

# Evaluation of the Interrelationship between Serum Osteocalcin Vitamin D and Parathyroid Hormone Levels and Glycemic Control in Type 2 Diabetes Mellitus in a Cross-Sectional Study

*by* Jana Publication & Research

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# Evaluation of the Interrelationship between Serum Osteocalcin Vitamin D and Parathyroid Hormone Levels and Glycemic Control in Type 2 Diabetes Mellitus in a Cross-Sectional Study

## Abstract

**Introduction:** Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by impaired insulin secretion and action, which influences not only glucose homeostasis but also bone metabolism. Emerging evidence indicates that bone-derived biomarkers such as osteocalcin, along with vitamin D and parathyroid hormone (PTH), may play a role in glycemic regulation.

**Materials and Methods:** This cross-sectional observational study was conducted at the Department of Medicine, Index Medical College, Hospital & Research Centre, Indore, from January 2023 to January 2024. A total of 200 participants were enrolled, including 100 diagnosed T2DM patients and 100 age- and sex-matched healthy controls. Fasting blood sugar (FBS), random blood sugar (RBS), and glycated hemoglobin (HbA1c) were measured as glycemic parameters. Serum osteocalcin, vitamin D, and PTH were analyzed as bone metabolism biomarkers using standard biochemical assays. Statistical analysis was performed to evaluate correlations between bone markers and glycemic indices.

**Results:** T2DM patients showed significantly lower serum osteocalcin and vitamin D levels compared to controls ( $p < 0.05$ ), while PTH levels were higher in diabetic individuals. Glycemic indices (FBS, RBS, and HbA1c) demonstrated inverse correlations with osteocalcin and vitamin D, whereas PTH levels were positively correlated with poor glycemic control. These findings suggest altered bone biomarker profiles in diabetic patients and their potential role in glucose dysregulation.

**Conclusion:** The study highlights the interrelationship between bone metabolism biomarkers and glycemic status in T2DM. Altered osteocalcin, vitamin D, and PTH levels may contribute to impaired metabolic regulation in diabetes. Monitoring these markers alongside routine glycemic indices could improve risk assessment and therapeutic strategies. Further longitudinal studies are warranted to establish causality and therapeutic implications.

**Keywords:** Type 2 diabetes mellitus; Osteocalcin; Vitamin D; Parathyroid hormone; Glycated hemoglobin; Bone metabolism biomarkers

## Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both, and is associated with disturbances in carbohydrate, fat, and protein metabolism (1). Type 2 Diabetes Mellitus (T2DM) accounts for over 90% of DM cases globally and has emerged as a major public health challenge. According to the International Diabetes Federation (IDF) Diabetes Atlas 2021, an estimated 537 million adults aged 20–79 years were living with diabetes worldwide in 2021, with projections indicating an increase to 643 million by 2030 and 783 million by 2045 (2). Over 75% of these cases occur in low- and middle-income countries. In India, the prevalence is particularly high; the Indian Council of Medical Research–India Diabetes (ICMR–INDIAB) study reported 62.4 million people with diabetes in 2011, expected to rise to 101.2 million by 2030 (3). By 2040, India is projected to have

123 million adults with diabetes, with millions more exhibiting impaired glucose tolerance, contributing substantially to morbidity and mortality (4).

The pathophysiology of T2DM extends beyond traditional disturbances in glucose and insulin dynamics to involve skeletal and mineral metabolism. Osteocalcin (OC), a non-collagenous protein secreted by osteoblasts, exists in carboxylated (cOC) and undercarboxylated (ucOC) forms. In addition to its role in bone mineralization, OC influences glucose metabolism by enhancing insulin secretion, improving insulin sensitivity, and regulating energy balance (5). Studies have shown that poorly controlled diabetes is associated with reduced OC levels, while improved glycemic control restores OC concentrations, suggesting a bidirectional bone–glucose axis (6,7).

Vitamin D, synthesized primarily in the skin upon ultraviolet light exposure, is essential for calcium and phosphate homeostasis but also plays a role in glucose metabolism by modulating  $\beta$ -cell function, insulin receptor expression, and inflammatory pathways (8,9). Vitamin D deficiency—affecting more than one billion people globally—is prevalent in India and has been linked to insulin resistance, T2DM risk, and poorer glycemic outcomes (10,11).

