IJPSR (2020), Volume 11, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 June 2020; received in revised form, 11 October 2020; accepted, 27 October 2020; published 01 November 2020

IN-VITRO INSIGHT INTO THE IMPACT OF DIABETES AND IMPLANT DEBRIS ON ORTHOPEDIC IMPLANT

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Keywords:

Implant detritus, Osteoclast, Osteolysis, Cytokines, TNF-α, IL-6

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ABSTRACT: Wear particle evoked lysis limits the long-run survivorship of total joint replacement (TJR) in polygenic disease. Monocyte / macrophages area the key cells of this adverse reaction. The aim of this study was to come up with useful human osteoclasts (OCs) *in-vitro* from sort a pair of diabetes (T2D) and health management to research the potential osteoclastogenic role of T2D and implant detritus in implant rejection. Long-run sterile failures of joint replacements area usually attributed to implant detritus evoked inflammation and lysis. Unhealthy cytokines are shown to extend bone cell activity that suggests that monocyte/macrophage exposure to each metal particles and soluble metal ions within the bone-implant interface seemingly contribute to implant debris-induced inflammation and later lysis. The current study may give insight into the potential role of implant debris on the etiology of bone loss in T2D. It concludes that Peripheral blood mononucleate cells (PBMCs) are primed for increased proinflammatory activity in patients with T2D and plays a central role in the rejection of the implant by increasing OC formation and organic bone process-bone resorption. Osteo-clastogenic role owed to implant detritus leads to the discharge of unhealthy cytokines-tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and interleukin-6(IL-6) in T2D subjects, and alteration of Bone mineral density (BMD) are key factors in implant rejection.

INTRODUCTION: Total joint replacement is becoming a more common practice over the years. Even though implantable medical devices are considered biocompatible by the Food and Drug Administration (FDA), the foreign body response and microbial infection leads to persistent local and systemic infections and exacerbates the fibrotic response [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(11).5923-31

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(11).5923-31

This effect can necessitate device removal and amputation, which leads to significant morbidity and cost. Diabetes mellitus was becoming increasingly prevalent worldwide and indicated as a risk factor for the infection of various orthopedic implants [2]. Some of the bone turnover changes are related to hyperglycemia, which is the principal common risk factor for T2D. TNF-α and other inflammatory mediators contribute to the development of insulin resistance inT2D.

Diabetes prevalence, increasing rapidly among the older population, has several orthopedic conse-quences. It has been reported that more than half of diabetes have arthritis and may eventually need a hip or knee replacement [3].

In total hip (THA) and total knee (TKA) arthroplasty patients, diabetes influences functional and perioperative clinical outcomes. Lovecchio *et al.,* and Wu *et al.,* reported a significantly higher risk of prosthetic joint infections in diabetic patients [4, 5]. Kaiser Permanente Registry also reported diabetes as a risk factor for aseptic loosening in TKA patients [6, 7]. Diabetes adversely affects bone, nerve, muscles, retina, cardiovascular system, and nephron [8]. Bone remodeling is a coupled process of bone resorption and formation that requires coordination of bone cells, namely osteocytes, osteoblasts, and osteoclasts [9]. Bone turnover is an integral part of calcium turnover in the body. Due to hyperglycemia, changes in bone turnover occur as the principal risk factor for type-1 diabetes (T1D) and T2D. Differentiation of PBMCs into OCs is a key process to understand bone loss associated with inflammatory conditions [10]. The relative amount of CD^{14+} OC precursor is elevated in human peripheral blood from patients with chronic inflammation characterized by OCs mediated bone erosion [11].

Cytokine-induced inflammatory state in implant rejection of total joint arthroplasties might be due to the close link between inflammation and diabetes [12]. Osteolysis is more challenging and poses higher risks for the diabetic patient with respect to the surgery and subsequent complications than primary joint replacement, which needs revision. All wear particles like metal, ultra-high molecular weight polyethylene (UHMWPE), polymethyl methacrylate (PMMA), ceramic, and hydroxyapatite can activate a macrophage [13].

