Association of vitamin D with deoxyribonucleic acid (DNA) damage: A systematic review of animal and human studies

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Vitamin D has anti-proliferative, anti-inflammatory, and apoptotic abilities. Vitamin D deficiency can induce deoxyribonucleic acid (DNA) damage. The aim of the study was to create a systematic review to analyze the relationship between vitamin D and DNA damage in various populations. PubMed, Scopus, EbscoHost, Google Scholar, and Epistemonikos were used to identify literature regarding the relationship between vitamin D and DNA damage. Assessment of study quality was carried out by three independent reviewers individually. A total of 25 studies were assessed as eligible and included in our study. Twelve studies were conducted in humans consisting of two studies with experimental design and ten studies with observational pattern. Meanwhile, thirteen studies were conducted in animals (in vivo). It is found that the majority of studies demonstrated that vitamin D prevents DNA damage and minimizes the impact of DNA damage that has occurred (p<0.05). However, two studies (8%) did not find such an association and one research only found a specific association in the cord blood, not in maternal blood. Vitamin D has a protective effect against DNA damage. A diet rich in vitamin D and vitamin D supplementation is recommended to prevent DNA damage.

Keywords: vitamin D, DNA damage, observational studies, in vivo studies, systematic review

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INTRODUCTION

Vitamin D and its receptors play an essential role in cancer development due to its anti-proliferative, anti-inflammatory, and apoptotic properties (Nair-Shalliker et al., 2012a; Deuster et al., 2017; Elhusseini et al., 2018). Endogenous synthesis of vitamin D begins with cholesterol oxidation, producing pro-vitamin D3. In the skin, Ultraviolet B (UVB) from sunlight converts pro-vitamin D3 to pre-vitamin D3. Then, isomerization of pre-vitamin D3 is done, with vitamin D3 (cholecalciferol) as its main end product (Osmancevic et al., 2015). Two hydroxylations by the enzymes vitamin D 25-hydroxy-
different populations of human studies and animal models.

METHODS

The manuscript was arranged using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines (Page et al., 2021). All members approved the review panel’s study procedure before conducting the literature search. The protocol has been registered in the International prospective register of systematic reviews (PROSPERO) with the registration number: CRD42023393054.

Literature retrieval

A comprehensive literature search was conducted across five databases to identify manuscripts on the relationship between vitamin D levels and DNA damage in human and animal subjects. The articles were discovered using Scopus, PubMed, EBSCOHost, Google Scholar, and Epistemonikos. Hand-searching was also conducted based on the included study bibliography to identify relevant publications that were not indexed in the previously reported databases (Umar et al., 2022). There will be no restrictions on geographical region or gender. However, we restricted our search to studies published between January 2012 and January 2023. The investigation was completed by January 19th, 2023. To ensure its validity, the study must be published in English. The search terms for this study are available as Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp/.

Study selection

We sought animal and human studies investigating the relationship between vitamin D levels and DNA damage. Studies must demonstrate the influence of a single vitamin D substance administration on DNA damage (rather than a formulation containing multiple compounds) to be considered. The study was deemed ineligible if its design consisted of a literature review (e.g., systematic review, narrative review, scoping review), opinion, book chapter, and editorial. Meanwhile, included studies must have access to the full text. As a result, we excluded conference abstracts, posters, and unretrieved complete records. Duplicates were removed from the literature retrieval. Three independent reviewers (VL, MK, and ZH) assessed titles and abstracts (primary screening) using a semi-automated process aided by Rayyan QCRI software (Ouzzani et al., 2016; Umar & Siburian, 2022). Following the completion of the first screening stage, the full text was assessed by two reviewers (IAL and TPU) to determine its eligibility for inclusion in the review. Any disagreements were discussed and resolved by a senior author (MIL) at any stage of manuscript evaluation.

