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Long-term outcome of total knee arthroplasty in patients with morbid obesity

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Abstract

Introduction Patients with morbid obesity and advanced painful knee osteoarthritis are considered as poor candidates for total knee replacement. Our aims were to evaluate the outcomes of TKR surgery and the risks for post-operative complications in patients with morbid obesity (BMI > 40 kg/m²) as compared with obese patients ($30 < BMI \le 40 \text{ kg/m}^2$) and non-obese patients, BMI < 30 kg/m²); to evaluate if there are differences between morbid-obese patients (BMI 40–49.99 kg/m²) and extreme morbid obese patients (BMI > 50 kg/m²); and to present some surgical tips which can improve the TKR outcomes in morbid obese patients.

Materials and methods There were successive 333 patients, of them 39 patients (11.7%) were lost for follow-up. So, this series included 292 patients -82 with bilateral TKR- and 374 TKR. The mean age was 64.3 years old (48–83 years) and the mean follow-up 10.8 years (4–17 years). The KSS and FKSS scores were calculated at the end of the follow-up period and compared to the pre-operative evaluation. Radiographic assessment at the end of follow-up included evaluation of implant position, alignment, and presence of radiolucent lines around the implants and was compared with the immediate post-operative radiographs. Statistical analysis was performed using SPSS v 22.0.

Results Our findings showed marked improvement following TKR of non-obese, obese, and morbid obese patients, regarding the KSS and FKSS. Significant change was observed between the non-obese and obese patients as compared to morbid obese patients. There were no significant differences between morbid obese patients with BMI > 40 versus those with BMI > 50. There was a slight increased risk of early complications following TKR in morbid obese patients such as skin necrosis and infection around the surgical incision.

Conclusions Marked improvement was observed in the three groups of patients after TKR, although non-obese and obese groups had better mean scores of KSS and FKSS than morbid obese patients. No significant differences were found within the morbid obese patients themselves. Therefore, we believe that morbid obese patients are appropriate candidates and can enjoy the benefits of total knee arthroplasty done with careful use of some surgical tips presented in our study.

Keywords Knee · Osteoarthritis · Total knee arthroplasty · Total knee replacement · Obesity · Morbid obesity

Introduction

Knee osteoarthritis (OA) is a leading cause of disability in the adult population [1]. Obesity is considered a significant risk

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as over-weight. According to the World Health Organization (WHO), patients with a BMI over 40 kg/m² are considered to be class III of obesity, also known as morbid obesity, and have significantly increased risk for developing immediate and late post-operative complications following TKR [9–12].

The aim of the present study is to evaluate the outcomes of TKR surgery and the risk for post-operative complications in patients with morbid obesity (BMI > 40 kg/m²) as compared with non-obese patient (BMI < 30 kg/m²) and obese patients ($30 < BMI \le 40 \text{ kg/m}^2$), to evaluate if there are differences between morbid-obese patients (BMI 40–49.99 kg/m²) and extreme morbid obese patients (BMI > 50 kg/m²) and to present our experience including some surgical tips which have been shown to improve the TKR outcomes in the super obese population.

Materials and methods

Clinical data

Following approval by the institutional review board (IRB), we retrospectively reviewed the prospective joint arthroplasty surgical database at the EMMS Hospital in Nazareth Israel for all consecutive patients who underwent a primary TKR performed by two experienced, high-volume orthopaedic surgeons from 1.1.95 to 31.7.12. The main indications for TKR were increased severe knee pain and decreased range of knee motion with progressive deterioration of the functional activity, without improvement following physiotherapy. Most of them were awakened by night pain and could walk only with the aid of a cane or crutches for a short distance and had a lot of difficulties in climbing steps. Inclusion criteria for this study were as follows: (1) patients with well-balanced hypertension or diabetes mellitus and other medical conditions, who received primary TKR for primary knee OA; (2) patients with BMI > 30 kg/m² on the day of surgery; and (3) patients with minimum follow-up of four years. Exclusion criteria were patients with secondary knee osteoarthritis as follows: (1) patients with post-traumatic knee OA, including previous fractures or dislocation, knee instability, and post-menisectomy; (2) patients with a history of various rheumatic diseases; and (3) patients with incomplete clinical or radiographic records.

