Probiotics' supplementation alleviates disease severity and improves postural balance by repairing intestinal leak in patients suffering from osteoarthritis: A double-blinded clinical trial.

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Abstract

Increased intestinal leakiness and associated systemic inflammation are potential contributors to osteoarthritis (OA) and postural imbalance in the geriatric population. To-date, no successful treatment to correct postural imbalance in OA is known. We aimed to explore the effects of a multistrain probiotic upon postural imbalance in OA-affected patients. In this randomized, double-blind trial with a placebo group, 147 patients suffering from knee OA (age-span = 64-75 years) were divided into placebo (n=75) and probiotics (n=72) study groups. Vivomix 112 billion, multistrain probiotic was given once a day for 12 weeks. The outcomes of study variables were determined first at baseline and later after 12 weeks of intervention. These were Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), knee flexion range-of-movement (ROM), pain intensity by visual analogue scale (VAS), handgrip strength (HGS), gait speed, and balance control assessed in standing, semi-tandem, and tandem stances. We determined plasma zonulin, to determine intestinal leak along with c-reactive protein (CRP) and 8-isoprostanes levels. A total of 136 OA patients taking placebo (n=71) and probiotics (n=65) were analyzed. The probiotics group exhibited a reduction in pain intensity, disease severity, and WOMAC scores along with improvement in balance scores, HGS, and walking speed ($p<0.05$ for all), no change in ROM, resting pain, and 8-isoprostanes levels. The correlation analysis revealed a robust association of balance scores with plasma markers of intestinal leakiness and inflammation in probiotics but not in the placebo group. Probiotics reduce postural imbalance in OA patients partly due to a reduction in intestinal leakiness.

Keywords: Probiotics, osteoarthritis, zonulin, postural imbalance, WOMAC index

Abbreviations:

OA, Osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; ROM, range-of-movement; VAS, visual analogue scale; HGS, handgrip strength; CRP, c-reactive protein; OKS, Oxford knee score

Introduction

Osteoarthritis (OA), in particular knee OA is a common cause of disability, especially frequent among older adults. OA is described as a degenerative disease of the whole organ, showing changes in nearly all the joint apparatus, namely joint ligaments, nerves, synovial membrane, capsule, meniscus, and muscles of the diseased joint[1-3]. There is mounting evidence in the literature suggesting the progression of degeneration of knee joint proprioception during the development of OA[4-6]. Therefore, joint proprioceptors (specialized nerve endings that sense position and movement) are impaired and convey inappropriate proprioceptive signals about joint movement and position[5]. Thus, defective proprioception causes loss in balance control, leading to postural imbalance, thereby augmenting the risk of falls[7-9]. Impaired proprioception is not the sole cause of postural imbalance. Several other factors, such as reduced muscle strength, knee instability, severe OA associated with pain, and improper joint support[10, 11] are additional contributing factors. These patients develop adaptations during walking to compensate for poor balance control. For example, they have reduced gait speed and range of motion (ROM)[12]. However, the pathophysiology of postural imbalance in OA has not been explored fully and remains elusive.

Under normal, homeostatically balanced conditions the gut microbiome maintains health and contributes to host defense. Recently, the role of altered gut microbiome/dysbiosis and associated altered immune responses along with chronic systemic inflammation was recognized as a contributing component in the pathophysiology of OA[13, 14], which has increased interest in the gut-joint axis for maintaining joint health. An intact intestinal mucosa limits the access of dangerous bacteria and their toxins into the general circulation [15]. Intestinal mucosal leakiness and gut dysbiosis lead to widespread inflammation in reaction to the harmful bacterial endotoxins[16]. Increased intestinal leakiness can be assessed by determining plasma levels as a biomarker for mucosal barrier damage[17]. Moreover, zonulin is considered a critical controller of intestinal permeability function and is potentially involved in the pathophysiology of various chronic inflammatory diseases[18]. Furthermore, zonulin inhibitors have been proposed for treating rheumatoid arthritis, thereby reinforcing the causative role of zonulin in altered intestinal leakiness[19]. We reported elevated plasma zonulin levels associated with various diseases of old age[20-22]. Moreover, evidence suggests the association of gut microbial dysbiosis, a common reason for higher intestinal leakiness, with the severity of postural imbalance and walking

problems in patients suffering from Parkinson's disease[23]. Taken together, these findings suggest the possible role of higher intestinal leakiness leading to postural imbalance. However, the existence of such a relationship in OA remains unidentified.