Parathyroid hormone (PTH), a key regulator of calcium homeostasis, is increasingly recognized for its effects on glucose metabolism. Elevated PTH levels, whether due to primary or secondary hyperparathyroidism, have been associated with reduced insulin sensitivity, impaired glucose uptake, and adverse cardiovascular profiles in T2DM patients (12,13).

Despite accumulating evidence on the individual roles of OC, vitamin D, and PTH in glucose regulation, limited research has examined these biomarkers collectively in T2DM. Understanding their combined interaction may enable early identification of metabolic alterations, enhance risk stratification, and inform personalized therapeutic strategies integrating bone and glucose metabolism (14,15). The present study was designed to evaluate the interrelationship between serum osteocalcin, vitamin D, and PTH levels and their association with glycemic control in patients with T2DM.

## Materials and Methods

### Study Design and Setting

This cross-sectional observational study was conducted in the Department of Medicine, Faculty of Medicine, Index Medical College, Hospital and Research Centre, Indore, Madhya Pradesh, India, over a 12-month period from January 2023 to January 2024.

### Study Population

A total of 200 participants were enrolled, comprising 100 patients with type 2 diabetes mellitus (T2DM) and 100 age- and sex-matched healthy controls. T2DM was diagnosed according to the American Diabetes Association (ADA) criteria [13]: fasting plasma glucose (FPG)  $\geq 126$  mg/dL,

glycated hemoglobin (HbA1c)  $\geq 6.5\%$ , or 2-hour plasma glucose  $\geq 200$  mg/dL after an oral glucose tolerance test.

**Inclusion criteria** were:

- Age  $\geq 42$  years.
- Confirmed T2DM attending the diabetic outpatient clinic.
- Healthy controls with FPG  $< 110$  mg/dL and HbA1c  $< 5.7\%$ .
- Written informed consent.

**Exclusion criteria** included:

- Osteoporosis, metabolic bone disease, or thyroid/parathyroid disorders.
- Medications affecting bone metabolism (e.g., corticosteroids, bisphosphonates).
- Vitamin D or calcium supplementation.
- Chronic kidney or liver disease, autoimmune or inflammatory disorders.
- Pregnancy or lactation.

### Clinical and Biochemical Assessment

<sup>35</sup> All participants underwent a structured clinical evaluation, including medical history, physical examination, and body mass index (BMI) measurement. <sup>30</sup> Following an overnight fast ( $\geq 8$  hours), 10 mL of venous blood was collected between 08:00 and 10:00 AM to minimize diurnal variation in biomarker levels [14].

#### 1. Osteocalcin Estimation

Serum osteocalcin, a bone-derived protein implicated in glucose metabolism, was quantified using a commercial <sup>22</sup> enzyme-linked immunosorbent assay (ELISA) kit with intra- and inter-assay coefficients of variation (CV) of  $< 8\%$  and  $< 10\%$ , respectively, ensuring assay precision. The ELISA method utilizes antigen–antibody specificity with enzymatic signal amplification for accurate protein quantification [15].

#### 2. Vitamin D and Parathyroid Hormone (PTH)

<sup>24</sup> Serum 25-hydroxyvitamin D [25(OH)D] and intact PTH levels were measured by chemiluminescent immunoassay (CLIA). CLIA offers high analytical sensitivity and specificity by detecting luminescent signals emitted during antigen–antibody binding, measured via luminometry [16,17].

#### 3. Glycemic Parameters

FPG and random blood sugar (RBS) were determined by the hexokinase enzymatic method, the reference standard for glucose estimation due to its high specificity [18]. <sup>17</sup> HbA1c was measured by high-performance liquid chromatography (HPLC), enabling precise separation from other hemoglobin fractions [19].

### Statistical Analysis

Data were entered into SPSS version 29.0 for analysis. Continuous variables were expressed as mean  $\pm$  SD or median (interquartile range, IQR) depending on distribution. Group comparisons were performed using the independent samples t-test or Mann-Whitney U test. Categorical variables were analyzed with the chi-square test. Pearson correlation was applied to examine associations between osteocalcin and glycemic parameters. A two-sided p-value  $<0.05$  was considered statistically significant[20].

### Ethical Considerations

The study protocol received approval from the Institutional Ethics Committee of Index Medical College (Approval No. MU/Research/EC/Ph.D./2022/336). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki [21].