Failure of orthopedic joint implants leading to pain, loss of function, and ultimately revision surgery. Failure may be due to infection, fracture, mechanical failure, or poor surgical technique. However, the most frequent cause of implant failure is aseptic loosening, which occurs in over 10% of cases within 20 years of primary hip arthroplasty.

Aseptic loosening by increased osteolytic activity at the bone implant interface leading to loss of fixation and potentially difficult revision surgery [14]. This research has provided a framework for studying the potential osteoclastogenic role of T2D and implant detritus in implant rejection.

MATERIALS AND METHODS:

Drugs and Reagents: Recombinant human soluble receptor activator of nuclear factor-kappa B ligand (sRANKL) and human soluble macrophage colony-stimulating factor(sM-CSF) were purchased from Bio Vision, Milpitas, CA, USA. Cobalt (II) chloride hexahydrate, Nickel (II) chloride hexa-hydrate, 3- [4, 5- dimethylthiazol-2-yl]- 2, 5-di-phenyltetrazolium bromide (MTT), dimethyl sulphoxide (DMSO), Leukocyte Acid Phosphatase Assay kit were purchased from Sigma Aldrich Co., USA. Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and Antibiotic-antimycotic solution were purchased from GIBCO, Grand Island, New York, USA.

Study Design: Study subjects were normal and T2D males and females between the ages of 30-75. They were recruited from the inpatient Department of Diabetes, Department of Orthopaedics, Rajiv Gandhi Government General Hospital, Chennai, India.

The study protocol was approved by the institutional ethical review board (IERB/6/2012), and written informed consent was obtained. The subjects were categorized into various groups.

Group IA: Normal subjects challenged with Cobalt (n =23 M/F: 11/12)

Group IB: T2D subjects challenged with Cobalt (n=23, M/F: 11/12)

Group IIA: Normal subjects challenged with Nickel (n =22, M/F: 11/11)

Group IIB: T2D subjects challenged with Nickel (n=22, M/F: 11/11)

Group IIIA: Normal subjects (n= 23 M/F: 11/12)

Group IIIB: T2D subjects (n= 23M/F: 11/12)

Inclusion and Exclusion Criteria: Inclusion criteria: T2D subjects without any systemic diseases, antibiotics, or an anti-inflammatory drug. Exclusion criteria: patients with endocrine diseases, bone diseases, and those who have received vitamin D3, calcium, Cathepsin K inhibitors, RANKL inhibitor, bisphosphonates were excluded from the study to avoid interference with bone integrity.

Demographic and Anthropometric Variables: Normal subjects matched with T2D subjects for age, height, weight, and body mass index (BMI) were chosen. Details like age, height, weight, BMI, blood pressure, duration of diabetes, medical history of diabetes were obtained by a written questionnaire from the study subjects.

BMD Measurements -Dual Energy x-ray Absorptiometry (dexa): BMD was measured by DEXA. in study subjects, using a Hologic QDR 4500A densitometer (HologicInc, Germany). BMD is defined as bone mineral content divided by the projected area of the scanned image. BMD = BMC/area (g/cm^2)

Isolation of Monocytes from Whole Blood: Peripheral blood was used to separate PBMCs. PBMCs were prepared by layering diluted peripheral blood over ficoll-Paque (Sigma St. Louis, MO, USA) solution, centrifuged at 1700 rpm, for 30 min at room temperature, washed and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. Subsequently, cells were counted in a haemo-cytometer using trypan blue.

Quantification of Osteoclast Precursors: Flow cytometry was used to determine the percentage of CD14 cells in PBMCs from T2D and normal groups. After cells were counted, 1×10^6 PBMCs were incubated in phosphate-buffered saline (PBS) with a dilution 1:10 of Fluorescein isothiocyanate (FITC) conjugated anti-human CD14 antibody for 30 min. Then cells were washed, suspended in PBS, and analyzed by flow cytometry using FAC Scan (Becton Dickinson, Mississauga, Canada).