The use of probiotics for the correction of gut dysbiosis has emerged as a promising approach to treating several chronic metabolic diseases[24]. Moreover, therapeutic lowering of plasma zonulin with probiotics supplementation may help restore intestinal leakiness, thereby preventing the entry of harmful bacteria into circulation. Manipulation of the gut microbiota has been proposed to decrease inflammatory levels in OA related to obesity phenotype[25]. Moreover, probiotics have been suggested to modify molecular pathways related to the gut-bone axis, exerting beneficial effects on bone health and OA-like degenerative changes in joints [26, 27]. However, no such role of probiotics in reducing postural imbalance related to OA has been explored. Our previous findings showed probiotics supplementation helped restore intestinal mucosal barrier function in patients suffering from chronic obstructive pulmonary disease[28]. Therefore, considering the possible link of intestinal leakiness with the development of postural imbalance in OA patients, we designed an investigation to determine the correlation of plasma zonulin with postural instability in OA patients treated with probiotics. Furthermore, we aimed to investigate the underpinning mechanism by evaluating the status of generalized inflammation and oxidative stress status in knee OA-affected patients.

Materials and Methods

Study layout and clinical evaluation of disease severity by Oxford knee score (OKS):

In this 12-week-long clinical trial, we used the double-blinded, randomized, placebo-control technique. We recruited clinically diagnosed osteoarthritis (OA) patients visiting the outpatients' Department of Trauma and Orthopedic Surgery of Rehman Medical Institute (RMI), Pakistan. This study was conducted according to the guidelines laid down in the Declaration of Helsinki[29] and all procedures involving human patients were approved by the Medical Research Ethics Committee of RMI (Ref. no. RMI-HEC-13-11-75, dated: 05/01/2021). Written informed consent was obtained from all patients. The primary demographic details of patients were recorded by filling in the datasheet. The patients were evaluated by taking a thorough history and detailed physical examination. The disease diagnosis and stage were confirmed by two orthopedic surgeons using OKS system. It is considered a well-established standardized measurement tool to determine pain intensity and functional ability for performing daily life

activities self-reported by OA patients. OKS consists of 12 questions, graded from 0 (poorest function) to 4 (maximal function), explained in detail already[30].

Patient selection criteria and randomization:

We enrolled 264 men and women patients aged 64-75 years. We included symptomatic patients with unilateral or bilateral knee OA with more than 6 months of pain. We excluded patients who met one or more of the following criteria: OKS score ≤ 6, intra-articular trauma or fracture, joint-related or knee replacement surgeries, intra-articular steroid injections, and treatment with OA disease-modifying agents. Moreover, patients with GI-related diseases taking probiotics or antibiotics were excluded based upon their past medical and drug-related history. All women were postmenopausal, and not taking any hormonal or bone-modifying medications. All patients had the same ethnic and geographic background.

A sample size calculation was performed to determine the optimal group size for this randomized control trial. To detect the anticipated 5% change in plasma zonulin levels with a desired statistical power of 80% (beta = 0.2) and a significance level of 0.05 (alpha), 63 participants per group were determined to be necessary. We assessed 264 enrolled patients; 147 fulfilled the inclusion criteria. 117 patients were excluded since 59 did not meet the criteria, 53 did not consent to participate, and five were excluded for variable reasons. Therefore, an independent analyst, not engaged in the disease assessment/diagnosis and determination of study outcome variables, performed computer randomization of 147 recruited OA patients. Likewise, the researchers were unaware of the randomization process. Patients were allocated randomized codes to receive the intervention. Therefore, allocation to placebo (n=75) and probiotics (n=65) groups was completely blinded. The intervention continued for three months (12 weeks), and during the follow-up period, none of the patients were lost to follow-up in the placebo group, and only one patient was lost in the probiotics group. Moreover, four and six patients discontinued intervention from the placebo and probiotics groups, respectively, and were excluded from the trial. Upon the completion of the trial, 136 patients in the placebo (n=71) and probiotics (n=65) groups were analyzed for outcome measurements **(figure 1)**.

Probiotic and placebo products:

In our previous study, we have already explained the treatment product details[31]. Patients received either probiotic or placebo capsules once daily for 12 weeks. Vivomix 112 billion is a high-potency probiotic capsule containing 112 billion live bacteria from eight specific

strains. These strains included Streptococcus thermophilus DSM 24731, Bifidobacterium longum DSM 24736, Bifidobacterium breve DSM 24732 and DSM 24737, Lactobacillus strains DSM 24735, DSM 24730, DSM 24733, and Lactobacillus delbrueckii subsp. bulgaricus DSM 24734. The capsule also contains maltose as an inactive ingredient and silicon dioxide as an anti-caking agent (Vivomix, UAE). Placebo capsules were isocaloric-matched ones containing no biologically active ingredient. Both placebo and probiotics were white powders that dissolved in water and were provided to patients in similar opaque containers with appropriate storage instructions. The study was designed to investigate the effect of probiotics independent of any lifestyle modifications such as diet or physical activity; therefore, patients were advised to maintain their routine lifestyle. Moreover, patients were instructed to continue the management of OA, which was already ongoing at the beginning of the trial.

