Experimental Research

Osteoblastogenesis of adipose-derived mesenchymal stem cells in chronic kidney disease patient with regular hemodialysis

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ABSTRACT

Background: Chronic kidney disease (CKD) is a health problem associated with high morbidity and mortality. Mineral and bone disorders are complications of CKD with a risk of fractures and cardiovascular disease. Osteoblast cells can differentiate into osteoblasts and regulate their regulation by a network of cytokines and transcription factors.

Objective: Analyzing differences in osteoblastogenesis of adipose mesenchymal stem cells in CKD patients and healthy people.

Methods: The study sample was adipose mesenchymal stem cells from CKD patient undergoing hemodialysis and healthy people. Osteoblastogenesis was assessed by measuring the concentrations of transforming growth factor-β1 (TGF-β1), bone morphogenetic protein-2 (BMP-2), and (DKK-1) in culture media. The Elisa method measured the concentration of these parameters on days 4, 7, 14, and 21. Data were analyzed using an independent t-test and post hoc test with p-value <0.05.

Result: There was a significant difference in CKD patients with increasing TGF-β1 on day 4 (t = 2.821; 95% CI = 30.498–199.727; p = 0.010) and decreased on day 14. In the BMP-2 parameter, there was an increase on day 7 (t = 4.291; 95% CI = 0.289–0.831; p <0.001). Similar conditions were also found in the DKK-1 parameter, increasing on the 7th day, but there was no significant difference (p = 0.583).

Conclusion: Osteoblastogenesis in adipose mesenchymal stem cells in CKD patients differs from that in healthy individuals. Osteoblasts fail in maturation and cause failure in matrix mineralization.

1. Introduction

Chronic kidney disease (CKD) is a health problem that covers 5–10% of the world’s population. As CKD is associated with high mortality rates, the challenge appears to be not only preventing the onset and progression of disease but also reducing morbidity and mortality. Patients with CKD usually die from sudden death, cardiac arrhythmias, acute myocardial infarction, peripheral arterial insufficiency, and stroke. Mortality in CKD patients can also be attributed to non-traditional risk factors such as inflammation, oxidative stress, anemia, and impaired bone and mineral metabolism associated with CKD [1,2]. The risk of hip fracture in men and women is 4 times higher in hemodialysis patients. Other studies suggest that the incidence of hip fracture is 1% and fracture elsewhere is 2.6%, whereas, in the general population, it is around 0.07–0.22% [3,4].

Kidney damage will cause a decrease in the number of functioning nephrons, resulting in impaired phosphate excretion. Impaired excretion will cause phosphate retention, which causes a response to increased levels of Parathyroid Hormone (PTH) and Fibroblast Growth Factor (FGF-23) in the circulation [5,6]. FGF-23 reduces phosphate concentration in the blood by two mechanisms: it reduces phosphate reabsorption by proximal tubular cells in the kidney and inhibits the enzyme 1 alpha-hydroxylase in the kidney, thereby reducing calcitriol synthesis [5,7]. Decreased calcitriol results in reduced absorption of calcium from the intestine and proximal tubule, resulting in a tendency toward hypocalcemia that is offset by increased production and secretion of PTH. The end effect is secondary hyperparathyroidism, which further exacerbates hyperphosphatemia (positive feedback). Secondary
hyperparathyroidism causes increased bone calcium mobilization, resulting in bone weakness and a tendency to fracture. Abnormal bone quality and quantity can lead to an increased risk of fracture in CKD patients [8]. Accumulation of uremic toxins in the blood of CKD patients, such as indoxyl sulfate, causes decreased Wnt/β-catenin signaling in osteoblasts and increased expression of Wnt signaling inhibitors such as sclerostin and Dickkopf-1 (DKK-1). The Wnt/β-catenin pathway has been recognized as a primary regulator of bone formation [9]. Plasma TGF-β levels were significantly higher in hemodialysis patients and patients with renal osteodystrophy. Several reports have revealed an interaction between TGF-β and Wnt signals on osteoblasts that causes high bone-turn over in CKD [10]. Cellular senescence is a common sign of ageing tissue and is usually defined as a dynamic process resulting from cell loss and affecting the ability to proliferate, and growth halted due to various stressors. CKD shares many phenotypic similarities with renal ageing, such as glomerular sclerosis, interstitial fibrosis, tubular atrophy and loss of repair ability, thus suggesting that CKD is closely correlated with cellular ageing. The most common markers for identifying cellular ageing include ageing-associated overexpression of -galactosidase [11]. Bone mineral disorder in CKD is a common complication in CKD patients. The pathophysiology is complex and still not fully understood. In vitro mesenchymal stem cell research has increased in recent years, which is used to study the cellular activity of primary cells to immortalized cells. Stroma/stem cells can differentiate into tissues under various stimuli outside the living organism. The use of MSCs in vitro helps study osteogenic processes, which can help simplify complex processes and help to study the processes that occur during osteogenesis. Most studies have used experimental models of CKD by observing treated experimental animals, local studies on bone and clinical studies by examining systemic osteogenesis markers [12]. Based on the description above, this study aimed to analyze the differences in osteoblastogenesis of adipose mesenchymal stem cells in CKD patients and healthy.