Cell Proliferation Assay: PBMCs were seeded in 96-well plates at a density of 5×10^3 cells/well in 200 µL DMEM containing 10% FBS and incubated overnight. Non-adherent cells were removed by gentle washing after 24 h. Then cells were replaced with serum-free medium with varying concen-trations of Co^{2+} (1-100 µM) and Ni^{2+} (1-100 µM). The cell number was assessed using MTT assay

Human Primary Osteoclasts Generation and Differentiation: PBMCs were plated in 24 well plates at 1×10^5 cells per well in 2 ml of DMEM medium containing 10% FBS, penicillin (100 µg/ml), streptomycin (100 µg/ml) and allowed to adhere for overnight. After overnight, the medium was semi depleted and replaced with fresh osteoclastogenic differentiation medium containing 25 ng/ml MCSF, 40 ng/ml RANKL and 1 µM dexamethasone, and 2 mM L-glutamine. The cells were re-fed every three days by semi-depletion. The cells were separated and treated with Cobalt (Co^{2+}) and nickel (Ni^{2+}) ions on the third day and cultured for 14 days. OCs were stained for TRAP using Naphthol AS-BI phosphate and Fast Garnet in the presence of sodium tartrate.

Measurement of Proinflammatory Cytokines by Enzyme-linked Immunosorbent Assay (ELISA): Levels of TNF-α, IL-1β, and IL-6 in mature monocyte and osteoclast culture supernatants were measured using ELISA (Legend max ELISA, USA). Absorbance was read at 450 nm on a micro-plate reader (Biotek, USA).

Statistical Analysis: Data were expressed as Mean ± SD and percentage, whatever appropriate. Statistical evaluation was performed using an unpaired student's t-test. p values of <0.05 were statistically significant. The analysis was performed with SPSS software.

RESULTS AND DISCUSSION:

Demographic and Anthropometric Variables: The baseline and biochemical characteristics of the diabetic subjects are shown in **Table 1**.

TABLE 1: BASELINE AND BIOCHEMICAL CHARACTERISTICS

Variables	Non-diabetic n = 23	Diabetic n=23
Mean age (years)	42.5 ± 5.191	41.4 ± 3.204
Male/Female ratio (n)	65/55	35/35
Duration of diabetes (Years)	-	8.7 ± 1.6
BMI (kg/m^2)	18.76 ± 2.10	19.44 ± 1.86*
Systolic blood pressure (mm Hg)	115 ± 4.91	118.72 ± 6.12 *
Diastolic blood pressure (mm Hg)	74.1 ±5.6	76 .11 ± 5.7*
FBG (mg/dl)	78.94 ± 9.42	153.71 ± 11.31 ***
HbA1c (%)	5.1 ± 0.38	7.7 ± 2.13***
Total cholesterol (mg/dl)	157 ± 17.3	185.2 ± 17.2***
Triglyceride (mg/dl)	142 ± 34.2	171 ± 57.3*
Calcium (mg/dl)	7.9 ± 0.8	8.1 ± 0.3*
Phosphate (mg/dl)	3.12 ± 0.82	3.8±0.41*

Significant associations for BMI ($p<0.05$), systolic blood pressure ($p<0.05$), diastolic blood pressure ($p<0.05$), fasting plasma glucose- FPG ($p<0.001$),

Glycated Haemoglobin- HbA1C % ($p<0.001$), total cholesterol ($p<0.001$) triglyceride ($p<0.05$), calcium ($p<0.05$) and phosphate ($p<0.05$) compared to non-diabetic subjects.

The interplay Between BMD and T2D with Different Age Groups: Table 2 revealed the correlation of BMD with T2D and non-diabetes according to age groups. BMD was significantly lower in T2D when compared to controls. P-value is <0.001 for all age groups of women. For men P-value is <0.01 for age groups 30-50 & 51-60, whereas for 61-75 P-value is <0.001. Our results showed decreased BMD for women in age groups 51-60 and 61-75 than 30-50.

For diabetes, early detection of this imbalance in bone metabolism may be helpful in reducing bone loss, fracture risk, and orthopedic implant failure. This study revealed that diabetic subjects had a high risk due to low BMD.

There was a negative correlation between T-scores and diabetes duration. Miso Jang *et al.,* reported a negative association between T2D and BMD. Our findings also suggested that age and hyper-glycaemic index were additional risk factors for developing aseptic loosening as BMD was inversely correlated with age and glycaemic control (HbA1c) **Table 1** and **2**. Abdulameer *et al.,* also reported a similar result in diabetic subjects [15].