Data extraction

The following information was extracted from the data: authorship, country of study, research participant data in the form of age, sex, and comorbidities (human), as well as experimental research data on animal type and age (animal studies), DNA damage parameters (comet tail length, tail DNA, and tail moment; phosphorylated Histone H2AX (γH2AX), 8-hydroxy-2'-deoxyguanosine (8-OHdG), chromosomal aberration, DNA damage score, micronuclei formation, telomere length, urinary cyclobutane thymine (T–T) dimer, and DNA repair indicator), and main findings. The information was recorded on the extraction sheet using Microsoft Office Excel 2019. Because of the vast diversity among included studies, the findings were presented as a qualitative synthesis rather than a meta-analysis.

Risk of bias analysis

The risk of bias (RoB) analysis was conducted for the animal studies using The Systematic Review Center for Laboratory Animal Experimentation’s risk of bias (SYRCLE’s RoB) tool. The RoB tool from SYRCLE contains ten items related to selection bias, performance bias, detection bias, reporting bias, and other biases (Hooijmans et al., 2014). Meanwhile, we used three different scales for human studies: the Newcastle-Ottawa Scale (for observational studies), the Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I), and Version 2 of the Cochrane risk-of-bias tool for randomized trials (ROB-2). The Newcastle Ottawa Scale is divided into three levels: high, moderate, and low. The ROBINS-I tool rates studies on seven domains, and ROB-2 rates on nine domains.

Figure 1. Study selection flow
into three major domains (selection, comparability, and outcome) (Liana et al., 2022). In contrast, the ROBINS-I (Sterne et al., 2016) and ROB-2 (Sterne et al., 2019) are divided into several parts within the pre-intervention, intervention, and post-intervention stages. The evaluation was carried out independently by two authors (IAL and TPU). In the event of a disagreement, the decision is made by a senior author (KM).

RESULTS

The search strategy identified a total of 942 studies. The search query found 551 studies on SCOPUS, 140 on Google Scholar, 28 on EbscoHost, and 12 on Epistemonikos. Following duplicate detection, 243 studies were excluded. Then, 699 studies entered title and abstract screening, where 56 studies were deemed eligible for full-text screening, which resulted in 24 studies being included in the final analysis. Meanwhile, six studies were identified from the citation search, and only one was finally contained. This process resulted in 25 selected studies (13 animal studies/in vivo and 12 human studies (two experimental studies and ten observational research)) considered in the final process of manuscript evaluation (Fig. 1).

Study characteristics

All of the included studies evaluated the association of vitamin D with DNA damage. Animal studies (Table 1) were done on the vitamin D-deficient diet (Chen et al., 2018; Elhusseini et al., 2018; Merino et al., 2018), hypertension (Machado et al., 2016, 2019), oxidative stress (Haq et al., 2019; Liu et al., 2019), and neurological disorder (Alfawaz et al., 2014; Mehri et al., 2020). Meanwhile, all of the following parameters were assessed only in one study: diabetes mellitus (Meerza et al., 2012), high-fat diet (Merino et al., 2018), kidney disease (Mohammed et al., 2019), aging (Qiao et al., 2020), and ovariectomy (Siebert et al., 2018) model. There are four main DNA damage detection methods, including immunohistochemistry (Chen et al., 2018; Elhusseini et al., 2018; Mohammed et al., 2019), comet assay (alkaline comet assay) (Alfawaz et al., 2014; Liu et al., 2019; Machado et al., 2016, 2019; Siebert et al., 2018), Enzyme-linked immunosorbent assay (ELISA) (Haq et al., 2019; Mehri et al., 2020), and micronuclei detection (Liu et al., 2019; Machado et al., 2016). Meanwhile, other detection methods are flow cytometry (Merino et al., 2018), gel electrophoresis (Haq et al., 2019), Western blot (Qiao et al., 2020) and reverse transcription polymerase chain reaction (RT-PCR) (Siebert et al., 2018). Rodents, both mice and rats, were used as

Figure 2. Risk of Bias for Animal Studies

Figure 3. Risk of Bias for Human Studies.