2. Method

2.1. Participant

Participants in this study were divided into 2 groups, such as CKD and control groups. Inclusion criteria in CKD group were patient chronic hemodialysis, undergoing procedures for making AV fistula cephalic or brachia-basilic, aged 20–60 years, serum Ca levels <8 mg/dL, serum phosphorus levels >4.5 mg/dL, iPTH levels >65 pg/dL, and body mass index (BMI) 18.5–30 kg/m². Minewhile, the exclusion criteria in the CKD group were patients diagnosed with diabetes mellitus, malignancy, rheumatoid arthritis, and consumed corticosteroids for a long time. Inclusion criteria in the control group included healthy people who underwent surgical procedures (in the upper arm, abdomen, or thigh area), aged 20–60 years, and had a BMI of 18.5–30 kg/m². Minewhile, the exclusion criteria in the control group were those being diagnosed with diabetes mellitus, malignancy, chronic kidney disease, rheumatoid arthritis, osteoporosis, consuming corticosteroids for a long time, and severe infection. The patient first read and signed the informed consent consciously and without coercion.

2.2. Design study

This study used a posttest-only control group design in vitro study to compare osteoblastogenesis in adipose mesenchymal stem cells in healthy individuals and CKD patients undergoing hemodialysis. Participants were divided into 2 groups, such as CKD and control groups. CKD group consisted of participant diagnosed with CKD, and the control group were healthy participant, which for each participant, 12 subjects were taken adipose-derived mesenchymal stem cells. There were 2 participants (CKD groups = 1 participant with 12 subject and control groups = 1 participant with 12 subject). Data collected in this study included TGF-β1, BMP-2, and DKK-1 levels. Before conducting the research, the researcher first conducted an ethical approval.

2.3. TGF-β1 examination

TGF-β1 signaling supports bone formation by promoting osteoprogenitor enrichment. TGF-β through TGF-βRI promotes pre-osteoblast commitment and early differentiation. TGF-β also inhibits latent osteoblast differentiation. TGF-β binds to its receptors (TGF-βRII and II) and activates Smad2 and Smad3 through phosphorylation. Examination using Sandwich ELISA for Human TGF-β1. Readings based on optical density plotting absorbance with 450 nm wave ELISA reader (colorimetric assay).

2.4. BMP-2 measurement

BMP-2 is a member of TGF beta and plays a role in osteoblast differentiation. BMP-2 activates Smad1/5/8 as R-Smad. The Smad1/5/8-Smad4 complex transcribes Runx2 expression and initiates osteoblast gene expression. Examination using Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for Human Dkk1. Readings using a 450 nm wave ELISA reader (colorimetric assay).

2.5. DKK-1 measurement

Dkk-1 is a regulatory gene that interferes with Wnt signaling by direct binding to LRPS/6. DKK-1 blocks Wnt/β-catenin signaling-induced osteoblastogenesis. Examination using Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for Human DKK1. Readings using a 450 nm wave ELISA reader (colorimetric assay).

2.6. Enzyme-linked immunosorbent assay examination

According to the manufacturer’s instructions, the concentration of osteoblastogenesis markers was measured using the Human Quantikine ELISA kit (Bio-Technology Laboratory, China). Briefly, 200 L was added to the excellent microplate pre-coated with monoclonal antibody to the desired factor and incubated for 2 h. The well plates were then washed with PBS, horseradish peroxidase-conjugated cytokines or growth factor-specific antibodies were added to each well, incubated for 2 h, and washed. Substrate solution was added and incubated for 30 min; The reaction was stopped by adding the stopping solution. Growth factor levels were determined by measuring the optical density at 450 nm with a microplate spectrophotometer (Bioassay Technology Laboratory, China).