TABLE 2: CORRELATION OF BMD WITH NON-DIABETIC AND DIABETIC

Study Subjects	Age Group (yrs.)	30 - 50	51 - 60	61 - 75
Non-Diabetic Women (Group IIIA)	BMD(g/cm^2)	0.869 ± 0.174	0.937 ± 0.124	0.98 ± 0.134
Diabetic Women (Group IIIB)	BMD(g/cm^2)	2.009 ± 0.683	2.230 ± 0.718	2.200 ± 0.515
Non-Diabetic Men (Group IIIA)	BMD(g/cm^2)	0.883 ± 0.207	0.890 ± 0.11	0.927 ± 0.134
Diabetic Men (Group IIIB)	BMD(g/cm^2)	1.750 ± 0.450	1.980 ± 0.396	2.167 ± 0.121

MTT Assay: The influence of metal ions in the cell proliferation was tested and analyzed by MTT assay. The effect was particularly strong for 100 µM concentration but not appreciable for the other concentrations of Nickel. For Cobalt ion, the highest concentration is inhibiting cell proliferation, and for low concentration 10 µM, it was observed a similar pattern to 100 µM Ni^{2+} and showed plenty of positive trap activity, indicating several studies reported a toxic effect of metal ions on osteoblasts and macrophages *in-vitro*.

The high concentration (100 µM) chosen for Cobalt ion resulted in lethal, which reduces the number of osteoclasts. However, for Nickel ion, it was found that is somehow inducing a delay in the osteoclast formation for an average of 4 days, later normal levels of osteoclast were reached. These findings suggest that Nickel is not inhibiting osteoclast formation and could permit an increase in cell proliferation during this delay, incrementing the pool of precursor cells, leading to increased osteoclasts in the periprosthetic tissue.

Due to this response, specific transcripts were found to be up-regulated either by Co^{2+} or Ni^{2+}. Even though metal ions at higher concentrations show inhibitory effect in mature osteoclasts, at systemic levels, their effect is stimulatory on developing osteoclasts.

The reason behind this is the substrate resorbing activity of the osteoclasts. High concentrations of Co^{2+} may trigger hypoxia-inducible factors (HIF) and initiate hypoxia-related gene expression, resulting in the activation of the HIF pathway, causing cytotoxicity [16]. Hence, for 24 h treatment, the final working metal ion concentrations for Co^{2+} and Ni^{2+} were confirmed as 1 µM, 10 µM, and 100 µM using MTT assays.

Effect of T2D on CD14+ Osteoclast Precursor: To examine whether the T2D has increased the osteoclast precursor pool, we studied the CD^{14+} cells isolated from the PBMCs using flow cytometry.

The study revealed that T2D subjects presented with a higher percentage of CD^{14+} OC precursors than the normal subjects (6.2 ± 0.8692; $p<0.01$) **Fig. 1A**. This finding is consistent with the observation of Cipolletta *et al.,* [17]

Effect of Metal Ions on Osteoclast Cell Number and Size: The present study evaluated the number and size disparity of osteoclast after treating PBMCs with metal ions. Higher numbers of multinucleated osteoclasts were significantly increased at the exposure of a high concentration of Co^{2+} ions (11.67 ± 3.3; $p<0.001$) than the Ni^{2+} ions in the T2D (9.7 ± 2.5; $p<0.01$) **Fig. 1B**.

On the other hand, the size of osteoclasts in the T2D samples was significantly increased in the presence of 100 μM of Co^{2+} ions (1733 ± 412 μm^2; $p<0.001$) and Ni^{2+} (1500 ± 243.3 μm^2; $p=0.01$) compared to non-diabetic **Fig. 1C**.