Assessment of knee joint range of movement (ROM):

Firstly, at the baseline and later after 12 weeks of treatment, knee flexion measurements were performed to determine the knee joint ROM. Measurements were performed with patients lying on the examination couch comfortably, following the protocol described[32]. A digital electronic goniometer (Meloq, Stockholm, Sweden) was used by trained staff to measure the ROM of the OA-affected joint without any forceful movements. We recorded three consecutive readings with brief periods of relaxation, and a mean value was considered for data analysis.

Assessment of knee pain intensity by visual analogue scale (VAS):

Patients rated their pain intensity using VAS, ranging from no pain (0) to worst possible pain (10) for knee pain rating. We followed the pain measurement scale already described elsewhere[33]. Pain intensity measurements were performed at the start (baseline) and end (after 12 weeks) of the trial, and patients were instructed to discontinue any analgesics three days before VAS measurements to avoid any masking effects of drugs. At each visit, patients were instructed to finish two VAS measurements, one at rest and the second while walking, to reflect pain intensity in resting and active states of the diseased joint.

Assessment of pain status and function by Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC):

WOMAC is a broadly utilized OA-specific questionnaire based on the self-reporting of symptoms by patients to evaluate underlying disease activity. The questionnaire has three sections, and the WOMAC score spans between 0-96 points. The three sections comprise questions for pain (total points = 20), stiffness (total points = 8), and functional deficiency (total points = 68). The total WOMAC count is obtained by adding the points from three categories; higher index values suggest severe symptoms and diminished joint function and vice versa[34]. In this trial, we measured the WOMAC index scores of our study patients at baseline and after 12 weeks of intervention.

Handgrip strength (HGS) and gait speed measurements:

HGS, an important tool to measure muscle strength, is associated with knee OA, especially with a classic radiographic feature of the disease, i.e., joint space narrowing[35]. We measured the HGS of our study patients by using a numerical hand dynamometer (CAMRY, USA), following the protocol already described by us[20, 28]. In brief, patients had to sit comfortably in the chair and hold the dynamometer in the dominant hand, squeezing it with maximum strength with the elbow flexed at 90º. The movements were ensured to be non-jerky, and three consecutive readings were recorded with brief intervals of relaxation in between. A mean value was calculated and used for analysis. Next, we determined the functional capacity of our study patients by recording their gait speed, following the protocol already described by us[36].

Assessment of postural balance:

At baseline and 12 weeks after the intervention, we determined the postural stability of our patients by assessing their body balance in standing posture. Patients were made to stand upright without support, and we assessed three balancing positions. Patients were made to put their feet together to assess side-by-side balance, a semi-tandem, and tandem stance for 10 seconds. The scoring system had a range of 0 (poor performance) to 4 (maximum performance), as described already[37]. One point was given to the patients able to balance their body with feet together and in semi-tandem positions for 10 seconds. Patients unable to maintain or not willing to try to maintain postural balance were allocated no points. Two points were allocated to patients capable of maintaining a fully tandem position for 10 seconds and able to maintain for 3-

9.99 seconds were given 1 point; however, if the time for maintaining this position was less than three seconds, then no points were allocated.

Circulating biomarkers' assessment:

3ml blood was drawn from the patients and stored in suitable vacutainers to obtain platelet-free plasma. Plasma was stored in Eppendorf tubes at -80ºC. We used commercially available ELISA kits and followed the manufacturer's protocol to determine levels of plasma zonulin (Cat # K5601, Immundiagnostik AG, Bensheim, Germany), c-reactive proteins (CRP) (R&D Systems, Minneapolis, MN, USA), and 8–isoprostanes (Cayman Chemical, Ann Arbor, MI, USA), as described previously[21].

Statistical evaluation:

The anthropometric measurements were given as mean ± standard deviation since the data met the criterion of normal distribution. Group-wise comparisons were done by using paired t-tests, and regression analysis was performed to study the correlation between various study variables. We used GraphPad Prism 8 (Graph Stats Technologies, India) for data analysis and creating graphs. A *p*-value of < 0.05 was regarded as statistically significant. $*p<0.05$ vs. baseline value of the same group.

Results

Basic demographic and medical features of patients:

We analyzed 136 patients in the placebo (n=71) and probiotics (n=65) groups for the outcome measures. The study patients were age, BMI, and gender-matched and had similar ethnicity **(table 1).** OA patients in both placebo and probiotics groups had comparable disease duration and percentages of affected knee joints. All the OA patients in this trial had almost the same WOMAC index scores, severity of the disease according to OKS, pain intensity according to VAS, and degree of knee flexion according to ROM. At the baseline, no significant difference existed between the clinical and biochemical parameters of the study patients. After 12 weeks of intervention, no changes were found in the placebo group in the studied outcome variables. Conversely, OA patients following treatment for 12 weeks with probiotics exhibited a reduction a marked reduction in OA-related symptoms and overall disease severity ($p < 0.05$). There was a substantial reduction in WOMAC index scores, pain intensity while walking, and consumption of analgesics (non-steroidal anti-inflammatory drugs) following treatment with probiotics ($p < 0.05$ for all). OA-affected patients also showed improvement in physical performance as

displayed by improvement in balance scores, HGS, and walking speed ($p < 0.05$ for all). Moreover, plasma zonulin and c-reactive protein (CRP) levels were found to be reduced in this group (all $p < 0.05$). However, we did not find any improvement in pain intensity at rest according to VAS, knee flexion ROM of diseased joints, and plasma 8-isoprostanes levels among patients in the probiotics group.