Overall, there had been successive 333 patients—who had fitted the inclusion criteria, 93 of them with bilateral TKRS, with 426 TKR. A total of 39/333 patients (11.7%) were lost for follow-up, either died or were not found. This series included therefore 292/333 patients – 82 of them with bilateral TKR, altogether with 374/426 TKR (87.8%) as follows: 11 TKR out of 15 Pts (73.3%) with a follow-up of 13–17 years, 44 TKR out of 52 Pts (84.6%) with follow-up of 9–12 years, 88 TKR out of 102 Pts (86.2%) with follow-up of six to eight years, and 231 TKR out of 257 Pts (89.8%) with

follow-up of four to five years. The mean age of these patients at operation was 64.3 years old (varied from 48 to 83 years) and the mean follow-up time was 10.8 years (varied from 4 to 17 years). Informed consent was obtained from each patient included in the study. All patients were examined at the end of follow-up and had updated radiographs of their knees between one and two years prior to the end of the study. All data like pre-operative anamnesis, physical examination, radiographic evaluation, pre-operative BMI, KSS and FKSS, operative procedures, complications, and follow-up after operations were collected using the institutional chart reviews of all patients and were recorded on excel.

The pre-operative BMI values of these 292 patients were done with a BMI measuring weight scale and height (Fig. 1) and were 21-68.2 kg/m², as illustrated in Tables 1 and 2. Of them, 37/292 patients were non-obese (BMI < 30 kg/m^2) and served as a control group, and one of them had bilateral TKR, seven of them with well-balanced hypertension (18%) and six of them with well-balanced non-insulin diabetes mellitus (16.2%). The remaining 255/292 obese patients served as a study group and were divided to two cohorts as follows: cohort A included 128 obese patients with BMI between 30 and 39.9 kg/m^2 before surgery—46 of them had bilateral TKR and the mean follow-up was 10.6 ± 2.8 years. Twenty-nine of them had well-balanced hypertension (23%) and 31 of them had well-balanced non-insulin diabetes mellitus (24.2%). Cohort B included 128 patients with morbid obesity and BMI between 40 and 68.2 kg/m² before surgery—35 of them had bilateral TKR (Table 1) and the mean follow-up was 10.1 \pm 3.2 years. Age and follow-up were similar in both groups. Forty-eight of them had well-balanced hypertension (38%) and 47 of them had well-balanced diabetes mellitus (36.7%)-2 of them treated with insulin. Among the 128 patients of cohort B, there were 103 patients with morbid obesity with BMI between 40 and 49.99 kg/m² and another 25



Fig. 1 Measurement of BMI of a 62-year female with morbid obesity (BMI 52.7 $\rm kg/m^2)$

Table 1 BMI measurementsdone before 374 total kneereplacement in 292 patientsaccording to the different groups	BMI	21–29.99 kg/m ² Non-obese Pts	30–39.9 kg/m ² Obese Pts	40–68.2 kg/m ² Morbid Obesity
	No. Patients (total 292, of them 82 with bilateral TKR) No. of TKRs (total 374)	37 38	128 174	127 162

patients with morbid obesity with $BMI > 50 \text{ kg/m}^2$, as illustrated in Table 2.

All together in this series, 374 TKR were performed, 38 of them in the non-obese control group of patients (12 males, 25 females), 174 TKR in the first study group of the obese patients (58 males and 116 females), and 162 TKR in second study group of patients with extreme obesity (27 males and 147 females) as illustrated in Tables 1 and 2. All details of preoperative demographic parameters and Knee Society Scores (KSS) and Functional Knee Society Scores (FKSS) were recorded for all patients, using the hospital database. Intraoperative measurement and post-operative outcomes and complications during the hospital stay and follow-up period were examined. Data regarding intra-operative tourniquet time and surgical incision measurement were collected.