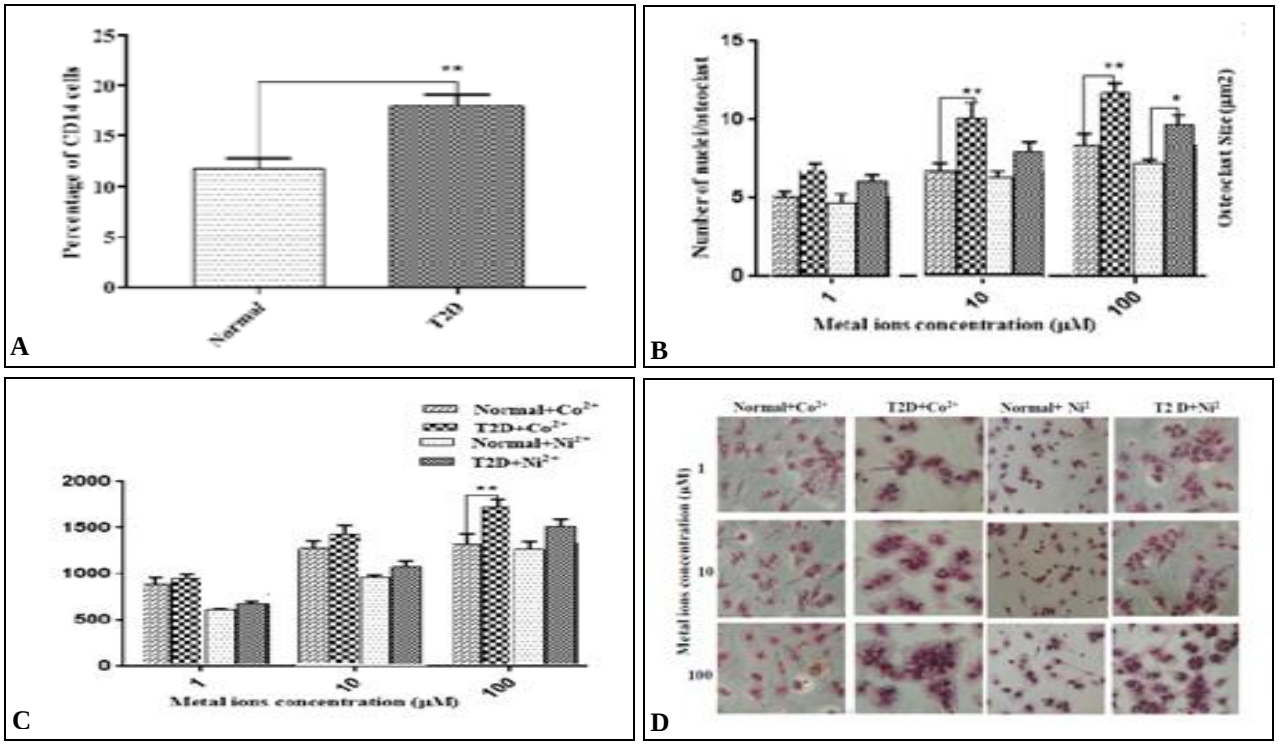

FIG. 1: CHARACTERIZATION OF OSTEOCLASTIN STUDY SUBJECTS. A. Percentage of osteoclast precursor Mononuclear cells were incubated with FITC-conjugated anti-CD14 antibody and analyzed by flow cytometry. **p<0.01, B and C: Osteoclast number and size osteoclast cells were treated with Ni^{2+} & CO^{2+} *p<0.05, **p<0.01, D. Image of TRAP-positive multinucleated osteoclasts. Cells were visualized under a phase-contrast microscope

Effect of Metal Ions on Osteoclasts Differentiation and Function in RANKL-Cultured PBMCs: To determine the effect of metal ions on osteoclastogenesis *in-vitro*, CD^{14+} monocytes were cultured using RANKL and M-CSF with varying concentrations of metal. They were then stained for TRAP. RANKL induced osteoclast formation from pre-osteoclast cells, and TRAP-positive multinucleated osteoclasts in Ni^{2+} and Co^{2+} treated T2D **Fig. 1D** compared to normally treated groups. Osteoclastogenesis is a multi-step process that includes the expansion of the progenitors, commitment to osteoclasts, and cell fusion. The study of TRAP-positive cell formation and activity is a well-known method of determining osteoclast formation and function [18].

