



# Medial plating versus newly designed intramedullary nail with distal interlocking system for distal tibia fractures: A biomechanical study with finite element analysis

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The intramedullary nail (IMN) method, which is frequently used in fractures of the diaphyseal region, is still known as a method with question marks in metaphyseal region fractures. The reason for this situation is that the cortical biomechanical support of the metaphyseal region is relatively low due to its anatomical structure, and the nail is insufficient to provide this support.<sup>[1]</sup>

The management of distal tibia fractures is controversial due to technical difficulties, with a high complication rate and a variety of treatment options depending on the type of fracture. Currently, the most widely used method

## ABSTRACT

**Objectives:** The aim of this study was to compare the biomechanical properties of a newly designed Tibial Intramedullary Nail-Distal Supportive Bolt Locking Screw (TIN-DSBLS) and medial anatomical plate in distal tibia fracture models using finite element analysis (FEA).

**Materials and methods:** Twelve medium-size, fourth-generation composite tibia models were used for testing. Models were divided into two groups as plate and nail group. A standardized osteotomy was made through each model. In the plate group, osteotomized models were fixed with distal tibia medial anatomic plate. Models in nail group were fixed with new design intramedullary nail with distal Bolt screw locking. All bone models were tested by an axial loading test machine to measure the force, maximum force, fracture site displacement and Stiffness. The second step of this study was FEA of the two models.

**Results:** The results of axial loading test results of two groups revealed that for distal tibia fracture, tibia nail which was locked with newly designed bolt screw showed superiority against plate-screw fixation about force ( $1597.76 \pm 85.54$  vs.  $830.74 \pm 146.7$  N), maximum force ( $3617.6$  vs.  $1512.42$  N), fracture site displacement ( $4.89 \pm 1$  vs.  $7.85 \pm 0.5$  mm) and stiffness ( $357.88 \pm 52.6$  vs.  $109.67 \pm 13.6$  N/mm). In FEA, maximum stress levels in implants were 1.7 time higher in nail group, bones were not exposed to higher levels of stress or deformation as in plate group. Maximum stress levels in bones of the nail group were 2.75 times lower than plate group. Total displacement in the plate group was more than two-fold compared to the nail group.

**Conclusion:** Our study results showed that the TIN-DSBLS provides more load transfer from the nail to the cortex in tibia distal metaphyseal fractures compared to traditional nails. Taken together, it is biomechanically superior to plate-screw fixation in fractures extending up to 2.5 cm proximal to the ankle joint.

**Keywords:** Biomechanic analysis, distal tibia fracture, finite element analysis, osteosynthesis.

Received: June 27, 2025

Accepted: September 16, 2025

Published online: December 29, 2025

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Doi: 10.52312/jdrs.2026.2459

**Citation:** Unal OK, Dagtas MZ, Najafov T, Demir C, Ugutmen E, Akpınar F. Medial plating versus newly designed intramedullary nail with distal interlocking system for distal tibia fractures: A biomechanical study with finite element analysis. Jt Dis Relat Surg 2026;37(2):477-488. doi: 10.52312/jdrs.2026.2459.

for distal tibia fractures is minimally invasive plate osteosynthesis (MIPO).<sup>[2]</sup> Since the distal tibia metaphysis area is wider than the diaphysis area, technical difficulties and deterioration in the tibial alignment can be seen in the application of IMNs, making them less preferred in this type fractures.

Plate using in distal tibia fractures provide anatomical reduction with reliable fixation which preserves the anatomical axis of the bone and allows early rehabilitation. However, it has been reported that wound problems, infections and reoperations are higher due to the thin subcutaneous tissue coverage and insufficient vascularization rather than the diaphysis area.<sup>[3,4]</sup> Due to such complications and despite insufficient support for the nail in the metaphyseal region, the use of nails in distal tibia fractures has started to be discussed again.<sup>[5,6]</sup>

The major challenge for nails used in distal tibia fractures is the stability of the distal fragment. In particular, in small diameter nails with small diameter screws, distal locking screw breakage can be seen with the failure of the fracture line.<sup>[7]</sup> Additionally, it is reported that fixation with three distal bicortical locking screws is more resistant to axial and varus loads than fixation with two distal bicortical locking screws in the literature. If nail is chosen for distal tibia fractures, it is recommended to use three distal locking screws.<sup>[8]</sup> The landmark for maximum nail depth for distal tibia is distal physeal scar<sup>[9]</sup> and this situation requires the fracture to extend at least 4 cm to the joint line in order to apply the nail with three distal locking screw. In fractures closer than 4 cm to the joint line, sufficient screws cannot be placed for distal fixation and stability of the fracture. The newly designed tibial nail (Tibial IMN-Distal Supporting Bolt Locking Screw [TIN-DSBLS]) can be fixed with a single screw (Bolt Locking Screw) for distal locking. This allows the IMN to be locked at a point closer to the joint line. The most significant advantages of the TIN-DSBLS are that it is more resistant to loads in this area, particularly fracture that is 2.5 cm distant to the joint line and transfers less load to the bone compared to traditional plate fixation. Furthermore, it minimizes skin complications by not causing skin irritation compared to plate applications.<sup>[10]</sup>

In the present study, we aimed to compare biomechanical properties of the medial anatomical plate and newly designed TIN-DSBLS in the distal tibia fracture model with biomechanical test and finite element analysis (FEA).