2.7. Statistical analysis

The data were presented in the form of descriptive data for the differentiation of adipose mesenchymal stem cells into progenitor cells. Quantitative data were presented in mean ± SD (standard deviation). The selection of statistical analysis was carried out after the data normality test was carried out with the Shapiro-Wilk test, and then the data were analyzed using independent t-tests or Mann-Whitney. Then, the measurement results were carried out by the Post Hoc Test Least Significant Difference/Games-Howell. The results of the analysis were declared significant if the p-value <0.05. Statistical analysis using SPSS version 23.0 software (IBM Corp., Armonk, NY, USA).

3. Result

3.1. Characteristic of participant

The participants aged in the CKD group and control group was 57 years and 44 years. The participants in CKD group were female, and in...
control group were male. BMI in CKD group was higher than in control groups. There was some increase in blood analysis in CKD group, including leukocytes, thrombocytes, neutrophiles, BUN, creatinine, fasting blood glucose, iPTH, and phosphorus (Table 1).

3.2. Comparison of TGF-β1 levels in CKD patients and healthy people

There was a significant difference in TGF-β levels in the control and CKD group on day 4 and day 14. The mean TGF-β level on day 4 in the control group and CKD group was 1285.89 ± 109.03 ng/L and 1401.00 ± 89.93 ng/L (t = 2.821; 95% CI = 30,498–199,727; p = 0.010), respectively. Meanwhile, the mean TGF-β1 level on day 14 in the control group and CKD group was 1295.08 ± 107.80 ng/L and the CKD group of 1216.22 ± 65.98 ng/L (t = 2.161; 95% CI = 3.191–154.530; p = 0.042; Table 2), respectively.

In the control group, there was only a significant difference in TGF-β1 levels on day 7 vs 21 (p = 0.007). In the CKD group, there were significant differences in several TGF-β1 levels, including day 4 vs day 14 (p = 0.001), day 4 vs day 21 (p = 0.001), day 4 vs day 21 (p = 0.001), day 7 vs day 14 (p = 0.001), and day 7 vs day 21 (p = 0.001). Meanwhile, in the comparison of control vs CKD group, there were some significant differences in the TGF-β1 levels which were assessed on day 4 (control group) vs day 4 (CKD group; p = 0.002), day 4 (control group) vs day 7 (control group; p = 0.017), day 7 (control group) vs day 14 (CKD group; p = 0.002), day 7 (control group) vs day 21 (CKD group; p = 0.001), day 14 (control group) vs day 4 (control group; p = 0.005), day 14 (control group) vs day 7 (control group; p = 0.027), day 14 (control group) vs day 14 (CKD group; p = 0.033), day 14 (control group) vs day 21 (CKD group; p = 0.009), day 21 (control group) vs day 4 (CKD group; p = 0.001), and day 21 (control group) vs day 7 (CKD group; p = 0.001; Table 3).

3.3. Comparison of BMP-2 levels in CKD patients and healthy people

There was a significant difference in BMP-2 levels in the control and CKD group on the 4th and 7th day. The mean BMP-2 level on day 4 in the control group and CKD group was 3.44 ± 0.23 ng/mL and 3.64 ± 0.28 ng/mL (t = 1.831; 95% CI = 0.375–4.221; p = 0.021), respectively. Meanwhile, the average BMP-2 level on day 7 in the control group and CKD group was 3.55 ± 0.39 ng/mL and 4.11 ± 0.24 ng/mL (t = 4.291; 95% CI = 0.289–0.831; p < 0.001; Table 2), respectively.