Figure 4. Risk of Bias Summary.
Table 1. Data extraction for animal studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Experimental animal</th>
<th>Disease model and inducer</th>
<th>Parameter</th>
<th>Method</th>
<th>Major findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Alfawaz et al., 2014)</td>
<td>Saudi Arabia</td>
<td>Western Albino rats (±21 days, n=28)</td>
<td>Neurotoxicity by PPA</td>
<td>Tail length</td>
<td>Comet DNA assay</td>
<td>There is a potential impact of vitamin D in protecting and treating PPA neurotoxicity, while ameliorating the DNA-damaging effects of PPA. Prevention impact of vitamin D against PPA-induced DNA damage is more profound than its treatment effect.</td>
</tr>
<tr>
<td>(Chen et al., 2018)</td>
<td>China</td>
<td>Vitamin D-deficient model</td>
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<td></td>
<td>There is an increase of DNA damage in 1,25(OH)2D3-deficient mice as measured by γH2AX and 8-OHdG on tumor cells according to immunohistochemistry.</td>
</tr>
<tr>
<td>(Elhusseini et al., 2018)</td>
<td>United States</td>
<td>Female C57BL/6 mice, (4–6 weeks old), control (n = 10), vitamin D-deficient diet group (n = 10)</td>
<td>Vitamin D-deficient model</td>
<td>DNA repair (RAD50 and RAD51)</td>
<td>Immunohistochemistry</td>
<td>Vitamin D-deficient mice showed increased γH2AX in myometrial tissue compared to healthy controls (P&lt;0.05). Vitamin D deficiency caused a significant reduction in the expression of DNA repair genes such as RAD50 (P&lt;0.005) and RAD51 (P&lt;0.0005) compared to healthy controls.</td>
</tr>
<tr>
<td>(Haq et al., 2019)</td>
<td>Saudi Arabia</td>
<td>Wistar Albino rats (1 week, n = 4)</td>
<td>Hydrogen peroxide (H2O2)</td>
<td>Chromosomal aberration (DNA fragmentation)</td>
<td>Comet assay</td>
<td>Vitamin D, which was given for 24 hours prior to the induced oxidative stress by H2O2 significantly (p&lt;0.001) reversed the deleterious and damaging effect of H2O2 alone as presented by DNA fragmentation percentage. Significantly lower value of 8-OHdG following the administration of vitamin D + H2O2 than H2O2 alone (p&lt;0.05).</td>
</tr>
<tr>
<td>(Liu et al., 2019)</td>
<td>China</td>
<td>Male mice (7–8 weeks)</td>
<td>CP</td>
<td>DNA damage score, total DNA in the tail (%), tail length, tail moment</td>
<td>Alkaline comet assay</td>
<td>Vitamin D3 suppressed CP-induced micronucleus formation in mice Buccal cells, with an alleviation range of 36.7–44.46% (p&lt;0.05).</td>
</tr>
<tr>
<td>(Machado et al., 2016)</td>
<td>Brazil</td>
<td>Male spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups</td>
<td>Hypertension model</td>
<td>DNA damage score, total DNA in the tail (%), tail moment</td>
<td>Alkaline comet assay</td>
<td>SHR rats with a vitamin D3 deficient diet showed a significant increase in the incidence of micronuclei formation in the bone marrow and peripheral blood (p&lt;0.05).</td>
</tr>
<tr>
<td>(Machado et al., 2019)</td>
<td>Brazil</td>
<td>Male Spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups</td>
<td>Hypertension model</td>
<td>Total DNA in the tail (%)</td>
<td>Alkaline comet assay</td>
<td>There was no significant difference in the percentage of DNA in comet tails (p&gt;0.05) in the vitamin D deficiency group when compared to the control group. Vitamin D3 supplementation or deficiency did not significantly affect cardiac genotoxicity.</td>
</tr>
<tr>
<td>(Meera et al., 2012)</td>
<td>India</td>
<td>Female albino mice</td>
<td>Diabetes model by alloxan (200 mg/kgBW)</td>
<td>DNA tail length</td>
<td>Comet assay</td>
<td>Vitamin D-supplemented group showed a significant decrease in liver (21.80 ± 2.40 μm) and pancreatic (19.25 ± 1.90 μm) DNA tail length in diabetic mice.</td>
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<tr>
<td>(Mehri et al., 2020)</td>
<td>Iran</td>
<td>Wistar rats (200–240g, n=48)</td>
<td>Alzheimer’s Disease by 5 μl of Aβ-containing solution</td>
<td>8-OHdG</td>
<td>ELISA</td>
<td>The level of DNA damage in Vitamin D and Aβ + Vitamin D groups in hippocampus and Vitamin D group of serum samples was significantly lower than that of the Aβ group (p &lt; 0.0001).</td>
</tr>
<tr>
<td>(Marina et al., 2018)</td>
<td>Chile</td>
<td>Male Sprague-Dawley rats (4 months, n=20 divided into four groups)</td>
<td>High-fat diet and vitamin D-deficient diet</td>
<td>DNA Fragmentation</td>
<td>Flow cytometry</td>
<td>Vitamin D supplementation results in lower DNA fragmentation, either in the control or experimental group (p&lt;0.05). The interaction between vitamin D deficiency and diet-induced obesity was significant in DNA fragmentation (p = 0.0359).</td>
</tr>
</tbody>
</table>
animal models in all studies. It was found that all DNA damage parameters are associated with vitamin D levels, except in one study which did not find significant difference in the percentage of DNA in comet tails in the vitamin D deficiency group when compared to the control group (Machado et al., 2019). Another study also showed that the preventive impact of vitamin D supplementation is better than its treatment effect to ameliorate DNA damage (Alfawaz et al., 2014).