## MATERIALS AND METHODS

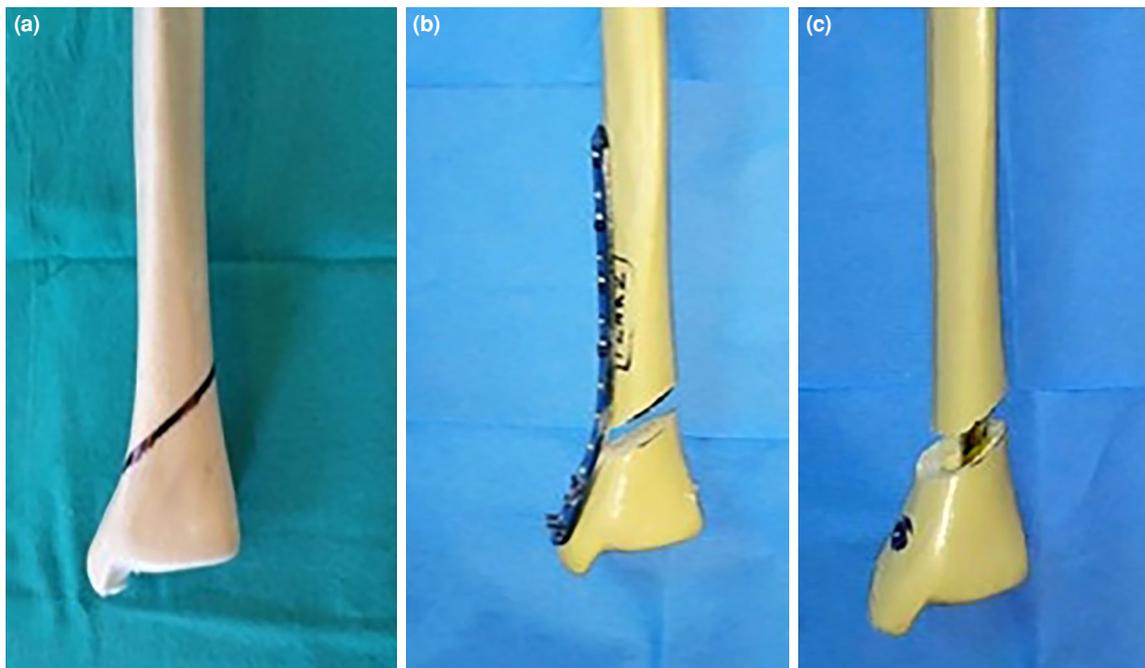
### Preparation of models and study groups

This study was conducted at Maltepe University, Faculty of Medicine, Department of Orthopedics and Traumatology and İstanbul Technical University, Department of Metallurgical and Materials Engineering between November 2020 and March 2021. The minimum sample size to statistically demonstrate a 10% damage effect at failure load in 80% of the samples is six samples per group. Twelve tibia models which were medium-size, fourth-generation composite tibia models (Model no: TB-01, Selbones Corp., Kocasinan, Kayseri, Türkiye) were used for testing. This validated bone model has been previously used for biomechanical studies, and its mechanical properties are similar to those of a healthy adult tibia.<sup>[11]</sup> Models were divided using a simple randomization method into two groups: plate and nail group. A standard osteotomy of each tibia, 45° oblique to the ground, was performed with an oscillating saw to create an Orthopedic Trauma Association type 43-A1.2 distal tibial fracture. Osteotomy was performed from the inferomedial to the superolateral cortex of the distal tibia, starting 2.5 cm proximal to the joint line, and each fracture was fixed with a plate or nail, leaving a 10-mm gap at the fracture site. In the plate group, osteotomized models were fixed with a distal tibia medial anatomic plate (left-sided MISS LC Distal Tibia Medial Plate, 17 holes, Model no: 34926710221, manufactured by TST Medical Devices Industry and Trade Limited Company, İstanbul, Türkiye) using nine locking screws. In the nail group, models were fixed with a tibia nail with proximal three locking screws (two oblique screws and one medial-to-lateral screw) and a new distal locking system (TIN-DSBLS, Model no: 815203408210) (Figure 1).

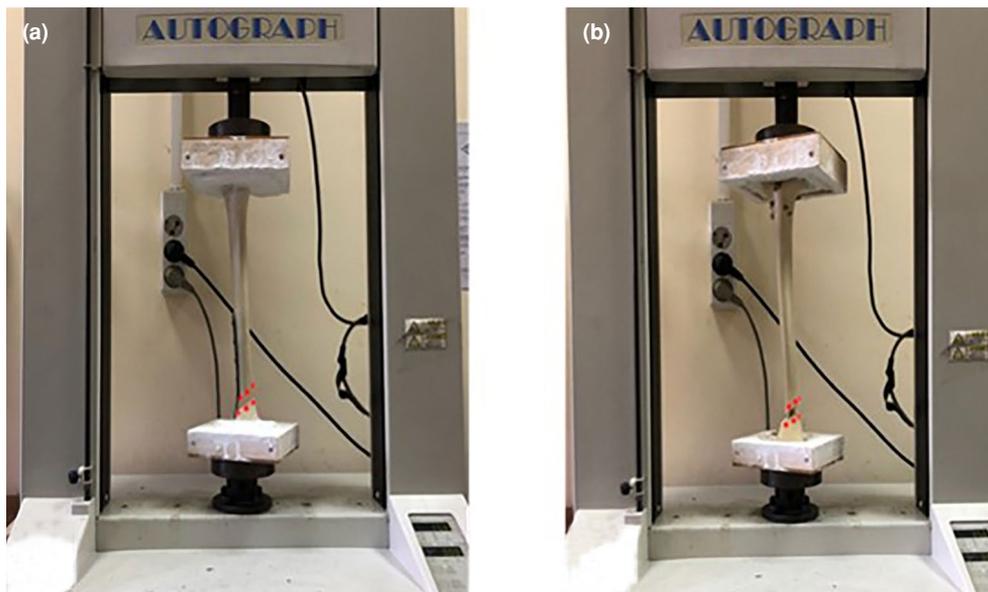
The proximal and distal ends of the models were secured with the anatomic polymethylmethacrylate (PMMA), which was fixed to the surface anatomies of the distal and proximal ends of the models to ensure identical alignment, loading points, and model orientation during all tests (Figure 2).