### Results

In this cross-sectional study, we evaluated the interrelationship between serum osteocalcin, vitamin D3, and parathyroid hormone (PTH) levels with glycemic control in patients with type 2 diabetes mellitus (T2DM) compared to healthy controls. A total of 200 participants were enrolled, comprising 100 patients with T2DM and 100 age- and sex-matched controls.

#### Demographic Characteristics

The two groups were comparable in terms of mean age ( $55.09 \pm 5.39$  vs.  $54.26 \pm 4.93$  years;  $t=1.135$ ,  $p=0.258$ ). However, the age distribution differed significantly ( $\chi^2=7.670$ ,  $p=0.022$ ). In the T2DM group, 58% were aged 51–60 years, 25% were  $\leq 50$  years, and 17% were  $>60$  years, compared to 70%, 25%, and 5% in controls, respectively (Table 1, Figure 1). Gender distribution was identical in both groups (62% female, 38% male;  $p=1.00$ ) (Table 2).

#### Glycemic Profile

Patients with T2DM had significantly higher glycemic indices compared to controls. Mean fasting blood sugar (FBS), postprandial blood sugar (PPBS), and glycated hemoglobin (HbA1c) were  $147.2 \pm 72.5$  mg/dL,  $338.4 \pm 74.4$  mg/dL, and  $7.22 \pm 1.4\%$ , respectively, versus  $86.0 \pm 17.0$  mg/dL,  $127.8 \pm 19.6$  mg/dL, and  $5.38 \pm 0.94\%$  in controls ( $p<0.001$  for all) (Table 3, Figure 2).

#### Bone Metabolism Biomarkers

The T2DM group demonstrated significantly reduced mean serum osteocalcin ( $4.64 \pm 1.4$  vs.  $7.26 \pm 2.5$  ng/ml;  $t=-8.983$ ,  $p<0.001$ ) and vitamin D3 levels ( $30.35 \pm 0.4$  vs.  $36.90 \pm 7.8$  ng/ml;  $t=-5.014$ ,  $p<0.001$ ), while PTH levels were markedly higher ( $70.18 \pm 32.8$  vs.  $38.4 \pm 19.8$  pg/ml;  $t=8.269$ ,  $p<0.001$ ) compared to controls (Table 4).

#### Biomarker Categories

Categorical analysis revealed significant between-group differences. Reduced osteocalcin levels ( $<3.7$  ng/ml) were more common in T2DM (22% vs. 10%), while none of the T2DM patients had elevated osteocalcin ( $>10$  ng/ml) compared to 3% of controls ( $p=0.018$ ). Vitamin D3 deficiency ( $<20$  ng/ml) was more prevalent in T2DM patients (15% vs. 0%), while sufficiency (20–50 ng/ml) was less frequent (79% vs. 93%;  $p<0.001$ ). Elevated PTH levels ( $>65$  pg/ml) were observed in nearly half of the T2DM cohort (49% vs. 12%;  $p<0.001$ ) (Table 5).

### Associations Within the T2DM Group

Within the T2DM group, osteocalcin categories were not significantly associated with FBS, PPBS, HbA1c, vitamin D3, or PTH (all  $p>0.05$ ) (Table 5). In contrast, vitamin D3 categories demonstrated significant associations. Patients with vitamin D3 deficiency had markedly higher HbA1c ( $9.3 \pm 1.1\%$ ) and PTH ( $117.6 \pm 30.8$  pg/ml) compared to those with sufficient or optimal vitamin D3 (HbA1c  $6.9 \pm 1.1\%$  and  $5.5 \pm 0.5\%$ , respectively;  $p<0.001$ ) (Table 7). Similarly, elevated PTH was associated with poorer glycemic control, reflected by higher FBS ( $162.0 \pm 95.2$  vs.  $132.9 \pm 35.8$  mg/dL;  $p=0.044$ ), higher HbA1c ( $8.23 \pm 1.3\%$  vs.  $6.25 \pm 0.71\%$ ;  $p<0.001$ ), and lower vitamin D3 ( $23.7 \pm 6.2$  vs.  $36.6 \pm 9.7$  ng/ml;  $p<0.001$ ) (Table 8).

No significant relationships were observed between osteocalcin and vitamin D3 or PTH categories in T2DM patients (Tables 9–10).