In the comparison between each BMP-2 level, there were some significant differences in control, CKD, and both groups. The control group had no significant difference in each BMP-2 level. In the CKD group, there was only a significant difference in BMP-2 levels on day 4 vs day 7 (p = 0.004). Meanwhile, in the comparison of control vs CKD group, there were some significant differences in the BMP-2 level values assessed on several days, including on day 4 (control group) vs day 7

Table 1

| Variable       | Groups | Age       | Sex | BMI     | Hb        | Leukocyte | Neutrophil | BUN | Creatinine | Fasting blood glucose | iPTH | Calcium | Phosphor |
|----------------|--------|-----------|-----|---------|-----------|-----------|------------|-----|------------|-----------------------|------|---------|----------|
|                | Control | 44 years old | Male | 24 kg/m² | 14.8 g/dL | 6940/mm³ | 389.000/mm³ | 11 mg/dL | 0.68 mg/dL | 114 g/dL | 12.58 pg/dL | 8.9 mg/dL | 3.9 mg/dL |
|                | CKD     | 57 years old | Female | 28.5 kg/m² | 10.3 g/dL | 14,650/mm³ | 370,000/mm³ | 52.7 mg/dL | 7.95 mg/dL | 125.0 g/dL | 7.3 mg/dL | 6.8 mg/dL |

Table 2

Comparison of TGF-β1, BMP2, and DKK1 levels in CKD patient and healthy people.

| Variable | Days | 4 | 7 | 14 | 21 |
|----------|------|---|---|----|----|
| TGF-β1 (ng/L) |   |   |   |    |    |
| Control  | 1285.89 ± 109.03 | 1334.44 ± 42.06 | 1295.08 ± 107.80 | 1232.99 ± 89.09 |
| CKD      | 1401.00 ± 89.93  | 1377.28 ± 42.06  | 1216.22 ± 107.80  | 1196.97 ± 89.09  |
| p-value  | 0.010* | 0.178 | 0.042* | 0.343 |
| BMP-2 (ng/ml) |   |   |   |    |    |
| Control  | 3.44 ± 0.23 | 3.55 ± 0.39 | 3.75 ± 0.23 | 3.43 ± 0.40 |
| CKD      | 3.64 ± 0.28 | 4.11 ± 0.24 | 4.05 ± 0.53 | 3.60 ± 0.48 |
| p-value  | 0.021* | <0.001** | 0.086 | 0.369 |
| DKK1 (ng/mL) |   |   |   |    |    |
| Control  | 34.70 ± 2.76 | 39.12 ± 1.35 | 28.31 ± 2.09 | 27.41 ± 3.31 |
| CKD      | 37.00 ± 1.64 | 39.44 ± 1.50 | 31.01 ± 1.57 | 31.27 ± 1.47 |
| p-value  | 0.081 | 0.583 | 0.002* | 0.001* |

Note: TGF-β = Transforming Growth Factor-β; BMP = Bone Morphogenetics Protein; DKK1 = Dickkopf-1; *significant <0.05; **significant <0.001.

3.4. Comparison of DKK-1 levels in CKD patients and healthy people

There was a significant difference in DKK-1 levels in the control group and CKD group on day 14 and day 21. The mean DKK-1 level on day 14 in the control group and CKD group was 28.31 ± 2.09 ng/mL and 31.01 ± 1.57 ng/mL (t = 3.575; 95% CI = 1.131–4.258; p = 0.002), respectively. Meanwhile, the mean DKK-1 level on day 21 in the control group and CKD group was 27.41 ± 3.31 ng/mL and 31.27 ± 1.47 ng/mL (t = 3.685; 95% CI = 1.684–6.019; p = 0.001; Table 2), respectively.

In the comparison between each DKK-1 level, it was found that most groups had significant differences in control, CKD, and both groups. In the control group, only day 14 vs day 21 did not significantly differ in DKK-1 levels (p = 0.991). In the CKD group, there was only an insignificant difference in the DKK-1 levels on day 14 vs day 21 (p = 1.000).

Meanwhile, in the comparison of control vs CKD groups, there were some insignificant differences in the DKK-1 levels, which were assessed on day 4 (control group) vs day 4 (CKD group; p = 0.265), day 4, 7 (control groups) vs day 7 (CKD group; p = 0.999), and day 21 (control group) vs day 14 (CKD group; p = 0.057; Table 3).

4. Discussion

TGF-β/BMP signalling is involved in most cellular processes throughout life. TGF-β/BMP has a widely recognized role in bone formation during mammalian development and exhibits versatile regulatory functions in the body. Signal transduction by TGF-β/BMP is specifically via Smad-dependent canonical pathways (ligands, receptors and Smads TGF-β/BMP) and Smad-independent non-canonical signalling pathways (e.g. the p38 mitogen-activated protein kinase, MAPK pathway). The Smad and p38 MAPK pathways converge at the Runx2 gene to control the differentiation of mesenchymal precursor cells induced by TGF-β/BMP. The coordinated activity of Smads activated by TGF-β/BMP and Runx2 is essential for skeleton formation [13].