### Mechanical test

All bone models were tested using an axial loading test machine (Shimadzu Autograph, AGS-J 10kN, Shimadzu Corp., Tokyo, Japan) with a capacity of 10 kN to measure the fracture site displacement (mm) and stiffness (N/mm). All models were attached to PMMA template within the wooden box and axial load was applied through the distal



**FIGURE 1.** (a) Orthopedic Trauma Association type 43-A1.2 distal tibia fracture model with 10 mm gap was (b) fixed with medial anatomic plate-screws and (c) newly designed tibia intramedullary nail (Distal Bolt Locking - DSBLs).



**FIGURE 2.** (a) Axial loading was applied to the Plate group and (b) Nail Group for mechanical testing.

and proximal ends of the machine. The proximal and distal loading points were chosen to mimic the anatomical loading of the tibia during walking as described in the literature (Figure 2).<sup>[12-14]</sup> Six samples from each group were tested under axial

loading. Specimens were first preloaded to 50 N and then loaded at a rate of 1 mm/s. Fragments' movement was recorded by the testing machine, and the displacement of the markers at the fracture site was recorded by the video camera system.

### Digital image acquisition

The three-dimensional (3D) geometry of models developed in this study was obtained using 64-slice spiral computed tomography (CT) (Toshiba Aquilion 64-slice spiral, Toshiba, Tokyo, Japan). All CT image data of osteotomized composite tibia models were used to reconstruct the 3D geometry of digital counterparts. The slice thickness and interval of CT images were 1.0 mm with a resolution of  $256 \times 256$  pixel. Images from CT were saved in Digital Imaging and Communications in Medicine (DiCOM) format.

### Three-dimensional modelling

The DiCOM dataset of CT images was imported into Mimics version 21.0 software (Materialize HQ, Leuven, Belgium) for segmentation of the surface geometry of the proximal and distal parts of the osteotomized tibia. During the segmentation process, pixel values representing a radiodensity greater than 300 HU were assigned to the bone.<sup>[15]</sup> Thus, the 3D models of the bone were created and saved as an MCS file. The 3D volumetric models were created and exported as polygon file format (PLY) files into ANSYS version 2019 R1 (Ansys Inc., Canonsburg, PA, USA).

Distal tibia medial anatomic plate, nine plate screws, locking nail, three proximal nail screws, a Bolt screw, and distal nail fixator screw were created in SolidWorks version 2016 software (Dassault Systèmes, Waltham, MA, USA) by measuring the real implants with a digital caliper. Plate thickness was 4.0 mm. The diameter of plate screws was 3.5 mm. and the diameter of nail was 10 mm. The diameter of proximal nail screws was 5.5 mm. The diameter of thick medial side of the bolt screw was 10 mm. The diameter of thin lateral part of the bolt screw was 6 mm. The diameter of the fixator was 4.3 mm. The 3D data of implants were saved in a STEP file and exported into ANSYS.

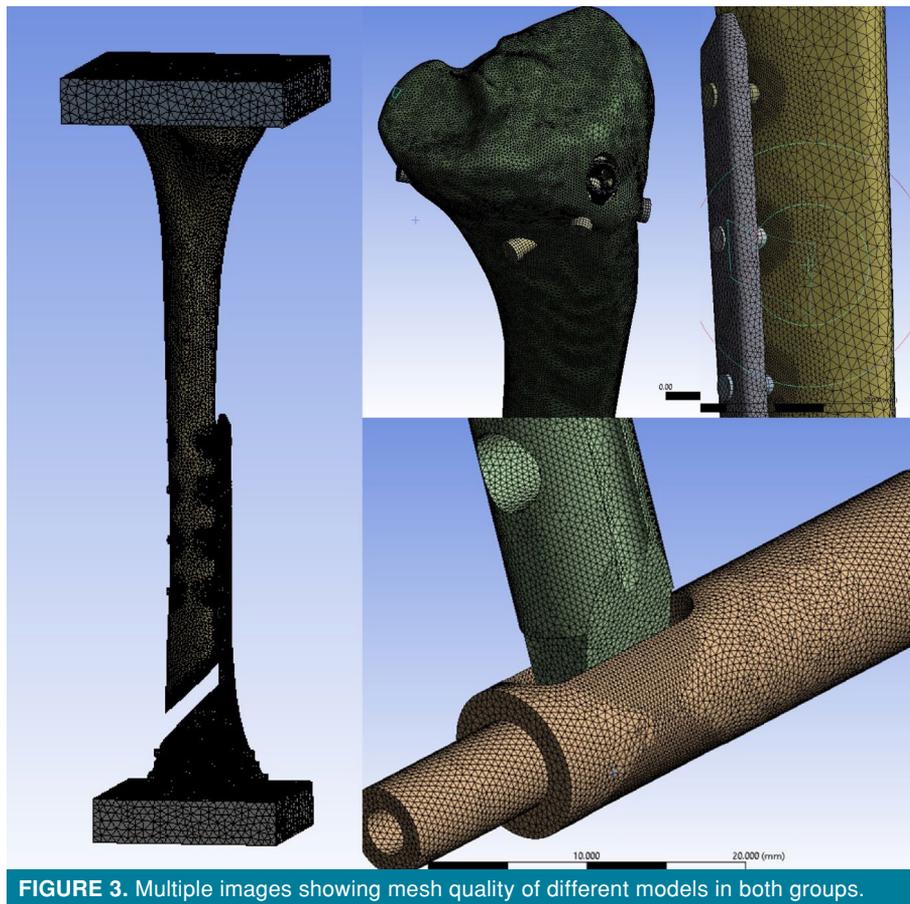
### Finite element modelling

We used two models of distal oblique tibia fracture osteosynthesis in this study. The first was a tibia fracture osteosynthesis with distal medial anatomical plate model and the second was distal tibia fracture with locking nail osteosynthesis model. Plate model was aligned with tibia model and screw models using SpaceClaim version 2019R2 software (Ansys Inc., Canonsburg, PA, USA). Four bone holes were created on the proximal tibia model and five bone holes on the distal tibia model, along the screw paths. The screw hole diameter was the