### Correlation Analysis

Correlation analyses revealed distinct associations among the studied biomarkers. HbA1c exhibited a strong inverse correlation with vitamin D3 in both groups (T2DM:  $r = -0.748$ ,  $p < 0.001$ ; controls:  $r = -0.379$ ,  $p < 0.001$ ) and a strong positive correlation with PTH (T2DM:  $r = 0.781$ ,  $p < 0.001$ ; controls:  $r = 0.847$ ,  $p < 0.001$ ), while no significant correlation was found with osteocalcin (Tables 11–12). Importantly, vitamin D3 showed a significant negative correlation with PTH in both groups (T2DM:  $r = -0.701$ ,  $p < 0.001$ ; controls:  $r = -0.301$ ,  $p = 0.002$ ), highlighting an inverse relationship between these two biomarkers irrespective of diabetic status (Table 13). These findings suggest that dysregulation of vitamin D3 and PTH may play a key role in glycemic imbalance, whereas osteocalcin appears to have limited direct correlation with HbA1c and other studied parameters.

### Summary of Findings

Collectively, these findings demonstrate that vitamin D3 and PTH are strongly interrelated and significantly associated with glycemic control in T2DM, while serum osteocalcin showed limited association with glycemic indices in this cohort.

### Discussion

This cross-sectional analysis of 200 participants, evenly split between T2DM patients and matched controls, highlights the intricate interplay between bone metabolism biomarkers and glycemic regulation. Our findings demonstrate significantly lower serum osteocalcin and vitamin D3 levels,

alongside elevated PTH, in T2DM patients compared to controls. Importantly, these alterations correlated with poor glycemic indices, underscoring the potential role of the bone–endocrine axis in glucose homeostasis.

### Osteocalcin and Glycemic Control

Osteocalcin, particularly in its undercarboxylated form (ucOC), has been implicated as an endocrine hormone influencing glucose metabolism by stimulating pancreatic  $\beta$ -cell proliferation, enhancing insulin secretion, and improving insulin sensitivity via adiponectin release and muscle glucose uptake [22,23]. Experimental and clinical studies suggest that ucOC supplementation improves glucose tolerance and insulin resistance [24]. However, in our study, total osteocalcin did not significantly correlate with glycemic indices in T2DM patients. This discrepancy may be attributable to limitations of conventional ELISA kits, which do not differentiate between bioactive ucOC and carboxylated osteocalcin [25]. Future work focusing on osteocalcin isoforms may provide greater clarity regarding its metabolic role.

### Vitamin D, PTH, and Insulin Sensitivity

Our results also demonstrated significantly lower vitamin D3 levels and higher PTH concentrations in T2DM patients, consistent with recent reports highlighting vitamin D deficiency and secondary hyperparathyroidism as contributors to impaired glucose metabolism [26,27]. Vitamin D is known to enhance insulin sensitivity by modulating calcium flux within pancreatic  $\beta$ -cells and suppressing systemic inflammation, while elevated PTH levels are associated with insulin resistance and  $\beta$ -cell dysfunction [28]. The strong negative correlation between vitamin D3 and HbA1c, and the positive correlation between PTH and HbA1c in our cohort, reinforce this biological link. These findings align with recent meta-analyses showing that vitamin D supplementation can modestly improve glycemic indices in T2DM [29].

### Bone–Glucose Axis–Clinical Implications

The reciprocal relationship observed between low vitamin D, elevated PTH, and poor glycemic control underscores the clinical significance of this endocrine triad. Correction of vitamin D deficiency may lower PTH levels and improve insulin sensitivity, suggesting a therapeutic avenue worth further exploration [30]. Moreover, persistently elevated PTH could serve as both a biomarker and mediator of metabolic dysfunction in T2DM. While osteocalcin's association with glucose metabolism remains less definitive in our study, future longitudinal investigations with ucOC measurements may help clarify its relevance to T2DM pathophysiology.

### Study Strengths and Limitations

The study's strengths include parallel measurement of multiple bone metabolism markers and well-matched controls, reducing confounding. However, limitations include its cross-sectional design, precluding causal inference, and the relatively modest sample size. Additionally, single-point biochemical assessments may not capture dynamic endocrine fluctuations. Prospective interventional studies focusing on vitamin D supplementation, PTH modulation, and specific osteocalcin isoforms are warranted to validate and extend these findings.