Changes in OKS, physical capacity, and circulating biomarkers according to balance scores in OA patients treated with probiotics:

Next, we categorized the study patients based on their balance scores into three groups with balance scores of 4, 3, and ≤ 2 to investigate whether study outcomes were affected by the altered postural balance of OA patients taking either placebo or probiotics. OA patients within three balance score groups in both placebo and probiotics groups showed similar OKS scores at baseline. After 12 weeks of trial, no change was observed in the OKS scores of patients in the placebo group. Conversely, the OA-affected patients having 4, 3, and ≤ 2 balance scores, after taking probiotics, showed a significant improvement in their OKS scores reflected as a reduction in disease severity when compared to baseline ($p < 0.05$ for all) **(figure 2A).** Additionally, the OA patients with balance scores 3 and ≤ 2 exhibited an increase in gait speed following treatment with probiotics in comparison to baseline (both $p < 0.05$) **(figure 2B).** Similarly, OA patients with a balance score of 3, after probiotics supplementation showed an improvement in HGS as compared to the baseline ($p < 0.05$) **(figure 2C).** Therefore, OA patients with a baseline balance score of 3 showed an overall improvement in functional capacity and a reduction in disease severity after probiotics treatment. The patients in the placebo group showed no changes in disease severity, gait speed, and HGS after 12 weeks compared to baseline.

We next investigated whether supplementation with probiotics affected intestinal leakiness and its associated inflammation in OA patients with various categories of balance scores. After 12 weeks of probiotics treatment, the OA patients within all three categories of balance scores showed a significant reduction in plasma zonulin (all $p < 0.05$) **(figure 2D)**, and CRP levels (all $p < 0.05$) **(figure 2E),** in comparison to the baseline, with no such changes observed in the placebo group. Moreover, plasma 8-isoprostanes levels remained unchanged in both placebo and probiotics groups **(figure 2F).**

Changes in balance scores, physical capacity, and circulating biomarkers according to OKS scores in OA patients treated with probiotics:

Next, we questioned whether disease severity affected the study outcomes in OA patients taking either placebo or probiotics for 12 weeks. Therefore, we grouped the patients into three categories (mild, moderate, and severe) by using the OKS scoring system. Patients with mild, moderate, and severe OA showed comparable balance scores at baseline in the study's placebo and probiotics groups. Following the intervention of 12 weeks, patients in the placebo group showed no changes in the balance scores compared to baseline. On the contrary, OA patients in moderate and severe categories taking probiotics treatment exhibited an improvement in their balance scores as compared to baseline (both $p < 0.05$) **(figure 3A).** Moreover, the gait speed of patients with mild and moderate OA improved as compared to baseline following probiotics treatment ($p < 0.05$) **(figure 3B).** HGS was improved in patients suffering from severe OA after 12 weeks of probiotics supplementation as compared to baseline ($p < 0.05$) **(figure 3C).** However, no changes in postural balance and functional capacity were observed in the OA patients taking a placebo. We further investigated whether probiotics affected plasma biomarkers among OA patients with varying degrees of disease severity. OA patients with the mild, moderate, and severe disease showed a reduction in plasma zonulin ($p < 0.05$) **(figure 3D)**, CRP levels were reduced among patients affected by OA with moderate and severe degrees (both $p < 0.05$) **(figure 3E),** no change observed in plasma 8-isoprostanes levels **(figure 2F)** when compared to baseline values. However, participants in the placebo group showed no changes in plasma biomarkers levels.

Correlation of plasma biomarkers with disease severity, physical ability, and postural balance in OA-affected patients treated with probiotics:

Lastly, we determined the correlations of plasma zonulin, an intestinal permeability marker, CRP an inflammatory marker, and 8-isoprostanes, a marker of systemic oxidative stress with severity of OA-related severity, physical capacity, and postural imbalance in placebo and probiotics groups **(table 2).** We found a significant correlation between WOMAC scores, gait speed, OKS, and balance scores with plasma zonulin levels in OA patients after probiotics treatment for 12 weeks. A robust correlation was observed with balance ($r^2 = 0.435$) and WOMAC index scores ($r^2 = 0.309$). We observed a significant correlation of plasma CRP levels with WOMAC scores, HGS, OKS, and balance scores among OA patients taking probiotics.