same as the screw diameter of 3.5 mm. This model was saved as an ANSYS file. The locking nail model was, then, aligned with tibia model, proximal nail screws, distal bolt screw, and fixator in SpaceClaim. Bone tunnel was created on proximal anterior nail entrance site of tibia model along the locking hole. The entrance diameter was 11 mm. Bone holes for proximal nail screws were, then, created along the screws. Proximal bone holes for proximal screws of locking nail were 5.5 mm in diameter. The bone hole for the bolt screw was created along the screw on distal tibia model. The diameter of the hole was 10 mm at the medial malleolus and 6 mm at the lateral side of the distal tibia model. The proximal template geometry was created by subtracting joint faces of proximal tibia from the box with dimensions of 4 cm in height, 14 cm in width and 16 cm in length in SpaceClaim. The distal template geometry was created by subtracting joint faces of distal tibia from the box with dimensions of 4 cm in height, 13 cm in width and 14 cm in length in SpaceClaim. All models were, then, remeshed with the ANSYS software. All models were discretized into 10-node quadratic tetrahedral elements. Tetrahedral elements were chosen over hexahedral elements because of their great flexibility in meshing complex curvilinear geometry. The total number of elements and nodes for the first group (plate model) was 541031 and 890149, for the second group (nail model), these values were 1324585 and 2091902, respectively. The element quality averages for the first and second groups were 0.8177 and 0.82357, respectively. These values revealed that the ANSYS analysis was extensively detailed (Figure 3).

### Material properties

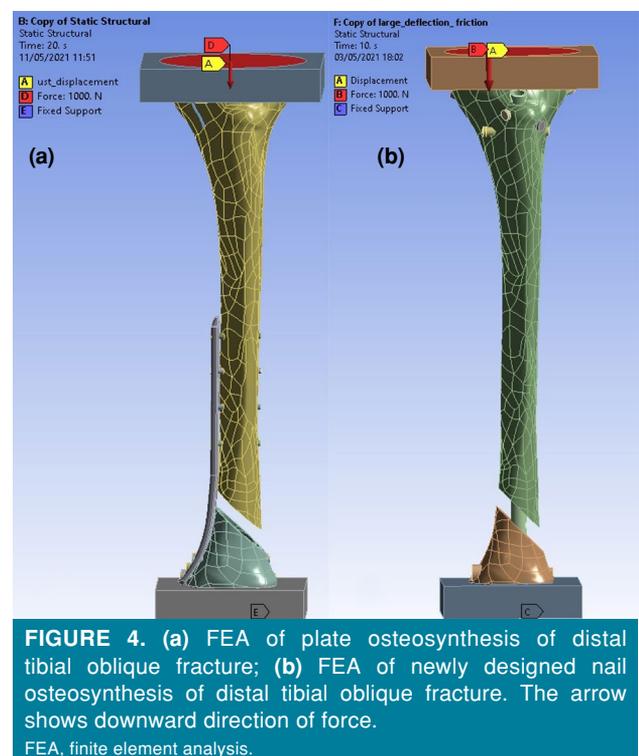
Bone material properties used in this study were determined according to the related literature. In several studies, the tibia bone was assumed as a rigid body.<sup>[16-18]</sup> In this study, tibia bone was defined as a linear elastic material with Young's modulus of 17 GPa for cortical bone and 5 GPa for cancellous bone. Poisson's ratio for both cortical and cancellous bones was 0.33.<sup>[19]</sup> A Young's modulus of 110 GPa and Poisson's ratio of 0.3 were set for the properties of Ti6Al4V titanium alloy that were assigned to the plate and nail models. A Young's modulus of 2.9 GPa and Poisson's ratio of 0.35 were set for the properties of PMMA that were assigned to the proximal and distal templates. All the materials, including the bone, metals and PMMA, were simplified as homogeneous, linear, and isotropic.



**FIGURE 3.** Multiple images showing mesh quality of different models in both groups.

### Boundary conditions and loads

In both groups the connection between templates and bones was considered as bonded constraints. In plate group, the connection between plate and screws was considered as bonded constraints. In plate group, the screws were connected as bonded constraint to proximal and distal parts of tibia bone. The screws were penetrated both cortices of the tibia. Contact between the plate and tibia bone was set as frictional constraint. In the nail group, connection between proximal and distal screws and bones was set as bonded constraint. In the nail group, the contact between screws and the nail was set as frictional constraint. The friction coefficient was set at a value of 0.64.<sup>[20]</sup> During simulation, translations of all implants and bones were free in all degrees of freedom, whereas translations of the proximal template were fixed in coronal and sagittal degrees of freedom and free in axial degree of freedom. Rotations of bones and implants were free in all degrees of freedom. In both groups distal template was fixed in all degrees of freedom. The 50N



**FIGURE 4.** (a) FEA of plate osteosynthesis of distal tibial oblique fracture; (b) FEA of newly designed nail osteosynthesis of distal tibial oblique fracture. The arrow shows downward direction of force. FEA, finite element analysis.