### Conclusion

The present study highlights the intricate relationship between bone metabolism markers and glycemic control in patients with type 2 diabetes mellitus. Our findings demonstrate that altered serum osteocalcin, vitamin D, and parathyroid hormone levels are significantly associated with impaired glucose homeostasis, underscoring the bidirectional link between bone and energy metabolism. These results suggest that monitoring bone biomarkers, alongside conventional

glycemic indices, may provide additional insights into the metabolic status of diabetic patients. Moreover, the study supports the growing evidence that bone-derived hormones and vitamin D play a role in insulin sensitivity and glucose regulation. Future longitudinal and interventional studies are warranted to clarify causal relationships and evaluate the therapeutic potential of targeting bone metabolism pathways in the management of diabetes.

## Additional Information

### Author Contributions

- **Concept and Design:** Anisha Tanwar,, Mohammad Nadeem Khan, Shreya Nigoskar
- **Data Presentation & Compilation:** Mohammad Nadeem Khan, Mandyal Jamatia, Ashok Kumar
- **Drafting of the Manuscript:** Anisha Tanwar, Mohammad Nadeem Khan,
- **Critical Review:** Anisha Tanwar, Mohammad Nadeem Khan, Shreya Nigoskar
- **Supervision:** Anisha Tanwar ,Shreya Nigoskar