Renal fibrosis is a common endpoint of various progressive kidney diseases leading to loss of nephrons and impaired renal function. Kidney function eventually results in end-stage renal disease (ESRD). Fibrogenesis involves tubulointerstitial tissue leading to tubulointerstitial fibrosis and glomeruli leading to glomerulosclerosis. Extensive studies have shown that fibrogenesis can be induced by several stimuli or
mediators, including growth factors, cytokines, toxins and lipid disturbances, and stress molecules, through several mechanisms and signaling pathways. Fibrosis is primarily driven by inflammatory cytokines, including members of the TGF-β1 superfamily, various interleukins and oxidative stress. TGF-β1 has played a crucial role in the pathogenesis of progressive renal fibrosis. TGF-β1 has been demonstrated to convert tubular epithelial cells into the extracellular matrix (ECM), producing fibroblasts or myofibroblasts and inducing epithelial-to-mesenchymal transition (EMT). TGF-β1 is a multi-functional mediator that regulates proliferation, differentiation, apoptosis, adhesion and migration in various cells such as macrophages, activated T and B cells, immature hematopoietic cells, neutrophils and dendritic cells [14].

Plasma TGF-β1 in hemodialysis patient found that patients with renal osteodystrophy (ROD) had significantly higher TGF-β1 levels than patients without ROD. They concluded that the pathological condition of ROD could stimulate increased production of TGF-β1 in patients undergoing hemodialysis [15,16]. Intense TGF-β1 expression in bone samples with osteitis fibrosa from patients with ROD is accompanied by intense TGF-β1 levels in hemodialysis patient found that patients with renal osteodystrophy (ROD) had significantly higher TGF-β1 levels than patients without ROD. They concluded that the pathological condition of ROD could stimulate increased production of TGF-β1 in patients undergoing hemodialysis [15,16].

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| Days | Control groups | CKD groups |
|------|----------------|------------|
|      | 4   | 7   | 14  | 21  | 4   | 7   | 14  | 21  |
| Control groups (TGF-β) | 0.990 | 0.946 | 1.000 | 0.608 | 0.001* | 0.040* | 0.967 |
| 4    | 0.003* | 0.001* | 0.001* | 0.265 | 0.001* | 0.015* | 0.025* |
| 7    | 0.001* | 0.001* | 0.001* | 0.041* | 0.999 | 0.001* | 0.001* |
| 14   | 0.991 | 0.001* | 0.001* | 0.032* | 0.001* | 0.057 | 0.035* |
| 21   | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 1.000 |
| Control groups (BMP-2) | 0.004* | 0.314 | 1.000 | 1.000 | 0.068 |
| 4    | 0.019* | 0.001* | 0.001* | 0.001* | 0.001* | 1.000 |
| 7    | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 1.000 |
| 14   | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| 21   | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| Control groups (DKK-1) | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| 4    | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| 7    | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| 14   | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| 21   | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |

Note: TGF-β = Transforming Growth Factor-β; BMP-2 = Bone Morphogenetics Protein-2; DKK-1 = Dickkopf-1; *significant <0.05; **significant <0.001.

The decrease in TGF-β1 concentration was caused by several mechanisms, namely due to inhibition of the TGF-β1 pathway in several places due to rescue efforts as a result of high concentrations or because iPTH levels were not too high compared to the condition of bone with high turnover. The R-Smads that respond to the TGF-β1 receptor are Smad2 and 3. Smad7 antagonists TGF-β1 signaling through several mechanisms, including interfering with R-Smad recruitment, promoting receptor dephosphorylation, recruiting the E3 ubiquitin ligase to induce receptor degradation, and blocking the functional Smad complex from interacting with DNA in the nucleus [13,19].

The cellular responses elicited by TGF-β1 are tightly controlled by multiple mechanisms at each step in their signalling pathway. BAMBI (BMP and activin membrane-bound inhibitor) is a transmembrane glycoprotein structurally associated with the type I TGF-β1 receptor but lacks an intracellular kinase domain. BAMBI functions as a type I receptor decoy that counteracts TGF-β1 family signals by preventing the formation of active receptor complexes upon ligand binding. Little is known about the physiological functions regulated by BAMBI or the pathological consequences of the imbalance between BAMBI and TGF-β1 signals [20].