Monocytes from T2D subjects, when exposed to Co^{2+} ions, exhibited a higher dose-dependent increase in the osteoclast cell number than the Ni^{2+} ions exposure. 100 μM of Co^{2+} increased the differentiation of osteoclast precursors into multinucleated osteoclasts significantly $p<0.0001$). The increase in osteoclast numbers in T2D subjects could be attributed to the prolonged hyper-glycemia, which elevates the expression of V-ATPase responsible for acidification of resorptive lacunae [19] and forms the advanced glycation end products (AGES) by augmenting RANK signaling [20]. It was also shown that OCs in the T2D group were larger than normal groups. It has been established that larger OCs are more active at bone resorption sites than smaller cells accounting for excessive bone loss [21].

Correlation of Pro-inflammatory Cytokine Secretion with the Effect of Implant Debris on Monocytes and Osteoclasts: The present study framed to assess the impact of implant debris Co^{2+} and Ni^{2+} on PBMCs derived from normal and T2D subjects, specifically on their differentiation into osteoclasts and pro-inflammatory response. In this investigation, we compared differential responses of OCs and monocytes with ions. Group IB and Group IIB OCs challenged with implant debris generally secreted much higher amounts of TNF-α

when compared to group 1A and Group IIA. However, insignificant amounts of IL-1β were secreted. IL-6 results were nearer to TNF-α. Thus 10 µM Co^{2+} triggered more pro-inflammatory cytokine secretion than 10 µM Ni^{2+}. Osteoclasts exhibited lesser cytokine secretion than monocytes, suggesting that while differentiating monocytes into osteoclasts, they lose their ability to induce a robust inflammatory response [5]. The ability of osteoclasts to produce an inflammatory response to metal ions is decreased as they are differentiated from monocytes [22]. Released cytokines recruit macrophages to the activation site, and a pro-inflammatory milieu develops. This may affect the biocompatibility and osseointegration of joint implants negatively [23].

Metal ions within the circulation may form a complex with serum proteins, which may be considered as antigens or free ions, could exhibit an inflammatory effect in lymphocytes, leading to bone resorption by increasing osteoclasts. Our results were consistent with the report of Csaba Vermes *et al.,* who stated that osteoclast and monocyte supernatants of debris challenged cells could promote osteoclast formation [24].