### Disclosure

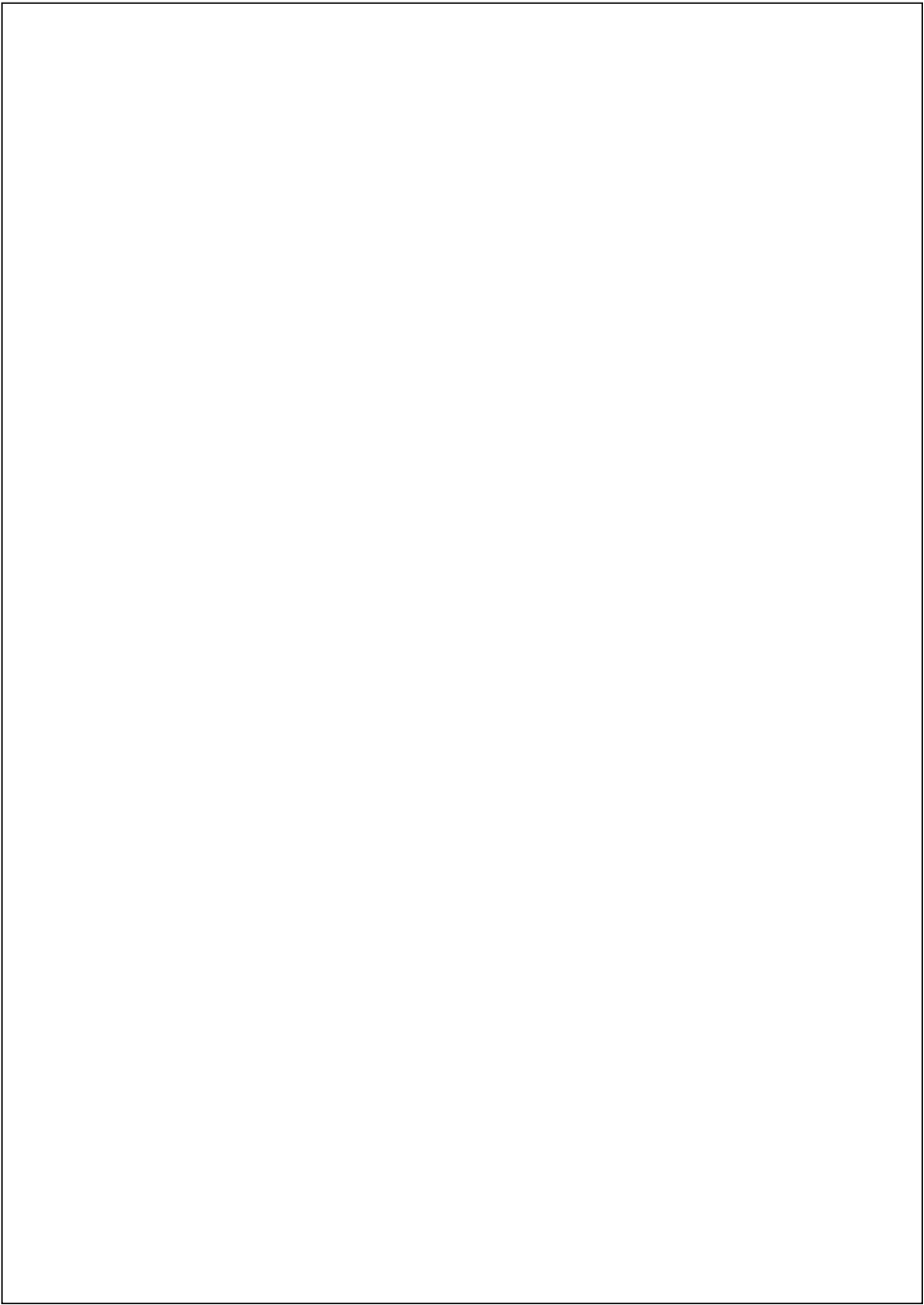
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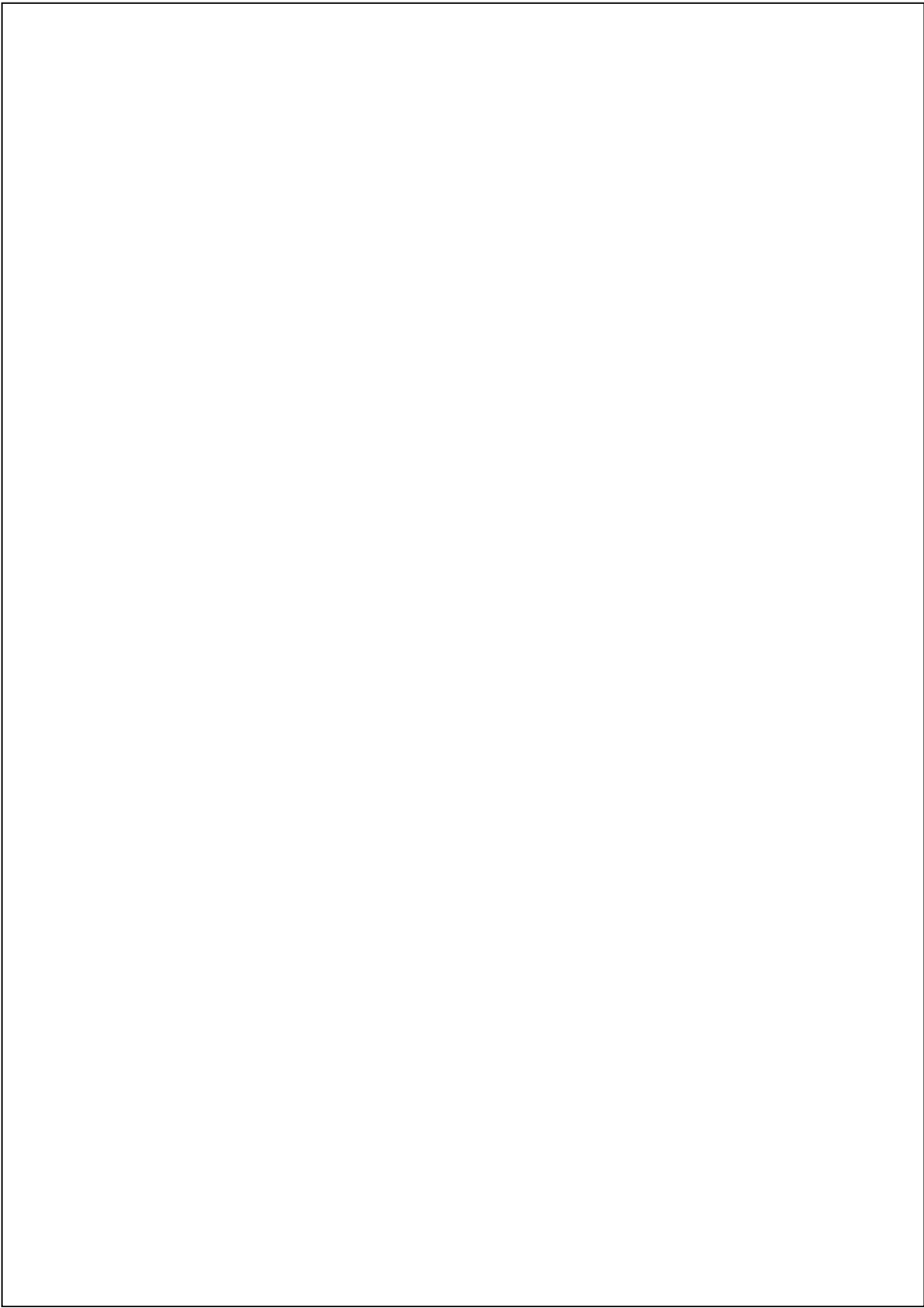