The BAMBI pseudoreceptors (BMP and membrane-bound activin inhibitors) inhibit TGF-β1 family signals by directly complexing with TGF-β1, activin, and/or BMP receptors to produce an inactive receptor complex [21] or by binding and enforcing an inhibitory effect. Smad7 on TGF-β1 signalling [13]. BAMBI expression is induced by Wnt/β-catenin signalling and suppressed by Toll-like receptor 4 (TLR4) signalling, thus allowing these pathways to cross-link with TGF-β1 signalling [22].

BMP is a multi-functional growth factor that belongs to the TGF-β1 superfamily. This protein is essential for many developmental processes, including cardiogenesis, neurogenesis, and osteogenesis. In particular, BMP-2 is the first BMP to be characterized and has been well studied within the BMP family. BMP-2 is essential during embryonic development, bone remodelling, and homeostasis in adulthood. Its specific

Table 3
Post hoc analysis of TGF-β1, BMP2, and DKK1 levels.
functions include finger formation and activation of osteogenic genes, such as Runt-Related Transcription Factor 2 (RUNX2) [23]. Serum levels of BMP-2 and BMP-4 in CKD were significantly increased compared with controls. Increased serum BMP-2 and BMP-4 expression levels were positively correlated with the aortic calcium content. Significantly increased serum BMP-4 levels were positively correlated with coronary artery calcium scores in patients with CKD and coronary artery calcification. Induction of inflammation, excessive oxidative stress, and apoptosis are currently considered the primary mechanisms by which BMPs are involved in vascular calcification. BMPs may play an essential role in the pathogenesis of vascular calcifications. BMP binds to serine-threonine kinase type II and type I (bone morphogenetic protein receptor-IA (BMPR-IA), BMPR-IB, activin receptor-like kinase-2 (ALK-2), and ALK1) to form specific complexes. The complexes regulate the phosphorylation of Smad1/5/8 and then combine with the Smad4 protein, which together translocate to the nucleus, where they are involved in osteogenesis and other biological processes. BMP-4 and BMP-2 are structurally similar and have osteogenic and ectopic bone formation activity [24].

BMP is a multi-functional growth factor belonging to the TGF-superfamily, so it can be assumed that BMP-2 secretion will also be increased in CKD-5D. TGF-β greatly enhances ectopic bone formation induced by BMP-2, with the resulting bone volume being five-fold more significant than BMP-2 alone [13].

DKK-1 is a Wnt inhibitor, mainly produced by osteoblasts and osteocytes. In normal subjects, DKK-1 is expressed at low levels in the skin, placenta, prostate, kidney, and platelets. DKK-1 expression is regulated by growth factors and hormones, including calcitonin, morphogenetic protein, PTH, and estrogen. Bone cells are considered the primary producing tissue, but DKK-1 expression increases during renal tubular epithelial proliferation and repair in early CKD [16]. CKD alters the production of Wnt inhibitors. Several studies have reported increased levels of sclerostin, DKK-1, SFRP1, and SFRP4 as CKD progresses. Serum phosphate is associated with levels of Wnt inhibitors. High serum phosphorous levels are a hallmark of CKD and are potentially the first to be responsible for the increase in Wnt inhibitors. Serum sclerostin and DKK-1 were positively correlated with phosphate levels in CKD patients. FGF-23 upregulates DKK-1, and high phosphorous levels are directly correlated with FGF-23, phosphorus can increase DKK-1 levels through FGF-23 [25].

5. Conclusion

The concentration of TGF-β1 in adipose mesenchymal stem cells in CKD patients increases significantly on day 7, which is the maturation phase. The concentration of TGF-β1 then decreases below the concentration of healthy people on observation day 14 and 21, significantly on day 14, which is the mineralization phase. The concentration of BMP-2 in adipose mesenchymal stem cells in CKD patients increases significantly on the 7th observation day, namely the maturation phase, compared to healthy people. The concentration of inhibitory factor DKK-1 in adipose mesenchymal stem cells of CKD patients increases significantly on the 7th observation day, such as the maturation phase, compared to healthy people.

Ethical approval

We have conducted an ethical approval base on the Declaration of Helsinki with registration research at the Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Sources of funding

None.