**TABLE I**  
Biomechanical results of the test samples

	1	2	3	4	5	6	1	2	3	4	5	6	p*
Force (mean ± SD)	1519.8 ± 239.4	1649.4 ± 314.1	1582.0 ± 167.4	1718.0 ± 276.8	1487.6 ± 198.4	1629.5 ± 201.8	1064.9 ± 474.9	695.5 ± 366.4	716.1 ± 371.1	938.8 ± 298.4	836.4 ± 302.1	732.5 ± 229.2	0.043
Force max. (N)	3611.5	3775.5	3954.5	3982.2	2919.5	3462.4	2056.5	1163.7	1126.9	1865.6	1596.5	1265.3	0.037
Fracture site displacement (mm) (mean ± SD)	5.80 ± 0.82	4.59 ± 0.91	6.16 ± 1.13	5.12 ± 0.73	3.45 ± 1.98	4.23 ± 1.28	8.65 ± 3.84	7.69 ± 4.44	7.74 ± 4.47	8.21 ± 3.95	7.81 ± 3.87	6.97 ± 4.12	0.021
Stiffness (N/mm) (mean ± SD)	362.34 ± 95.66	408.12 ± 105.1	286.60 ± 73.80	302.86 ± 78.23	375.74 ± 96.34	411.63 ± 99.41	133.53 ± 64.38	104.23 ± 45.32	94.16 ± 44.69	102.8 ± 52.41	115.59 ± 31.28	107.71 ± 43.29	0.039

SD, standard deviation; N, Newton; \* Mann-Whitney U test.

preload was applied at the top of proximal template in axial downward direction. For transparency of validation process, in the simulation, loading increased up to 1000 N for 20 sec according to mechanical test results (Figure 4).

**Statistical analysis**

Statistical analysis was performed using the IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were presented in mean ± standard deviation (SD) or median (min-max), where applicable. The Mann-Whitney U test was used to compare the biomechanical test results (force, maximum force, stroke, stiffness) between the two implants in the tibia fracture pattern. A p value of < 0.05 was considered statistically significant.

**RESULTS**

**Biomechanical test results**

The previously defined plate and nail groups were tested with axial loading, and some mechanical parameters were compared (Table I). According to the results, the mean value of the force was 830.74 ± 146.7 N in plate group and 1597.76 ± 85.54 N in nail group. The mean value of the maximum force 1512.42 N in plate group and 3617.6 N in nail group. Another parameter was fracture site displacement which was 7.85 ± 0.5 mm in plate group and 4.89 ± 1 mm in nail group. The mean value of the stiffness was 109.67 ± 13.6 N/mm in plate group and 357.88 ± 52.6 N/mm in nail group. The results of axial loading test results of two groups revealed that for distal tibia fracture, tibia nail which was locked with newly designed bolt screw has shown superiority against plate-screw fixation about force (1597.76 ± 85.54 vs. 830.74 ± 146.7 N), maximum force (3617.6 vs. 1512.42 N), fracture site displacement (4.89 ± 1 vs. 7.85 ± 0.5 mm) and stiffness (357.88 ± 52.6 vs. 109.67 ± 13.6 N/mm) (Table I).

**Stress and displacement values of Plate Group**

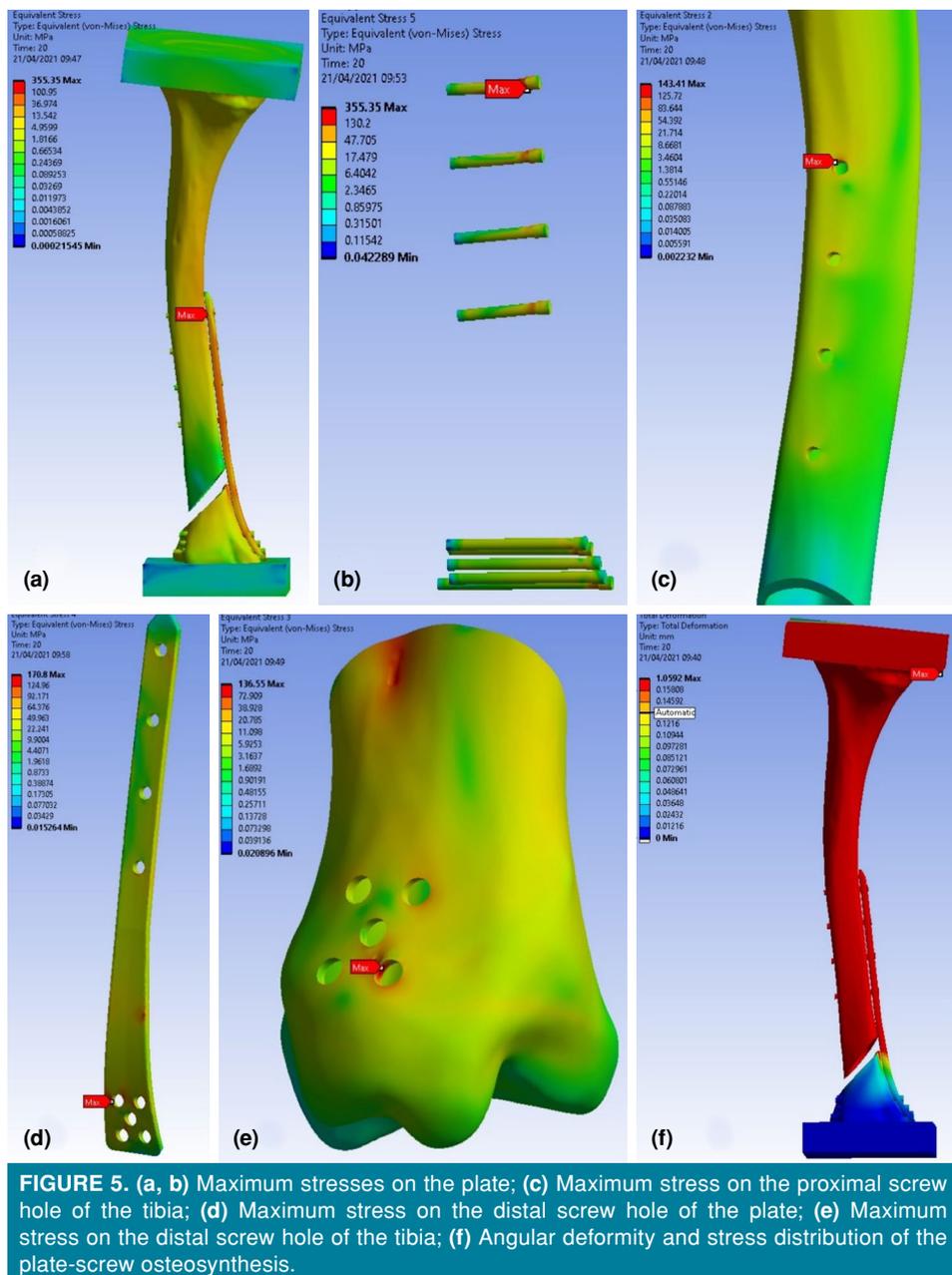
Maximum von Mises (MPa) stress of 355.35 in this group emerged at the junction point of most proximal screw with plate. Maximum von Mises stress at the proximal part of tibia bone was 143.41 MPa, which was seen in the screw hole of most proximal screw. Maximum von Mises stress at the plate was 170.8 MPa seen in the posterior screw hole of proximal row for distal bone fragment fixation. Maximum von Mises stress at the distal part of tibia bone of 136.55 MPa was seen in the posterior most distal screw hole. A maximum