Human research (Table 2) were conducted in experimental (randomized and non-randomized clinical trial) (Wenclewska et al., 2019; Gungor et al., 2022) and observational (cross-sectional and cohort) (Ladeira et al., 2015; Lan et al., 2014; Nair-Shalliker et al., 2012b; Najeeb et al., 2020; Ng et al., 2021; O’Callaghan-Gordo et al., 2017; Petersen et al., 2014; Usman et al., 2021; Wang et al., 2016; Fagundes et al., 2019) design. In human studies, the most commonly employed parameter is comet assay (comet tail length, DNA damage score, and percentage of DNA in comet tail) (Fagundes et al., 2019; Lan et al., 2014; Najeeb et al., 2020; Ng et al., 2021; Wang et al., 2016; Wenclewska et al., 2019) and micronuclei formation (buccal or lymphocyte) (Fagundes et al., 2019; Ladeira et al., 2015; Nair-Shalliker et al., 2012b; O’Callaghan-Gordo et al., 2017; Usman et al., 2021). Other parameters are aniline blue staining (sperm DNA damage) (Gungor et al., 2022), thymine dimer (Petersen et al., 2014), and ELISA (urinary 8-OHdG) (Usman et al., 2021). Several conditions were observed in the included studies, such as diabetes mellitus, cancer, obesity, vitamin D-deficient state, and infertility. A similar finding was also observed in animal studies when the majority of the investigations revealed a significant association between vitamin D and DNA damage status. However, a study on workers occupationally exposed to formaldehyde (Ladeira et al., 2015) and on the general population (Nair-Shalliker et al., 2012b) did not show a significant association between vitamin D level and micronuclei formation, while another study only found the association in cord blood but not in maternal blood (O’Callaghan-Gordo et al., 2017). Another research also did not present any association between vitamin D level and comet assay result as the DNA damage marker in the general population (Wang et al., 2016).

### Risk of Bias

All of the animal studies included in the analysis followed a similar pattern (Figs 2 and 4), with a low risk of bias on baseline characteristics, random outcome assessment, selective outcome reporting, and other biases. However, it is noteworthy that allocation concealment, random housing, and intervention blinding were not met by all studies, resulting in a high risk of bias. Meanwhile, only four studies (Liu et al., 2019; Mehri et al., 2020; Mohammed et al., 2019; Siebert et al., 2018) have a low risk of bias for outcome assessor blinding, and only two studies (Mehri et al., 2020; Siebert et al., 2018) have a low risk of bias for incomplete outcome data analysis.