According to our measurements—in non-obese patients— 34% had valgus knees (mean 3.7°), 58% had varus knee (mean 6°), and 18% neutral alignment (°), in obese patients—38% had slight valgus knee (mean 3.9°), 53% had varus knee (mean 5.9°), and 9% had neutral alignment (0°), in morbid obese patients, of BMI 40–49.99—40% had valgus knees (mean 3.5°), 47% had varus knee (mean 9.6°), and 13% neutral alignment (°), and in extreme morbid obesity of BMI > 50, 53% had valgus knees (mean 3.8°), 37% had varus knee (mean 9.6°), and 10% neutral alignment (°).

Early post-operative complications as surgical site infection, skin necrosis around surgical wound, thromboembolic events, peripheral nerve injuries, and low haemoglobin levels necessitating blood transfusion were recorded. Late complications as late infections, knee manipulations, implant loosening, and revision for any reason were recorded. The KSS and FKSS scores were calculated at the end of the follow-up period and compared to the pre-operative evaluation. Radiographic assessment included evaluation of implant positioning and alignment on standing position of both knees)

Table 2BMI measurements, before surgery of 127 patients withmorbid obesity, 35 of them had bilateral TKR, with BMI between 40and 49.99 kg/m² and BMI > 50 kg/m² according to the different groupsof BMIs

BMI > 40 kg/m ²	40-49.99 kg/m ²	50–68.2 kg/m ²
Morbid obesity		
No. Patients (total 127, of them 35 with bilateral TKR)	103	24
No. of TKR (total 162)	137	25

and the presence of radiolucent lines around the implants suggesting the possibility of mechanical loosening, as compared with immediate post-operative radiographs.

Statistical analysis

All statistical analysis was performed using SPSS v 22.0 (SPSS Inc., Chicago, Illinois) by one of the authors (CM). To evaluate the difference between the cohorts regarding demographic data, KSS score, and complications rate, Chi square test, the Student's *t* test, and univariate analysis of variance (ANOVA) were used. 95% confidence interval (CI) and effect sizes (calculated using Cohen's D or partial Eta square) are presented. A *p* value < 0.05 was considered statistically significant. Power calculations revealed that in order to detect a medium-size difference (medium effect size) between the three groups, a sample size of 52 participants in each study group would provide power of .80 using a one-tailed alpha of .05.

Surgical technique

All operations were done under spinal and epidural anaesthesia using a tourniquet. Pre-operative antibiotic was applied 1 h before operation. Based on our experience there are some surgical principles that are used routinely during TKR in most patients, but should be done very carefully in patients with morbid obesity as follows: the incision should be planned and marked in knee extension and lateralized relative to the tibial tubercle. It should be done with the knee in flexion (90°- 110°). By flexing the knee, the skin and subcutaneous fat are gently retracted. The dissection must be continued directly and sharply with minimal damaging of the subcutaneous tissues until the level of the arthrotomy. In morbidly obese patients, incision and subcutaneous dissection while the knee is flexed prevent devascularization of skin flaps and decrease the risk for fat necrosis and infections. Lateralization of the skin incision decreases the size of the lateral flap, which has a limited blood supply and is believed to reduce the risk for skin infection and necrosis (Fig. 2).

Implant oversizing in TKR may lead to pain, limited range of motion, and poor knee scores and loosening too quickly and early failure [13]. In morbidly obese patients, an oversized implant will have no bone support in the peripheral zone and the enormous moment forces across the unsupported peripheral part of the implant. Multiple drilling on a sclerotic bone



Fig. 2 Demonstrating necrosis of the medial side of the lateral flap in a patient with extreme obesity $(BMI-52 \text{ kg/m}^2)$

(Fig. 3) during total knee arthroplasty improves the depth of cement penetration and reduces the appearance of radiolucent lines [14]. In morbidly obese patients, the sclerotic bone of tibia and patella is wide and thick; therefore, multiple drillings are necessary to ensure cement penetration and proper fixation of the implants to the bone. Patellar osteonecrosis was



Fig. 3 Demonstrating multiple drilling of sclerotic bone before insertion of the implant

reported as a potential complication following extensive lateral release in TKR [15, 16]. During lateral release, the superior lateral geniculate artery can be sacrificed [17, 18]. In morbidly obese patients, an effort should be made to preserve vascular supply as much as possible, and extensive patellar release may devascularize the anterior knee tissues resulting in fat and skin necrosis.