displacement in this group of 1.06 mm was seen in the proximal part of tibia bone. The proximal part of tibia was angulated just proximal to the proximal end of plate, leaving the plate system on the concave site. The deformation appeared exactly in the same direction and extent as in mechanical testing.

**Stress and displacement values of Nail Group**

Maximum von Mises stress of 603.58 MPa in this group emerged at the junction point of the nail with its distal bolt screw. Maximum von Mises stress at the proximal part of tibia bone was 45.16 MPa

seen in most distal screw hole of proximal locking screws. Maximum von Mises stress at distal part of tibia bone was 52.06 MPa which seen in the screw hole on the lateral side of the bone, namely, the screw hole of thinned tip of distal bolt screw. The maximum von Mises stress of the proximal locking screws was 212.53 MPa, observed in the contact area of the screws with the nail. The maximum von Mises stress of the distal bolt screw was 542 MPa, which was seen in the area around the nail tip locking hole. Although the stress was high in this region, it was homogeneously distributed. The maximum



**FIGURE 5.** (a, b) Maximum stresses on the plate; (c) Maximum stress on the proximal screw hole of the tibia; (d) Maximum stress on the distal screw hole of the plate; (e) Maximum stress on the distal screw hole of the tibia; (f) Angular deformity and stress distribution of the plate-screw osteosynthesis.

von Mises stress of the distal bolt screw fixator was 574.46 MPa, observed in the contact area between the nail and the fixator. Maximum von Mises stress of nail was 603.58 MPa seen at the junction point of the nail with its distal bolt screw. A maximum displacement in this group of 0.49 mm was seen in the proximal part of tibia bone (Figures 4 and 5). No significant deformation was observed in any bone or implant in this group.

Comparing the maximum stress values in both implants, the maximum stress was found to be 1.7 times higher in the nail group. The maximum stress levels on the bone at the implant-bone junction points were compared between the two groups, it was seen that the maximum stress levels in the bones in the nail group were 2.75 times lower than in the plate group. Total displacement in the plate group was more than two-fold compared to the nail group.

## DISCUSSION

In the present study, in which two different fixation methods were compared in the distal tibia fracture model, and we chose a distal tibia valgus oblique fracture model to simulate a fracture that would cause stress on the implant with axial loading. Our study results revealed that the newly designed tibia IMN with distal bolt screw fixation was similar to medial plating and even superior in some results.

The plate-screw fixation of the distal tibia fractures has many advantages, such as direct visualization of the fracture line, ensure correction of the alignment and reduction, compressive fixation of the fragments etc. However, recently, many studies reported that plate fixation, especially in the medial side, may cause soft tissue problems.<sup>[21]</sup> While plate-screw systems are used as a standard for fixation in distal tibia fractures, after the development of nails providing distal multi-planar fixation, nails are also used in such fractures.<sup>[22]</sup> By this way, both plate-related complications were minimized, and a more stable fracture fixation was achieved. However, in cases of fractures resulting in malunion or nonunion, this has been reported as movement between fracture fragments due to inadequate fixation.<sup>[23]</sup> One of the main principles of preventing malunion or failure after fracture fixation is stability. Therefore, angled fixed lock systems have been developed to provide rigid fixation at the distal end of the nail in distal tibia fractures and to minimize movement in the fracture gap.<sup>[24]</sup> However, more distal fractures could not be fixed with this system

