.
3. I am so worried about my physical problems that I cannot think of anything else.