Results

As illustrated in Tables 1 and 2, there were in cohort A 128 obese patients ($30 < BMI \le 40 \text{ kg/m}^2$), who had 174 TKR (104 females and 70 males) with a mean BMI of $35.2 \pm 2.6 \text{ kg/m}^2$ on the day of surgery. The cohort B (BMI > 40 kg/m^2) included 127 morbid obese patients who had 162 TKR, in 96 females and 66 males, with a mean BMI of $46 \pm 4.9 \text{ kg/m}^2$ on the day of surgery.

As illustrated in Table 3, blood transfusion in PC-s was 2.74 in the non-obese group, 2.75 in cohort A, and 2.88 in cohort B (p = 0.276). The mean tourniquet time in the nonobese group was 81.1 ± 13.8 , in cohort A was 85 ± 13 minute, with no significant difference compared to the 86 ± 16 minute tourniquet time in cohort B (p = 0.97). The surgical incision in the morbidly obese cohort (B) was 19 ± 2.7 cm, significantly higher than the incision length of 17.4 ± 2.4 cm in cohort A (p = 0.001) and 16.8 cm in the non-obese group. During the early post-operative period, one patient of the non-obese group, two patients in cohort A, and four patients in cohort B suffered from superficial wound infection, which was treated by antibiotics and resolved completely. No cases of skin necrosis or nerve palsy were reported in cohort A. In cohort B, one patient suffered from skin necrosis around the surgical incision (Fig. 2) and was treated with surgical debridement and secondary closure. Also, one patient of the morbid obesity group suffered from transient peroneal nerve palsy. One patient in cohort A and three patients in cohort B sustained a venous thromboembolic event. One patient from cohort A and one patient from cohort B experienced limited range of motion and underwent subsequent knee manipulation under anaesthesia.

The late post-operative complications included patellar clunk syndrome in one patient of the non-obese group and in two patients in of cohort B. One patient from the non-obese group and one from cohort A had aseptic loosening (7 years and 5 years, respectively, after surgery) and underwent revision total knee arthroplasty. There were two patients that had aseptic revisions in cohort B (22 and 50 months after surgery). One patient from cohort B suffered from late deep infection (34 months after surgery) and was treated with two-stage revision surgery. The overall rate of complications was significantly higher in cohort B (p = 0.018).

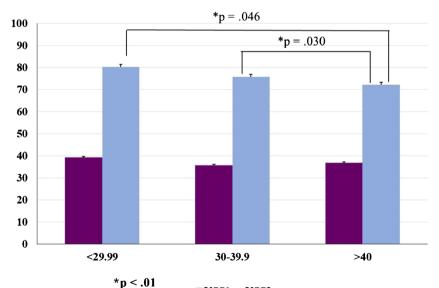
Table 3 Surgical details and post-op complications

		Non-obese-control BMI < 30 Pts37 (38 TKR)	Cohort A30 < BMI ≤ 40128 Pts (174 TKR)	Cohort B BMI > 40 128 Pts (162 TKR)	P value
Surgical details:					
Blood transfusion (PCs)	Mean	2.74	2.75	2.88	0.276
	SD	0.93	0.92	0.98	
Tourniquet time (minutes)	Mean	84.8	85.73	85.58	0.97
• · · ·	SD	13.8	13.5	15.7	
Surgical incision length (cm)	Mean	16.8	17.4	19	0.001
	SD	2.6	2.5	2.7	
Post-op complications:					
Superficial infection		1	2	4	
Skin necrosis		0	0	1	
Transient peroneal palsy		0	0	1	
Thromboembolic events		0	1	3	
Limited range of motion needed knee manipulation		1	1	1	
Patellar clunk syndrome		1	0	2	
Late deep infection		0	0	1	
Revision (5)					
Aseptic revision		1		2	
Septic revision		0	1	1	
Overall complications					0.018