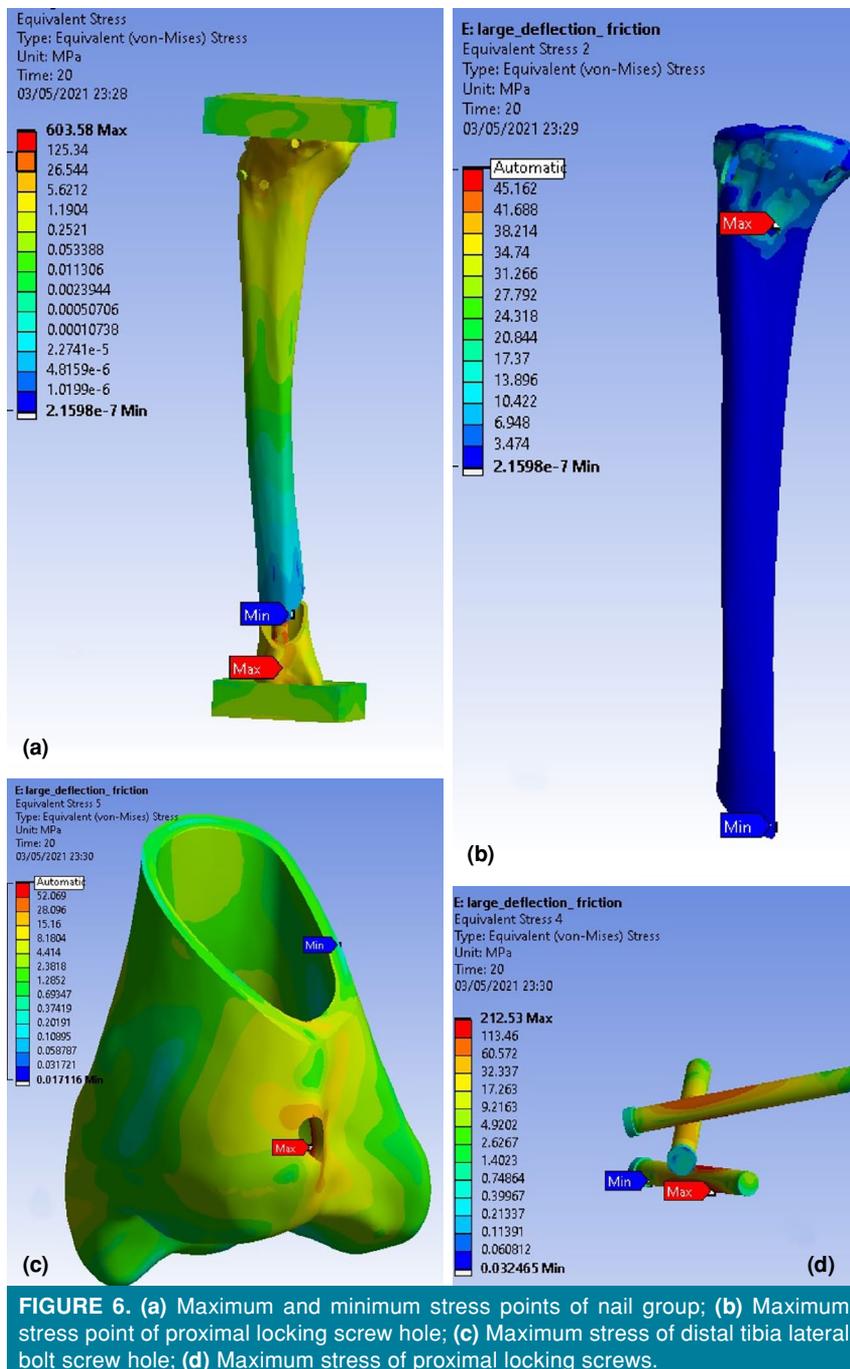
due to insufficient bone stock for implantation of distal screws. The new IMN system (TIN-DSBLS), which has a distal bolt screw and locking system, has a different design than the classical ones and has the claim of providing rigid fixation even in fracture that is 2.5 cm distant to the joint line, being a strong alternative to the plate screw system at this point.

Current studies have compared nail and plate-screw in terms of axial stiffness and interfragmentary movement in distal tibia metaphyseal fractures.<sup>[24-26]</sup> This is because callus formation during the secondary fracture healing process is mostly dependent on interfragmentary movement and structural stability. In the biomechanics and FEA study by Baseri et al.<sup>[27]</sup> showed that fixation of the tibia with the conventional nailing method will lead to a stiffer bone-implant construct compared to plate-screw fixation. They also found that the interfragmentary movement, which had a negative effect on the bone union process, was smaller in the bone-nail structure than in the bone-plate complex. However, some studies have shown that nail fixation for distal tibia extraarticular fracture has disadvantages. In fractures close to the distal tibia metaphyseal area, the mechanical load cannot be transferred from the nail to the cortical bone due to the weak contact between the nail and the cortical bone, and most of this load is carried by the nail and transferred to the distal screws. As a result, the distal screws are loaded more than their strength and the stability of the entire system is reduced. This situation may cause failure, malalignment and malunion.<sup>[28,29]</sup> According to the results of our biomechanical study comparing the TIN-DSBLS nail which is stated to provide more load transfer from the nail to the cortex in tibial distal metaphyseal fractures and the plate-screw combination, the new design nail showed superiority to plate-screw fixation in terms of strength, maximum strength, fracture site displacement and stiffness in fracture that is 2.5 cm distant to the joint line. In this context, the axial loading required for the deterioration of fracture fixation stability in TIN-DSBLS was found to be statistically higher than in the plate-screw system.

Despite maximum stress levels in implants were 1.7 time higher in nail group, bones were not exposed to higher levels of stress or deformation as in plate group. Maximum stress levels in bones of the nail group were 2.75 times lower than plate group. When the amount of displacement at the

fracture line was examined in both groups, the total displacement in the plate group was more than twice as much as in the nail group. In the current study, we did not calculate the stiffness of these two groups in FEA simulation, since our aim was to dive deep in the understanding the mechanism of fixation superiority of the nail system over the plate system. During the simulation process we discovered that the nail system makes more stress

on the implant and less stress on the bone (Figure 6). This is possible for two main reasons: First - no cantilever force is applied to the bone, as the nail passes through the mechanical axis of the tibia, the second reason - the elasticity of the titanium alloy is much higher than the bone; therefore, it can absorb a large amount of energy until it becomes plastic. There is another discovery about the higher amount of stress in the nail group. The reason of