We assessed the risk of bias in human studies (Fig. 3) using three scales: NOS, ROBINS-I, and ROB-2. Most studies (10/12; 83.33%) have moderate/some concerns about bias. A non-randomized study, on the other hand, runs the risk of bias due to insufficient intervention classification. Meanwhile, there is only one study (O’Callaghan-Gordo et al., 2017) that has a low overall risk of bias. According to the summary graph (Fig 4) on observational studies, 70% have a high risk of bias on comparability due to a lack of explanation on confound-
Table 2. Data extraction for human studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Location</th>
<th>Design</th>
<th>Age</th>
<th>% Male</th>
<th>Population</th>
<th>Method</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Fagundes et al., 2019)</td>
<td>Brazil</td>
<td>Prospective cohort</td>
<td>62.11</td>
<td>37</td>
<td>75 patients with type 2 diabetes mellitus who were given supplementation of vitamin D₃ 400 IU/day for 8 weeks</td>
<td>Comet assay; Buccal micronucleus cytome assay</td>
<td>Decreased DNA damage index (comet assay) (p&lt;0.05) and micronuclei formation (p&lt;0.05) following supplementation with vitamin D₃ and a wash-out period There is a negative correlation between DNA damage index and vitamin D levels (r=−0.2569; p&lt;0.0001) but not in micronuclei.</td>
</tr>
<tr>
<td>(Gunor et al., 2022)</td>
<td>Turkey</td>
<td>Non-RCT</td>
<td>34.67</td>
<td>100</td>
<td>58 men with unexplained infertility (+50 controls)</td>
<td>Aniline blue staining (sperm DNA damage)</td>
<td>There was a negative and significant correlation between vitamin D levels and sperm DNA damage (r = −0.605, p&lt;0.001)</td>
</tr>
<tr>
<td>(Ladeira et al., 2015)</td>
<td>Portugal</td>
<td>Cross-sectional</td>
<td>39.64</td>
<td>65.45</td>
<td>55 workers occupationally exposed to formaldehyde (+80 controls)</td>
<td>CBMN assay (buccal and lymphocyte)</td>
<td>Vitamin D has no association with the frequency of micronuclei (lymphocytes or buccal cells) in workers exposed to formaldehyde</td>
</tr>
<tr>
<td>(Lan et al., 2014)</td>
<td>China</td>
<td>Cross-sectional</td>
<td>50 ± 10</td>
<td>50</td>
<td>16 patients with severe asthma and vitamin D &lt;30ng/ml (+16 controls and 16 patients with vitamin D &gt;30ng/ml)</td>
<td>Comet assay (DNA damage score)</td>
<td>The total DNA damage score for a subject with Vitamin D deficiency was significantly increased compared to the scores in Vitamin D sufficiency (p = 0.002).</td>
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<tr>
<td>(Nair-Shalliker et al.,</td>
<td>Australia</td>
<td>Cross-sectional</td>
<td>46.0</td>
<td>NA</td>
<td>207 participants</td>
<td>CBMN assay</td>
<td>There is no association between log serum 25(OH)D concentration and log-transformed frequency of any CBMN-cyt assay biomarker (p=0.3)</td>
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<td>2012b)</td>
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<tr>
<td>(Najeeb et al., 2020)</td>
<td>Iraq</td>
<td>Cross-sectional</td>
<td>61.87</td>
<td>48.89</td>
<td>45 cancer patients (+35 controls)</td>
<td>Comet assay</td>
<td>Correlation between Tail DNA% and plasma vitamin D is significant in cancer patients (r=−0.3707; p&lt;0.0001) and control (r=0.2824; p&lt;0.001) with higher damage at lower vitamin D level</td>
</tr>
<tr>
<td>(Ng et al., 2021)</td>
<td>Malaysia</td>
<td>Cross-sectional</td>
<td>29.96</td>
<td>0</td>
<td>134 participants (47 obese, 87 non-obese)</td>
<td>Alkaline comet assay</td>