However, a robust association was found with balance scores (r^2 = 0.412). Plasma 8-isoprostanes levels were significantly correlated with WOMAC scores, HGS, and OKS scores in OA patients taking probiotics, with the highest association observed with HGS (r^2 = 0.375).

Discussion

Our results indicate that the administration of probiotics reduced pain and disease severity in patients suffering from knee OA. These effects were accompanied by improvement in gait speed and HGS in the probiotics group after 12 weeks of intervention as compared with the baseline. There was a significant reduction in plasma zonulin and CRP levels, while no changes were observed in 8-isoprostanes levels following probiotics supplementation in OA patients. Moreover, we report an improvement in balance scores in these patients. A reduction in plasma zonulin and inflammatory markers may partly describe intestinal mucosal repair as the possible underlying mechanism for the beneficial effects of probiotics in OA patients with postural imbalance. Additionally, plasma zonulin can potentially serve as a marker to assess postural imbalance and functional compromise in these patients.

Our findings support the existence and functioning of the gut-joint axis for the maintenance of joint-related health[38]. However, the correlation of intestinal permeability and associated gut dysbiosis with postural imbalance in OA was deficient. We have reported that probiotics intake can alleviate postural imbalance in older adults with OA. The role of gut dysbiosis in developing postural instability and gait difficulty phenotypes has been reported in neurodegenerative diseases, such as PD[39] and Multiple sclerosis[40]. Our observations extend the role of gut dysbiosis in developing postural imbalance in OA patients. Moreover, beneficial effects of probiotics in improving postural instability and gait difficulty have been observed in a mouse model of PD, suggesting the role of the gut-brain axis in preventing deterioration of motor functions in these patients[41], which further supports our findings.

The postural imbalance is coupled with declining muscle strength, proprioception, and neural control of muscles and joints. Although there is very limited evidence regarding the association of increased intestinal leakiness with postural imbalance in OA patients, this mechanistic factor has been proposed in developing several other chronic inflammatory diseases. High plasma zonulin, a marker of intestinal leakiness, has been reported to be associated with a decline in muscle strength and functional performance. Furthermore, we have previously reported the association of high plasma zonulin levels with muscle weakness and impaired

functional capacity in several age-related diseases[20, 21, 42]. Several previous studies have also reported the beneficial effects of probiotics intake in reducing plasma zonulin, discussed in detail elsewhere[43]. These previous reports align with our findings that probiotics supplementation lowers plasma zonulin levels, and is partly involved in intestinal repair, thereby improving postural stability in OA patients.

Previous studies have reported the beneficial effects of probiotics use in knee OA patients and have suggested this effect to be due to suppression of inflammation. Moreover, clinical studies have shown lower CRP levels in OA patients, a marker of inflammation following probiotics treatment[44]. These observations support the findings of our trial, where probiotics lowered CRP levels among patients in the probiotics group. We found improved HGS after probiotics intake, as observed previously[45]. We have also reported a reduction in pain sensitivity among the OA patients following probiotics use, which is in line with previously reported randomized controlled trials[46]. Interestingly, in an OA experimental model, lactobacilli's beneficial effects on cartilage health have been reported[47], which was also the component of our multistrain probiotic. Therefore, the marked reduction in pain sensitivity and improvement in disease severity observed after probiotics intervention in our study cohort reinforces the existence of the gut-joint axis and its potential to be manipulated therapeutically.

Since relevant studies on this topic are very limited, we suggest replication on a larger sample size. Additionally, understanding the role of individual strains of bacteria in improving joint health warrants more investigations. Furthermore, detailed molecular analysis of the blood of patients taking probiotics can help identify individual bacterial components responsible for improving the balance status of these patients.

This study has certain limitations. We need to expand our understanding by conducting such studies on a varied population regarding ethnicity and background. We have not considered any changes in the lifestyle and food habits of our studied population during the trial, which can be a potential confounding factor. We have not studied microbiome composition, which can potentially modify probiotics' effects. The major strength of this trial was its randomization to rule out the investigator's bias. Moreover, our patients had the same ethnic and geographic background, thereby reducing gut microbiome variability[48]. The study tools were simple, inexpensive, convenient to use, and easily accessible in any clinical setup.

Conclusions

Altogether, our findings reinforce the impact of the gut-joint axis and its potential therapeutic manipulation on the pathophysiology of postural imbalance in OA patients. We have proposed that increased intestinal leakiness and associated widespread inflammation augment OA symptoms, mainly pain and functional performance. Moreover, a leaky gut is a primary contributing factor in accelerating postural instability in OA patients. This is the first clinical study to report the beneficial effects of probiotics intake in improving balance in OA patients. We propose high plasma zonulin levels as a potential biomarker for the clinical evaluation of postural balance in OA patients. Lastly, our work suggests the beneficial effects of probiotics to be used as a therapeutic option to reduce postural imbalance in old-age patients suffering from OA.