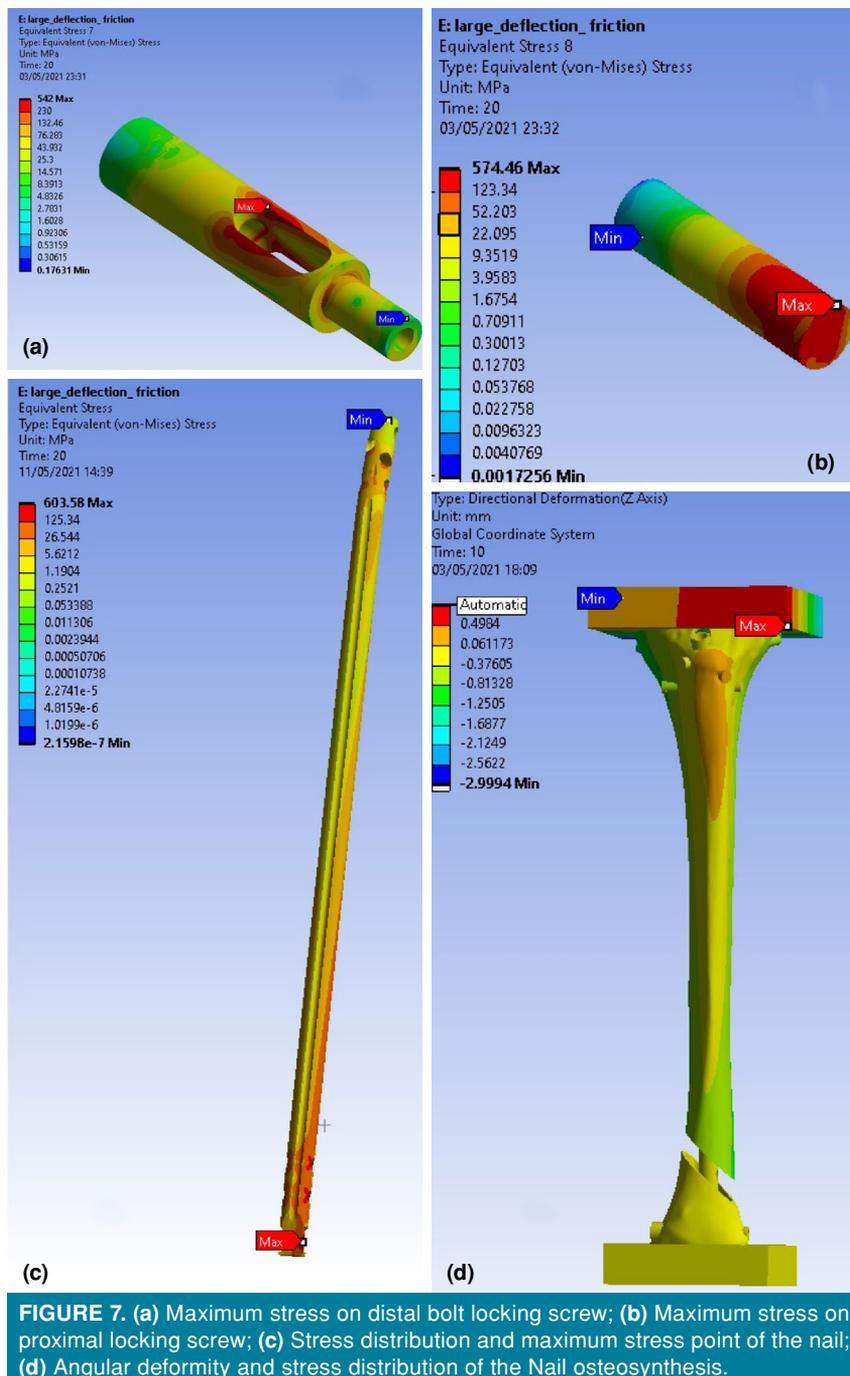


**FIGURE 6.** (a) Maximum and minimum stress points of nail group; (b) Maximum stress point of proximal locking screw hole; (c) Maximum stress of distal tibia lateral bolt screw hole; (d) Maximum stress of proximal locking screws.

high stress levels is the sharp-edged geometry of distal bolt screw nail sitting hole. We believe that if the hole edges could be rounded, the stress could be decreased either in the bolt screw and in the nail tip (Figure 7).

Nonetheless, this study has certain limitations and strengths. An inherent limitation to biomechanical studies is that biological processes cannot be fully simulated. The fact that the study was

conducted in a laboratory environment prevents the evaluation of the effects of non-implant factors such as the surgical environment, the surgeon's experience, and infection, which affect the success of such operations. Furthermore, the study examined the effect of axial loading on implants, and torsional and bending forces also have an effect on implant failure. Another limitation may be physicians' unfamiliarity with the FEA rules



and software technicians' inability to interpret such medical studies. In addition, obtaining consistent results in tests performed according to the sample size shows that the reliability of the test results is high. As a result, this study can be considered as a pilot study to investigate the usability of the new-generation implant (TIN-DSBLS) in complex distal tibia fractures that may push the limits in orthopedic surgery and to estimate its advantages and disadvantages compared to traditional plate-screw fixation.

In conclusion, thanks to technological developments, surgeries with high risk of complications are becoming more comfortable, safe and successful with new design implants in the field of orthopedics. At this point, this study determined that the TIN-DSBLS, designed for the purpose of fixation in complex distal tibia fractures, is biomechanically superior to the traditionally used tibia medial anatomical plate-screw system. The most realistic reason for this superiority is that it provides fixation in a larger area of the distal fragment and with more cortex contact. This study may encourage larger-scale studies to be conducted in the future.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** Conception and design of the study: O.K.U., M.Z.D., E.U., F.A.; Study selection, data extraction, and methodological quality assessment of each included study (performed independently): O.K.U., M.Z.D., T.N., C.D., E.U., F.A.; Manuscript preparation: O.K.U., M.Z.D., T.N.; Equal contribution: O.K.U., M.Z.D., T.N., C.D., E.U., F.A.; Final approval of the article: O.K.U., M.Z.D., T.N., C.D., E.U., F.A.

**Conflict of Interest:** The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

**Funding:** The authors received no financial support for the research and/or authorship of this article.

**AI Disclosure:** The authors declare that artificial intelligence (AI) tools were not used, or were used solely for language editing, and had no role in data analysis, interpretation, or the formulation of conclusions. All scientific content, data interpretation, and conclusions are the sole responsibility of the authors. The authors further confirm that AI tools were not used to generate, fabricate, or 'hallucinate' references, and that all references have been carefully verified for accuracy.

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