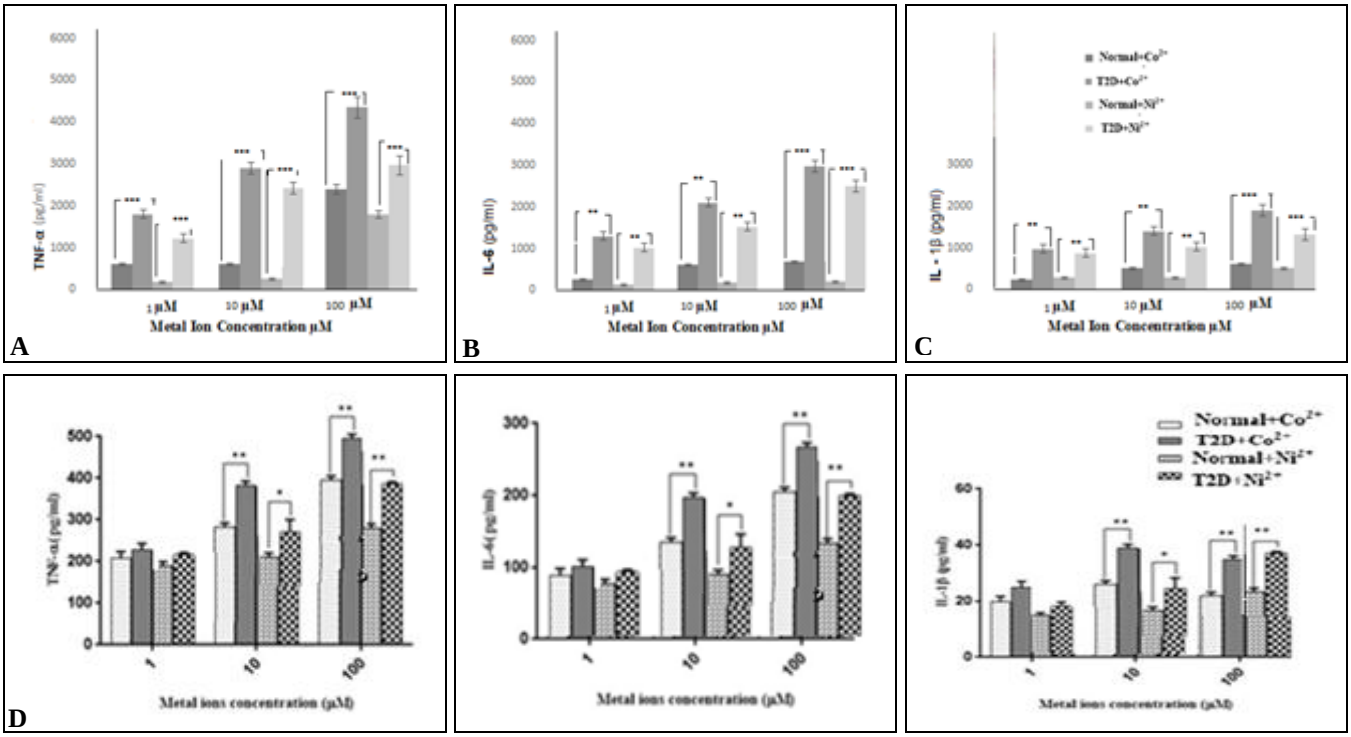

FIG. 2: A: LEVEL OF CYTOKINES IN MONOCYTES SUPERNATANT AT 4TH DAY AFTER 24 HOURS METAL CHALLENGE: GROUP IA COMPARED WITH GROUP 1B AND GROUP IIA COMPARED WITH GROUP IIB FOR IL-1B, TNFA, IL-6 SECRETION FROM MATURE MONOCYTES. P-Value is < 0.0001*** for IL-1β; for TNF- α P-Value is < 0.0001***; for IL-6 P Values is 0.001** B: Level of cytokines in osteoclast supernatant at 14th day after 24 hours metal challenge Group IA compared with Group 1B and Group IIA compared with Group IIB for IL-1β, TNFα, IL-6 secretion from osteoclast supernatant at 14th day after 24 hours metal challenge. P-Value is < 0.0001*** for TNFα; P-Value is < 0.0001*** for IL-6; P-Value is 0.001** for IL-1β

Implant debris induced cytokine profile of study subjects were assessed. Levels of IL-1β, IL-6, and TNF-α were monitored with increasing doses of ions like Co^{2+} and Ni^{2+} in osteoclasts & monocytes using ELISA. **Fig. 2A** & **B** revealed that IL-1β, IL-6, and TNF-α levels were increased dose-dependently in the T2D compared to normal metal challenged groups. Among them, TNF-α was highly expressed ($p<0.0001$ for Co^{2+}; $p<0.001$ for Ni^{2+}), whereas IL-1β ($p<0.0001$ for Co^{2+}; $p<0.01$ for Ni^{2+}) was detected at low levels, and IL-6 was moderately expressed ($p<0.0001$ for Co^{2+}; $p<0.01$ for Ni^{2+}) at the highest concentration of the metal ion. IL-6 is an important marker for chronic inflammatory processes and is assumed to cause osteoclast activation. Vermes *et al.,* confirmed that IL-6 is secreted by metal challenged cells, which are critical in the pathogenesis of the periprosthetic osteolysis [25]. Stea *et al.,* demonstrated a positive correlation between wear debris and the cytokines

IL-1β, IL-6, and TNF-α, with osteolysis [26, 27]. TNF-α is more prominent in osteoclast cultures in the present study, which states that TNF-α is a fundamental cytokine in "mediating particle-driven osteoclastogenesis and osteolysis [28]. Hence, by triggering pro-inflammatory cytokines, IL-6, IL1β, and TNF-α, osteoclastic cells may attract more inflammatory cell infiltrates and contribute to their migration into the peri-implant tissue. Thus enhancing the inflammatory reaction to ortho implants.