<td>Multivariate analysis revealed that individuals with serum 25(OH)D level of ≥ 31 nmol/L had a significantly lower tail moment (1.06 ± 0.22 mmol/L vs. 2.37 ± 0.60 nmol/L, p = 0.029) and tail olive moment (2.36 ± 0.24 mmol/L versus 3.46 ± 0.60 mmol/L, p = 0.031) compared to those with lower serum 25(OH)D level, in the obese group</td>
</tr>
<tr>
<td>(O’Callaghan-Gordo et al.,</td>
<td>Spain</td>
<td>Cross-sectional</td>
<td>39.74</td>
<td>7.22</td>
<td>344 participants (173 mothers and 171 newborns)</td>
<td>CBMN assay</td>
<td>The association between cyclobutane thymine dimers (T–T dimers) and vitamin D is significant (r=−0.76; p&lt;0.0001), strongly indicating that the harmful DNA effects of ultraviolet radiation are unavoidable</td>
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<td>2017)</td>
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<tr>
<td>(Petersen et al., 2014)</td>
<td>Denmark</td>
<td>Cross-sectional</td>
<td>46.48</td>
<td>71</td>
<td>171 newborns</td>
<td>Urinary cyclobutane thymine (T–T) dimers</td>
<td>The association between cyclobutane thymine dimers (T–T dimers) and vitamin D is significant (r=−0.3707; p&lt;0.0001) and control (r=0.2824; p&lt;0.001) with higher damage at lower vitamin D level</td>
</tr>
<tr>
<td>(Usman et al., 2021)</td>
<td>United Kingdom</td>
<td>Cross-sectional</td>
<td>14.60</td>
<td>45.28</td>
<td>132 adolescents (53 obese, control: 59 non-obese)</td>
<td>Buccal micronucleus cytome assay (buccal epithelial cells) ELISA (measures 8-OHdG from urine)</td>
<td>Vitamin D has significant correlation with 8-OHdG (r=−0.245; p&lt;0.001) and buccal micronuclei (r=−0.305; p&lt;0.01)</td>
</tr>
<tr>
<td>(Wang et al., 2016)</td>
<td>China</td>
<td>Cross-sectional</td>
<td>20.69</td>
<td>36.36</td>
<td>121 participants (44 males, 77 females)</td>
<td>Comet assay IV Lite scoring system</td>
<td>The percentage of DNA in the tail decreased in the intervention group when compared to the control group, either with or without T2DM (p&lt;0.05) DNA oxidative parameters (Fpg) decreased in the intervention group (113.63 ± 4.26 vs. 104.19 ± 3.06, p&lt;0.05), especially in the T2DM group when compared to the control group (p&lt;0.01).</td>
</tr>
<tr>
<td>(Wenclewska et al., 2019)</td>
<td>Poland</td>
<td>RCT</td>
<td>63.43</td>
<td>29.17</td>
<td>92 people with vitamin D deficiency (intervention: 48 people, control: 44 people (14 with T2DM, 30 healthy)</td>
<td>Comet assay (Peripheral lymphocyte)</td>
<td>The percentage of DNA in the tail decreased in the intervention group when compared to the control group, either with or without T2DM (p&lt;0.05) DNA oxidative parameters (Fpg) decreased in the intervention group (113.63 ± 4.26 vs. 104.19 ± 3.06, p&lt;0.05), especially in the T2DM group when compared to the control group (p&lt;0.01).</td>
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Abbreviation: 25(OH)D, 25 hydroxy vitamin D; 8-OHdG, 8-Hydroxyguanosine; T–T dimers, cyclobutane thymine dimers; CBMN, cytokinesis-block micronuclei; Cyp27b1, Cytochrome p450 27B1; DNA, Deoxyribonucleic acid; ELISA, Enzyme-linked immunosorbent assay; H₂O₂, Hydrogen peroxide; IU, International unit; IRR, Incidence rate ratio; RCT, Randomized controlled trial; T2DM, Type 2 diabetes mellitus. Data was presented as: (a) mean ± SD or (B) median (min-max), (c) exposed mean, (d) overall mean.
ing control. However, regarding selection, 70% of the studies have a low risk of bias, and 20% of the included research has a high risk of bias. Most studies have a moderate risk of bias in outcome assessment, primarily due to non-blinding outcome assessment.