At the end of the follow-up period, all groups of patients had a marked improvement of the Knee Society Score (KSS) following TKR as compared to the pre-op findings in non-obese patients from 43.41 ± 6.4 to 85.75 ± 5.52 , in obese patients from 45.3 ± 5.8 to

84.87 ± 5.9, and in patients with morbid obesity (BMI > 40) from 45.85 ± 4.26 to 83.77 ± 7.29 (Fig. 5). The improvement was higher in the non-obese patients as compared to the obese and patients with morbid obesity (*F* = 8.89, *p* < .001).

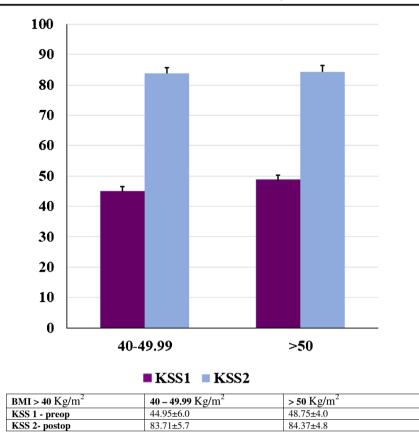
Fig. 4 Pre-operative KSS 1 and post-operative KSS 2 according to BMI, demonstrating significant changes between non-obese and obese patients to morbidly obese patients





BMI	< 29.99 Kg/m ²	30 – 39.9 Kg/m ²	> 40 Kg/m ²
KSS 1 - preop	43.41±6.4	45.30±5.8	45.85±4.3
KSS 2 - postop	85.75±5.5	84.87±5.9	83.77±7.3

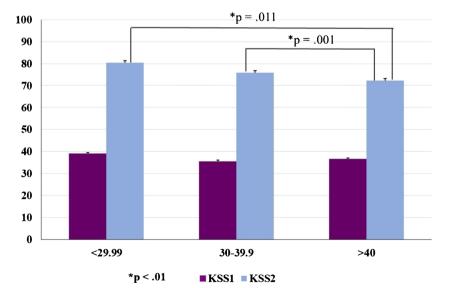
Fig. 5 Pre-operative KSS (1) and post-operative KSS (2) of obese patients with BMI > 40 kg/m², demonstrating none significant changes between Pts with BMI < 50 and over 50



Comparison of the post-op KSS scores between patients with BMI < 30, 30–40, and over 40 shows a significant difference ($F_{(2,270)} = 3.35$, p = .37, 95%CI = 82.67, 84.59), with a small effect size (partial eta square = 0.02). Post hoc pairwise comparison shows a significant difference only between the first two groups—non-obese and obese patients with BMI <

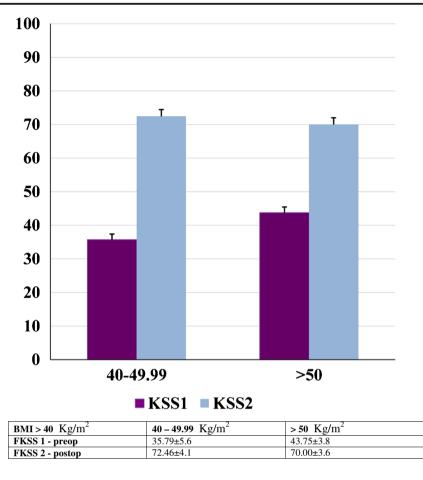
30, 30–40, as compared with the morbid obese with BMI > 40 (p = .046 and .030, respectively), without any significant differences between the first two groups themselves (p = .530). Splitting the morbid obese group into two groups (Fig. 6), BMI 40–49.9 and of over 50 indicates that mean post-op scores did not differ between the two groups ($F_{(1,120)} =$