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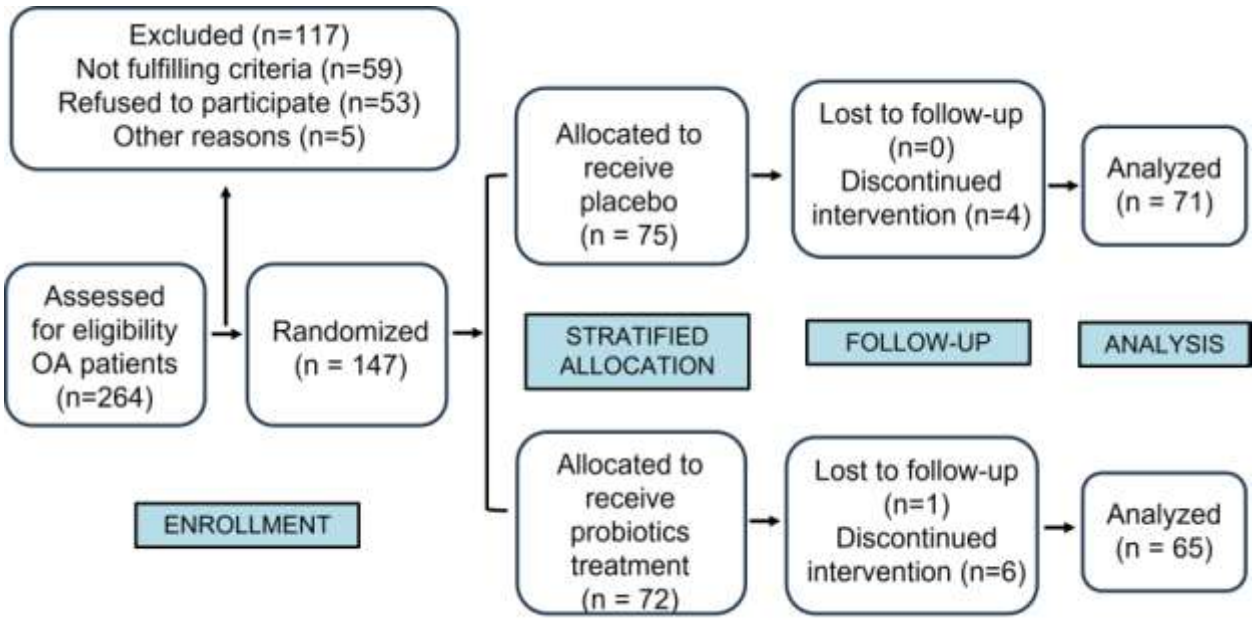