**DISCUSSION**

DNA damage can be divided into two types, endogenous and exogenous. Endogenous DNA damage stems from chemically active DNA involved in hydrolytic and oxidative reactions with air and reactive oxygen species (ROS), which are naturally present in cells. On the contrary, exogenous DNA damage occurs due to the involvement of environmental, physical, and chemical substances such as UV and ionizing radiation, alkylating agents, and cross-linking agents (Chatterjee & Walker, 2018). Vitamin D is regarded as an essential factor in the status of DNA damage (Najeb et al., 2020). Vitamin D deficiency (plasma 25(OH)D <50 nmol/l) and severe deficiency (<30 nmol/l) have been associated with elevated oxidative stress, DNA damage promotion, and overall mortality (Wang et al., 2016). The impact of vitamin D on DNA damage is prominent in several disorders, including hyperglycemia and cancer (Gabryanczyk et al., 2021).

Hyperglycemia increases the production of free radicals and also induces DNA damage (Giacco & Brownlee, 2010). Studies conducted in patients with type 2 diabetes mellitus (T2DM) showed that vitamin D significantly prevented DNA damage and oxidative stress in patients with T2DM (p<0.05). A vitamin D-responsive element has been identified in the promotion region of the insulin receptor gene in human (Gikas et al., 2009). Pancreatic cells express the nuclear receptor for 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), which modulates insulin action (Bland et al., 2004). Furthermore, vitamin D minimizes insulin resistance by its effect on calcium and phosphorus metabolism along with the upregulation of the insulin receptor gene, as well as suppression of the synthesis of proinflammatory cytokines that contribute to insulin resistance, including interleukins and TNF-α due to its antioxidative properties (Wenclewski et al., 2019; Maestro et al., 2002; Talaei et al., 2013).

In malignancy, vitamin D had positive functions as anti-proliferative, proapoptotic, anti-inflammatory, anti-angiogenesis, anti-metastatic, and anti-invasion as well as estrogen signaling inhibitor (Vuolo et al., 2012; Deuster et al., 2017; Wacker & Hollack, 2013). Calcitriol (the active form of vitamin D) inhibits the proliferation of many malignant cells by inducing cell cycle arrest and cell accumulation in the G0/G1 phase of the cell cycle. In cells, calcitriol causes G1/G0 arrest in a p53-dependent manner by increasing the expression of the cyclin-dependent kinase inhibitors p21Waf/Cip1 and p27Kip1, decreasing the activity of cyclin-dependent kinase 2 (CDK2), and causing hypo-phosphorylation. Calcitriol also increases the expression of p73, a homolog of p53, which is associated with the induction of apoptosis in several human and murine tumor systems. Suppression of p73 abrogates calcitriol-induced apoptosis and reduces the ability of calcitriol to enhance the cytotoxic effect of agents such as gemcitabine and cisplatin in a squamous cell carcinoma (SCC) model (Krishnan & Feldman, 2010; Khrisnan et al., 2012). In the previous study, it was found that vitamin D deficiency is a risk factor for malignancy (cancer) and accelerates the invasion process (Najeb et al., 2020; Migliaccio et al., 2022). The population of the 25 studies in this systematic review is diverse. Twelve studies conducted in humans analyzed DNA damage in patients with comorbid diseases such as T2DM, obesity, infertility, cancer patients, and the general population. Meanwhile, thirteen in vitro studies analyzed DNA damage using animal models with hypertension, ovariectomy, nephrotoxicity, vitamin D deficiency, and oxidative stress.

Several studies on vitamin D indicated that the vitamin has a beneficial impact on all organ systems of the human body. Both 25(OH)D and its hormonally active form, 1,25(OH)2D are vital for physiological functions, especially to reduce inflammation and excessive cellular oxidative stress. The 1,25(OH)2D hormone or calcitriol modulates cell proliferation through direct and indirect pathways, such as by the inhibition of the transcription factor, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which is associated with an elevation of oxidative stress and cellular response to inflammation and injury (Tilstra et al., 2012). Due to suppression of NF-κB activation, calcitriol helps to reduce chronic inflammation (Myszka & Klinger, 2014). However, more research into the relationship between DNA damage, oxidative stress, and vitamin D is required.