Fig. 6 Pre-operative FKSS (1) and post-operative FKSS (2) according to BMI, demonstrating significant changes between nonobese and obese patients to morbidly obese patients



BMI	< 29.99 Kg/m ²	30 – 39.99 Kg/m ²	>40 Kg/m ²
FKSS 1- preop	39.16±6.8	35.63±5.1	36.69±4.0
FKSS2 - postop	80.33±5.7	75.77±5.5	72.18±5.1

Fig. 7 Pre-operative FKSS (1) and post-operative FKSS (2) of patients with morbid obesity according to BMI, demonstrating nonsignificant changes between Pts with BMI < 50 and over 50



0.79, p = .377, 95%CI = 79.83, 83.83, effect size = .007). The mean KSS score on the follow-up periods in the non-obese patients was 85.75 ± 5.5 and in cohort A (obese patients) was 84.87 ± 5.9 with a nonsignificant difference between themselves (p = 0.87, as compared to the 83.77 ± 8.2 KSS score in cohort B (Fig. 4)). Similar results with nonsignificant differences at the end of the study were observed in patients with morbid obesity in the groups with BMI from 40 to 49.88 and the patients with BMI over 50 (Fig. 5).

The FKSS scores showed similar tendency of marked improvement following operations in all groups of patients (Fig. 6). Comparison of the post-op functional KSS scores between patients with BMI < 30, 30–40, and over 40 shows a significant difference ($F_{(2,270)} = 7.12$, p = .001, 95%CI = 72.73, 82.60), with a medium effect size (partial eta square = 0.05). Post hoc pairwise comparison shows a significant difference only between the first two groups—non-obese and obese patients with BMI < 30 and BMI 30–40, as compared with the

Fig. 8 Radiographs of a 67-yearold female with BMI of 43 after 11 years with excellent results





Fig. 9 Radiograph of a 65-year-old male with BMI of 48 after 16 years with excellent results

morbid obese patients with BMI > 40 (p = .011 and .001, respectively). There were not any significant differences between the first two groups of non-obese and obese patients themselves (p = .700). Splitting the morbid obese group into two groups (Fig. 7), BMI 40–49.9 and BMI over 50 indicates that mean post-op scores did not differ between groups (F ($_{1,120} = 0.12$, p = .124, 95%CI = 65.54, 72.09, effect size = 0.02). On the whole, there were no marked changes between the different groups of obese patients, but we observed some FKSS better results for non-obese patients with BMI < 30 (Figs. 6 and 7). There was not any significant difference between the KSS and FKSS results between the groups of patients related to percentage of diabetes mellitus.

Based on our clinical experience in patients with bilateral knee osteoarthritis that need bilateral TKR, we recommend performance of the second TKR within six to nine months following the first TKR, and that in order to enable the best rehabilitation for such patients (Figs. 8 and 9).

Discussion

The findings of this study showed marked improvement of the non-obese, obese, and morbid obese patients, according to the KSSS and FKSS following TKR. There was significant change between the control non-obese (BMI < 30 kg/m²) and the obese patients (BMI $< 40 \text{ kg/m}^2$) as compared to the patients with morbid obesity with $BMI > 40 \text{ kg/m}^2$. However, there were not any significant changes between the different types of morbid obesity of patients with BMI 40-49.99 kg/m² as compared to those with BMI over 50 kg/m². It was also found that there is an increased risk for early complications following TKR in patients with morbid obesity (BMI > 40) as compared with obese patients (BMI < 40) and non-obese patients (BMI < 30). These complications are mainly related to skin necrosis and infection around the surgical incision. There were similar tourniquet time for both cohorts, but the surgical incision needed for TKR in patients with morbid obesity was significantly longer than that needed in non-obese and obese patients. Longer incision may be a co-factor that increases the risk for superficial infections.