The results of this study demonstrated that the presence of cobalt and nickel ions in the osteoclastic cells belong to diabetes groups induced potent expression of pro-inflammatory cytokines IL-6, IL-1β, TNFα in a dose-dependent manner compared to cells in the normal groups, which leads to chronic inflammatory response and further induces resorption of the peri-implant bone. Eger *et al.,* reported that in nonclinical animal models, suppression of inflammatory cytokine signaling might have clinical utility in preventing or limiting maladaptive osteolytic responses to metal implants [29].

Several studies have demonstrated persistent inflammatory response that may also lead to increased osteoclastic activity in a hyperglycaemic state [30]. Hence, inflammatory osteolysis in T2D is the most important factor limiting the longevity of total joint arthroplasty leading to aseptic loosening **Fig. 2A**. The findings in this work demonstrated that monocytes have a much stronger inflammatory response by releasing high amounts of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. We found that CD^{14+} monocytes encourage OCs formation, which is confirmed by increased TRAP-positive staining.

In-vitro studies have shown that IL-1β, IL-6, and TNF- α can influence osteoclasts directly through their specific receptors located on osteoclasts or by modulating the RANK/RANK ligand RANKL / osteoprotegerin (OPG) pathway. Higher levels of osteoclastogenic cytokines IL-6 and IL-1β could enhance osteoclastogenesis, leading to increased bone loss **Fig. 2B** [31]. In this study, the authors investigated the stimulatory effect of implant debris on OC differentiation in T2D and non-diabetic *in-vitro*.

It is known that implant debris increases the risk of fractures in T2D. A net increase in bone resorption is responsible for skeleton weakening and fracture risk. Our study focused on the inflammatory responses of diabetic and non–diabetic OCs and monocytes to wear debrisin terms of cytokines produced. Monocytes significantly induce cytokine secretion more strongly than OC because monocyte polarization is the first step in any immune response. Hence diabetes may have direct effect on bone turnover, which leads to the development of inflammatory milieu in diabetic patients with an implant. Hyperglycaemia affects bone homeostasis by inhibiting osteoblast differentiation and altering the response of the parathyroid hormone that regulates the metabolism of phosphorus and calcium [32]. Alikhani *et al.,* investigated AGEs that generate reactive oxygen species, which leads to oxidative stress and cell death.

AGEs in diabetic patients increased inflammation via the up-regulation of TNFα and IL-1β in monocytes and macrophages. It affects adherence, growth, and accumulation of extracellular matrices [11]. Hence, revision surgery is required. These studies strongly support the hypothesis that increased recruitment of osteoclast precursors contributes to osteolysis around loose orthopedic implants.

CONCLUSION: Findings of the study leads to the conclusion that wear particles induce osteolysis by stimulating both the recruitment of osteoclast precursors and their subsequent differentiation into mature osteoclasts.

Cellular mechanisms involved in aseptic loosening suggest that the development of therapeutic interventions that reduce either recruitment or differentiation of osteoclast precursors would improve the performance of orthopedic implants.

Revision surgery not only increases the cost but also increases the chances of morbidity. The present study had been an approach to give some information about the cellular responses to the implant debris in T2M.Osteolysis research needs further investigation for the development of specific anti-inflammatory therapeutic interventions to prevent periprosthetic bone loss. Reducing severe inflammatory responses in T2M with implants may

be best addressed by targeting glycation products, hyperglycemia, and osteoclast precursors. Our research might play an essential role in the screening process for the susceptibility of orthopedic implant-induced metal hypersensitivity and rejection in inflammatory diseases before joint replacement, providing the new idea for prevention and throw lights on treatment.

FUNDING: The research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

ACKNOWLEDGEMENT: Nil

DECLARATION OF COMPETING INTEREST: All authors declare that they have no financial interests relevant to this study.

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How to cite this article:
Thenmozhi A, Shila S, Nagalakshmi K and Rasappan P: *In-vitro* insight into the impact of diabetes and implant debris on orthopedic implant. Int J Pharm Sci & Res 2020; 11(11): 5923-31. doi: 10.13040/IJPSR.0975-8232.11(11).5923-31.