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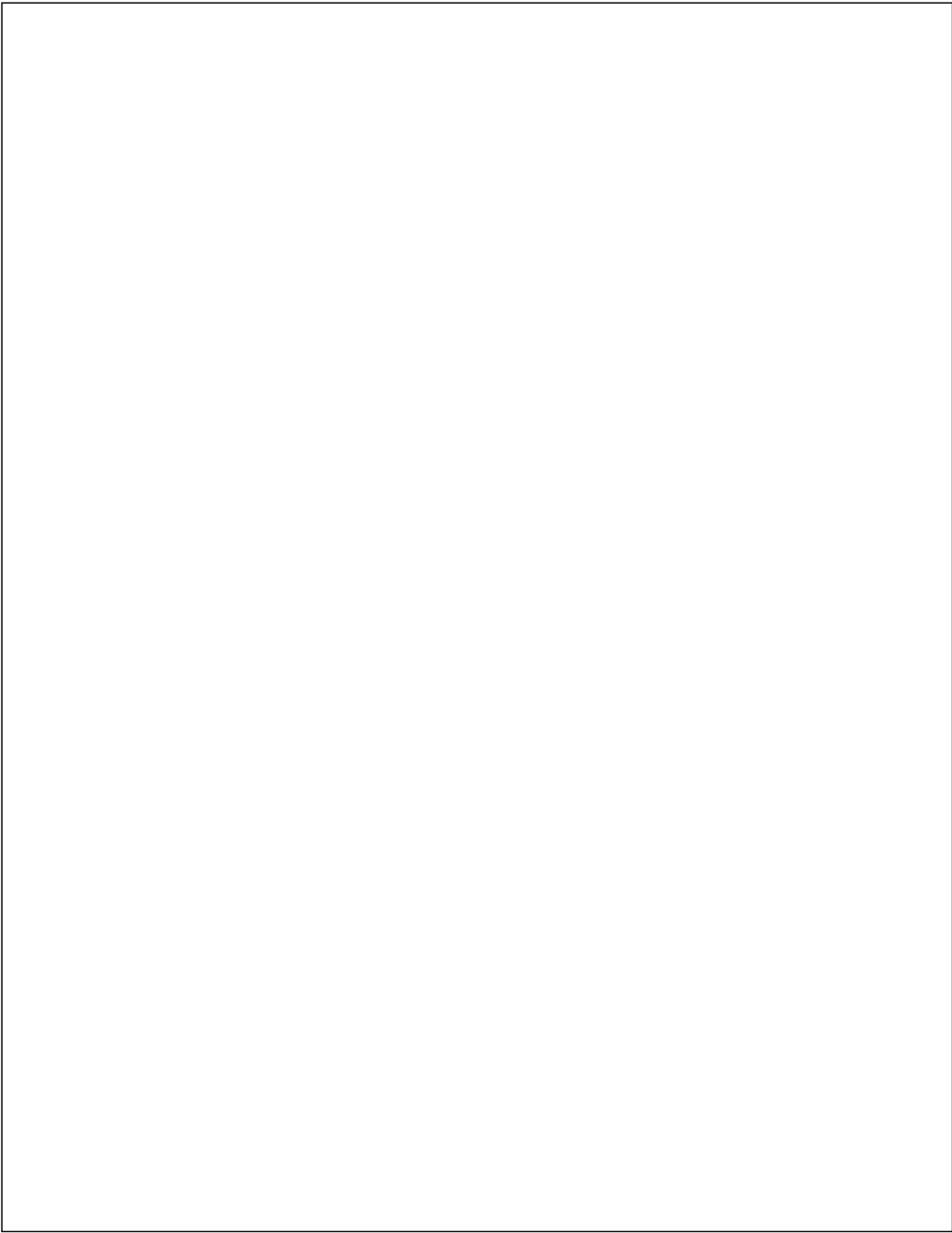
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