Author contribution

Please specify the contribution of each author to the paper, e.g. study concept or design, data collection, data analysis or interpretation, writing the paper, others, who have contributed in other ways should be listed as contributors.

All authors contributed toward data analysis, drafting and revising the paper, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

Consent

All participants are required to fill out an informed consent.

Registration of research studies

1. Name of the registry: Health Research Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
2. Unique Identifying number or registration ID: 0264/KEPK/IX/2021.
3. Hyperlink to your specific registration (must be publicly accessible and will be checked): -.

Guarantor

Artaria Tjempakasari is the person in charge of the publication of our manuscript.

Declaration of competing interest

Artaria Tjempakasari, Heri Suroto, and Djoko Santoso declare that they have no conflict of interest.

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References

[1] M. Kahdina, N. Mardiana, D. Fauziah, Levels of hemoglobin, leukocytes, and platelets of chronic kidney disease patients undergoing hemodialysis in Surabaya, Biomol. Health Sci. J. 1 (1) (2018) 29–33, https://doi.org/10.20473/bhsj.v1i1.8190.
[2] S.J. Chadban, C. Ahn, D.A. Axelrod, B.J. Foster, B.L. Kasiske, V. Kher, et al., Summary of the kidney disease: improving global outcomes (KDIGO) clinical practice guideline on the evaluation and management of candidates for kidney transplantation, Transplantation 104 (4) (2020) 708–714, https://doi.org/10.1097/tp.0000000000003137.
[3] P.D. Miller, Chronic kidney disease and the skeleton, Bone Res. 2 (2014), 14044, https://doi.org/10.1038/bone-res.2014.44.
[4] P.A. Abadini, M. Thaha, A. Mustika, 8-Hydroxydeoxyguanosine urine and total nitric oxide serum in chronic kidney disease, Folia Medica Indonesiana 58 (2) (2022) 137–140, https://doi.org/10.20473/fmi.v58i2.31814.
[5] K.A. Huruka, T. Sugatani, O. Agapova, Y. Fang, The chronic kidney disease - mineral bone disorder (CKD-MBD): advances in pathophysiology, Bone 100 (2017) 80–86, https://doi.org/10.1016/j.bone.2017.01.023.
[6] M.J. Sarnak, K. Maenn, S.Bangalore, J.L. Cavaclante, D.M. Charytan, J.C. Craig, et al., Chronic kidney disease and coronary artery disease: JACC state-of-the-art review, J. Am. Coll. Cardiol. 74 (14) (2019) 1823–1838, https://doi.org/10.1016/j.jacc.2019.08.1017.
[7] A.R. Ardhany, S.D. Suryantoro, M. Thaha, D. Santoso, A rare case: vesicoureteral reflux in Indonesian young adult with neurogenic bladder and chronic kidney disease stage 4, 2022, Ann. Med. Surg. 74 (2012), 103267, https://doi.org/10.1016/j.amsu.2022.103267.
[8] A. Dunso, E.A. González, K.J. Martin, Vitamin D in chronic kidney disease, Best Pract. Res. Clin. Endocrinol. Metabol. 25 (4) (2011) 647–655, https://doi.org/10.1016/j.beem.2011.05.005.
[9] X. Liu, K. Liu, Q. Sun, Y. Wang, J. Meng, Z. Xu, et al., Efficacy and safety of febuxostat for treating hyperuricemia in patients with chronic kidney disease and in renal transplant recipients: a systematic review and meta-analysis, Exp. Ther. Med. 16 (3) (2018) 1859–1865, https://doi.org/10.3892/etm.2018.6367.
[10] M. Liu, X.C. Li, L. Lu, Y. Cao, R.R. Sun, S. Chen, et al., Cardiovascular disease and its relationship with chronic kidney disease, Eur. Rev. Med. Pharmacol. Sci. 18 (19) (2014) 2918–2926.
[11] T. Wang, Y. Xi, R. Lubwama, H. Hannanchi, K. Inlay, C. Koro, Chronic kidney disease among US adults with type 2 diabetes and cardiovascular diseases: a national estimate of prevalence by KDIGO 2012 classification, Diabetes Metabol. Syndr. 13 (1) (2019) 612–615, https://doi.org/10.1016/j.dsx.2018.11.026.