Figure 1: Flow chart of the study

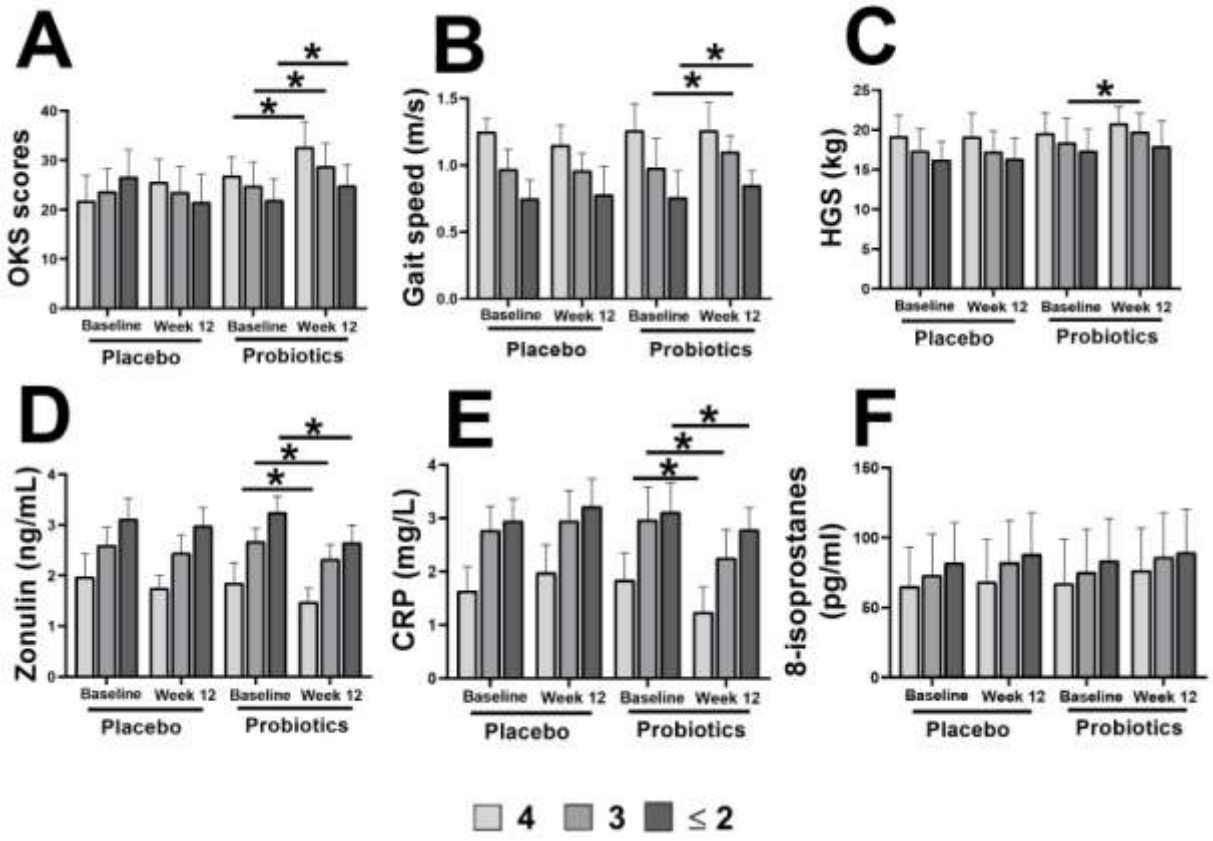

Figure 2: (A) OKS scores, (B) gait speed, (C) HGS, (D) plasma zonulin, (E) CRP, and (F) 8-isoprotanes levels in placebo (n = 71) and probiotics groups (n = 65) according to balance scores, *p < 0.05. (OKS; Oxford knee score, HGS; handgrip strength, CRP; c-reactive protein, OA; osteoarthritis).

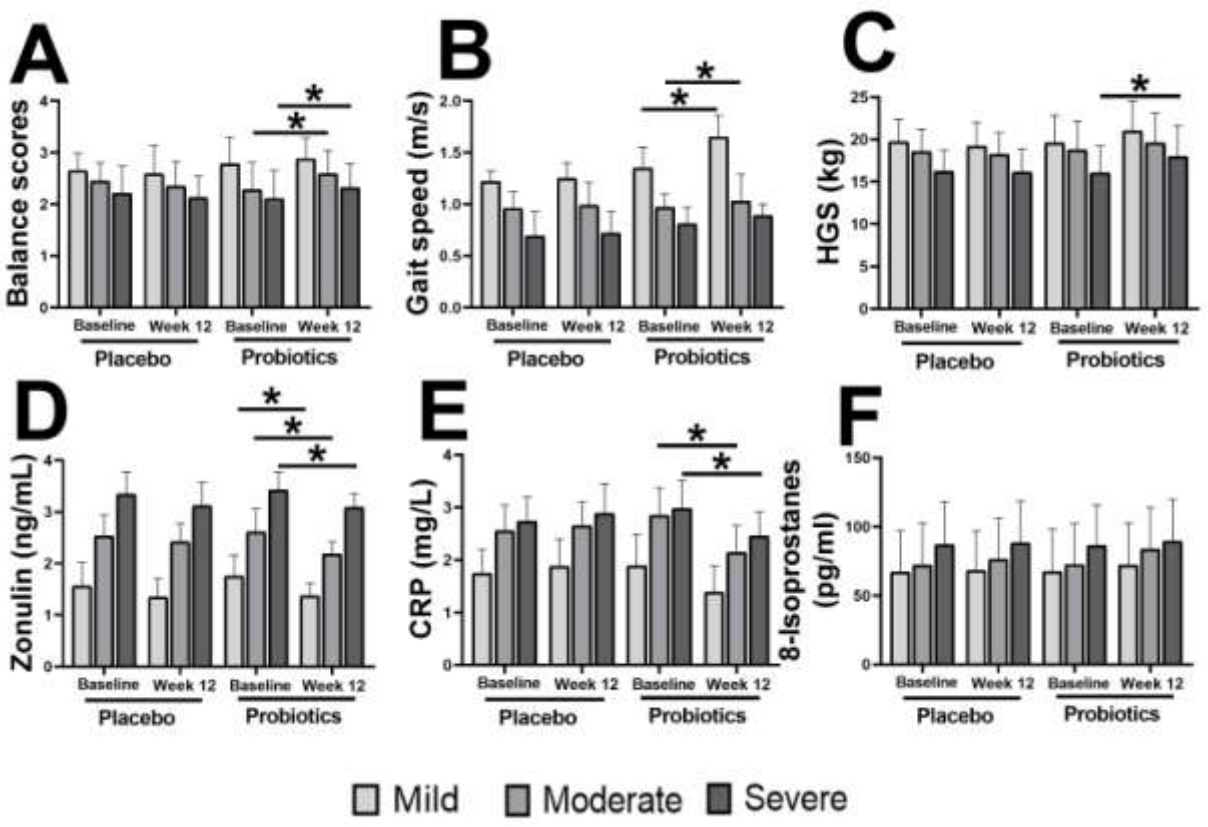

Figure 3: (A) OKS scores, (B) gait speed, (C) HGS, (D) plasma zonulin, (E) CRP and (F) 8-isoprotanes levels in placebo (n = 71) and probiotics groups (n = 65) according to balance scores, *p < 0.05. (OKS; Oxford knee score, HGS; handgrip strength, CRP; c-reactive protein, OA; osteoarthritis).