Vitamin D receptor (VDR) is found in testicular tissue, prostate, and spermatozoa. In addition, the intense metabolism of vitamin D in the male reproductive system and increased expression of VDR in the neck of the sperm cause males to require vitamin D for functionally active sperm (Jensen et al., 2011). Incubation of semen samples with vitamin D for 30 minutes led to a significant increase in sperm velocity parameters. This progressive increase in motility is due to vitamin D-dependent calcium release and subsequent cyclic AMP/protein kinase A (cAMP/PKA) activation and Adenosine triphosphate (ATP) production (Gunter et al., 2004). There was a significant negative correlation (p>0.05) between vitamin D and sperm DNA damage in this systematic review. After binding to the VDR receptor, vitamin D initiates slow genomic effects by stimulating the release of ligand-activated transcription factors in the nucleus. In unexplained infertile patients with vitamin D deficiency, sperm DNA damage may occur due to delayed genomic effects (Jurutka et al., 2001).

Studies in various animal models showed that vitamin D exerts a protective effect on DNA. Vitamin D can reduce the DNA damage index (percentage of DNA in comet tails assessed from comet tests). In addition, in genomic instability animal models induced by cyclophosphamide, vitamin D can reduce the frequency of micronuclei formation (a marker of DNA damage). Besides the percentage of DNA in comet tails and the frequency of micronuclei, DNA damage can also be assessed by the levels of 8-OHdG, a marker of oxidative DNA damage (Smith et al., 2005). Elevated levels of 8-OHdG in rat animal models are also associated with a complete loss of VDR expression. Because the expression of VDR depends on the availability of 1,25(OH)2D, the loss of VDR suggests that there may be a role for 1,25(OH)2D in protecting cells against hyperproliferation and oxidative DNA damage (Kállay et al., 2002; Nair-Shalliker et al., 2012a). Decreased levels of 8-OHdG after vitamin D supplementation was proven in the animal model studies (Haque et al., 2019; Mohammed et al., 2019). The results of studies in animal models (in vivo) are in line with those of studies in cells (in vitro) (Chen et al., 2018; Liu et al., 2019). These outcomes can confirm that vitamin D has a protective effect on DNA.
Various parameters of oxidative stress are also presented in this systematic review. In human studies, vitamin D supplementation led to a significant decrease in NO and total thiols and an increase in the concentration of reduced glutathione (GSH) leading to a decrease in oxidative processes in cells (Fagundes et al., 2019). In a study of animal models with hypertension, vitamin D was not significantly associated with DNA damage. However, vitamin D3 deficiency alters the level of Thio-barbituric Acid Reactance Substance (TBARS) in a mouse model of spontaneous hypertension, which is an indicator of Reactive Oxygen Species (ROS)-initiated peroxidation of unsaturated fatty acids in membrane lipids and alters the permeability, fluidity, and integrity of the plasma membrane (Potter et al., 2011). Lipid peroxidation predisposes patients to conditions such as hypertension and thrombomembolic (Yavuzer et al., 2016). Vitamin D can reduce oxidative stress that occurs in cells thereby reducing DNA damage.

This systematic review proved that vitamin D protects against DNA damage. However, there are some limitations to this systematic review. First, the study populations are largely heterogeneous with different diseases and DNA damage parameters; thus, the results can be biased. In addition, human studies are still sparse (only one randomized controlled trial/RCT) and mainly with a moderate RoB. Consequently, the application of the results to humans still needs to be considered. Further studies with randomized controlled trial designs are expected in the future to increase the strength of evidence.

CONCLUSION

There is a significant association between vitamin D and DNA damage. However, although the majority of studies have found that vitamin D has a protective effect against DNA damage, other research found contradictory findings. Thus, the need for further investigations with stricter criteria must be commenced. Nevertheless, it is safe to conclude that a diet with sufficient vitamin D content and supplementation (more than 1000 IU/day, preferably about 2000–5000 IU/day) is recommended to prevent DNA damage and oxidative stress in cells.

REFERENCES