[12] R.M. Moyses, S.C. Schiavi, Sclerostin, osteocytes, and chronic kidney disease - mineral bone disorder, Semin. Dial. 28 (6) (2015) 578–586, https://doi.org/10.1111/sdi.12915.

[13] Y. Chen, C. Di, X. Zhang, J. Wang, F. Wang, J.F. Yan, et al., Transforming growth factor β signaling pathway: a promising therapeutic target for cancer, J. Cell Physiol. 235 (3) (2020) 1903–1914, https://doi.org/10.1002/jcp.29108.

[14] F.J. Lopez-Hernandez, J.M. Lopez-Novoa, Role of TGF-β in chronic kidney disease: an integration of tubular, glomerular and vascular effects, Cell Tissue Res. 347 (1) (2012) 141–154, https://doi.org/10.1007/s00441-011-1275-6.

[15] X. Jiang, H. Kanai, T. Shigehara, A. Maezawa, S. Yano, T. Naruse, Metabolism of transforming growth factor-beta in patients receiving hemodialysis especially those with renal osteodystrophy, Ren. Fail. 20 (1) (1998) 135–145, https://doi.org/10.3109/08860229809045096.

[16] Y. Iwasaki, H. Yamato, M. Fukagawa, TGF-beta signaling in bone with chronic kidney disease, Int. J. Mol. Sci. 19 (8) (2018), https://doi.org/10.3390/ijms19082352.

[17] V. Szegeczki, H. Perényi, G. Horváth, B. Hannah, A. Tamáš, Z. Radák, et al., Physical training inhibits the fibrosis formation in alzheimer’s disease kidney influencing the TGFβ signaling pathways, J. Alzheim. Dis.: JAD. 81 (3) (2021) 1195–1209, https://doi.org/10.3233/JAD-201206.

[18] A. Tjempakasari, H. Suroto, D. Santosio, Mesenchymal stem cell senescence and osteogenesis, Medicine 58 (1) (2021), https://doi.org/10.3390/medicine58010061.

[19] P.M. Tang, Y.Y. Zhang, T.S. Mak, P.C. Tang, X.R. Huang, H.Y. Lan, Transforming growth factor-β signalling in renal fibrosis: from Smads to non-coding RNAs, J. Physiol. 596 (16) (2018) 3492–3503, https://doi.org/10.1113/jp27492.

[20] J. Krstic, D. Trivanovic, S. Mojsilovic, J.F. Santibanez, Transforming growth factor-beta and oxidative stress interplay: implications in tumorigenesis and cancer progression, Oxid. Med. Cell. Longev. 2015 (2015), 654594, https://doi.org/10.1155/2015/654594.

[21] T. Sekiya, S. Adachi, K. Koku, T. Yamada, O. Higuchi, Y. Furukawa, et al., Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor-beta signaling, as a target of the beta-catenin pathway in colorectal tumor cells, J. Biol. Chem. 279 (8) (2004) 6840–6846, https://doi.org/10.1074/jbc.M310876200.

[22] Q. Meng, H. Guo, L. Xiao, Y. Cui, R. Guo, D. Xiao, et al., mTOR regulates TGF-β1-induced epithelial-mesenchymal transition in cultured human lens epithelial cells, Graefe’s archive for clinical and experimental ophthalmology – Albrecht von Graefes Archiv fur Klinische und experimentelle Ophthalmologie. 251 (10) (2013) 2363–2370, https://doi.org/10.1007/s00417-013-2435-z.

[23] D. Halloran, H.W. Durban, A. Nohe, Bone morphogenetic protein-2 in development and bone homeostasis, J. Dev. Biol. 8 (3) (2020), https://doi.org/10.3390/jdb8030019.

[24] F. Wei, Y. Zhou, J. Wang, C. Liu, Y. Xiao, The immunomodulatory role of BMP-2 on macrophages to accelerate osteogenesis, Tissue Eng. 24 (7–8) (2018) 584–594, https://doi.org/10.1089/ten.TEA.2017.0232.

[25] K.J. Sellers, C. Elliott, J. Jackson, A. Ghosh, E. Ribe, A.I. Rojo, et al., Amyloid β synaptotoxicity is Wnt-PCP dependent and blocked by fasudil. Alzheimer’s & dementia, the journal of the Alzheimer’s Association. 14 (3) (2018) 306–317, https://doi.org/10.1016/j.jalz.2017.09.008.