Table 1: Demographic characteristics of the OA patients corresponding to the placebo and probiotics treatment over a period of 12 weeks. Values are expressed as mean ± SD, paired t-test. *p < 0.05 *vs.* baseline of the same group (n = 65-71 / group). (BMI; body mass index, VAS; visual analogue scale, WOMAC; Western Ontario and McMaster Universities Arthritis Index, NSAIDs; non-steroidal anti-inflammatory drugs, OKS; Oxford knee score, ROM; range of movement, HGS; handgrip strength, CRP; c-reactive protein, OA; osteoarthritis).

	Placebo		**Probiotics**	
	Baseline	**12 weeks**	**Baseline**	**12 weeks**
Age (years)	69.1 ± 4.6	69.2 ± 3.9	69.3 ± 5.5	69.5 ± 5.1
BMI (kg/m^2)	25.38 ± 5.5	26.68 ± 4.8	25.21 ± 4.2	24.96 ± 3.5
Gender n (%)				
Male	24 (32)	21 (29.5)	23 (31.9)	19 (29)
Female	51 (68)	50 (70.4)	49 (68)	45 (69.2)
Duration of arthritis (years)	12.5 ± 4.5	12.7 ± 4.2	13.5 ± 7.2	13 ± 7.5
Affected joint side (%)				
Left	47.7	47.7	47.3	47.3
Right	52.3	52.3	52.1	52.1
Pain intensity (VAS)				
Resting	4.25 ± 3.6	4.28 ± 3.2	4.58 ± 3.3	4.21 ± 2.5
Walking	6.8 ± 4.6	6.9 ± 3.5	7.6 ± 2.8	6.1 ± 2.2*
WOMAC index	47.2	47.5	47.8	44.5*
Taking NSAIDs for OA treatment (%)	74.5	74.5	75.6	72.8*
OKS	23.80 ± 5.46	23.20 ± 5.21	24.68 ± 6.85	28.80 ± 7.55*
Balance scores	2.39 ± 0.34	2.25 ± 0.50	2.36 ± 0.52	2.55 ± 0.40*
Knee flexion ROM	121.5 ± 6.8	121.3 ± 6.5	122.2 ± 5.7	123.7 ± 5.2
HGS (Kgs)	17.22 ± 2.65	17.14 ± 2.81	18.21 ± 3.25	19.56 ± 3.56*
Gait speed (m/s)	0.94 ± 0.10	0.92 ± 0.15	0.95 ± 0.20	1.05 ± 0.21*
Plasma zonulin (ng/ml)	2.68 ± 0.45	2.56 ± 0.36	2.73± 0.41	2.25 ± 0.25*
8-isoprostanes (pg/ml)	73.20 ± 30.2	82.50 ± 28.80	75.40 ± 31.2	85.24 ± 30.5
CRP (mg/L)	2.67 ± 0.45	2.85 ± 0.52	2.75 ± 0.60	2.30 ± 0.50*

Table 2: Coefficients of determination of plasma zonulin, CRP, and 8-isoprostanes with indexes of WOMAC, functional performance, disease severity, and balance scores in OA patients according to placebo and probiotic treatments. *p < 0.05 (n = 65-71 / group). (HGS; handgrip strength, OKS; Oxford knee score, CRP; c-reactive protein, OA; osteoarthritis).

	Placebo		**Probiotics**	
	Baseline	**12 weeks**	**Baseline**	**12 weeks**
Zonulin				
WOMAC	0.156	0.176	0.256*	0.309*
HGS	0.276*	0.298*	0.319*	0.415*
Gait speed	0.064	0.068	0.104	0.128*
OKS	0.106	0.101	0.146*	0.241*
Balance scores	0.265*	0.287*	0.351*	0.435*
CRP				
WOMAC	0.245*	0.306*	0.389*	0.399*
HGS	0.276*	0.285*	0.329*	0.388*
Gait speed	0.078	0.085	0.134*	0.108
OKS	0.112	0.097	0.159	0.257*
Balance scores	0.254*	0.187*	0.399*	0.412*
8-isoprostanes				
WOMAC	0.124	0.098	0.126	0.256*
HGS	0.265*	0.248*	0.308*	0.375*
Gait speed	0.112	0.098	0.115*	0.126
OKS	0.075	0.099	0.153*	0.177*
Balance scores	0.115	0.179*	0.059	0.086