The reports regarding the relationship between obesity and the outcome of TKR are inconsistent. Numerous studies have shown inferior results of knee scores in obese patients as compared with non-obese patients following TKR [11, 19]. Collins et al. in a long follow-up of 445 consecutive primary total knee replacements of nine years, based on evaluation of Knee Society Score, peri-operative complications, and implant survival, did not find differences in the overall complication rates or implant survival between non-obese and obese patients [20]. These authors described a small but significant adverse effect on clinical outcome, with highly obese patients showing lower function scores than non-obese patients. However, in this study, significant improvements in outcome are sustained in all groups 9 years after TKR with low perioperative complications and revision and the conclusion was that there is no reason to limit access to TKR in obese patients [20].

Similar results were also observed by other authors suggesting that obesity does not lead to poor results [21-27]. Wooten and Cutin suggested that patients with morbid obesity should optimize their condition prior to TKR [25]. Martin et al. in their review on morbid obesity and total knee arthroplasty supported this view and recommended pre-operative optimization of the nutritional status before TKR, such as safe weight loss strategies and if needed bariatric surgery, though it is difficult to determine if a weight limit should be enforced when evaluating candidacy for TKA 26]. Similar results to those of our study were also reported by Li et al. that found that six months after total joint replacement in 2040 patients who had undergone THR and 2964 who had undergone TKR, severely or morbidly obese patients reported excellent pain relief and substantial functional gain that was similar to the findings in other patients. While obesity is associated with a greater risk of early complications, obesity in itself should not be a deterrent to undergoing TJR to relieve symptoms [27-29]. However, our results are based on much longer follow-up of four to 17 years (mean 10.8 years). In cohort A, the mean follow-up was 10.6 (± 2.8) years and in cohort B 10.1 (± 3.2) years. Other papers that described similar results were also reported by Affatato et al. [29] and by Zingg et al. [30]. However, Girardi et al. describe that TKA and THA patients with higher BMI required significantly longer operation-related times and had higher to-tal length of hospital stay. Higher BMI patients also carried higher odds of readmissions within 30 days in both TKA and THA groups [31].

Based on our experience, there are some surgical principles that are used routinely during TKR in most patients, but should be done very carefully in patients with morbid obesity as follows: the incision in bone knee flexion (90° to 110°) with minimal subcutaneous dissection until the level of the arthrotomy. In morbidly obese patients, this may reduce the risk of devascularization of skin flaps, fat necrosis, and infections. Lateralization of skin incision relative to the tibial tubercle decreases the size of the lateral flap, which has a limited blood supply and may reduce the risk for skin infection and necrosis. Implant oversizing in TKR may lead to pain, limited range of motion, and poor knee scores [13]. In morbidly obese patients, an oversized implant will have no bone support in the peripheral zone and the enormous moment forces across the implant may lead to fast loosening and early failure. It is also advised to use multiple drilling on of the tibia and patellar sclerotic bones during TKR to ensure cement penetration and proper fixation of the implants to the bone. It is also suggested to perform minimal marginal patellar release in morbidly obese patients in order to preserve vascular supply and prevent patellar osteonecrosis and skin necrosis.

In conclusion, this study highlights a very important issue regarding knee arthroplasty, particularly with the epidemiologic changes of obesity and the growing rate of knee osteoarthritis in the morbidly obese population. Marked improvement of KSS and FKSS was found in the three groups of patients between pre- and post-op; however, the non-obese and obese groups had better mean scores of KSS and functional KSS than the morbid obese patients. No significant differences were found within the morbid obese patients themselves (BMI 40-49.99 to BMI 50-68.2). Therefore, we believe that the morbidly obese patients are appropriate candidates for TKR. Our data suggests that despite the increased risk for peri-operative complications after TKR in morbidly obese patients, the results are similar to those of obese patients. Therefore, we believe that morbidly obese patients can enjoy the benefits of total knee arthroplasty done with careful use of some surgical tips presented in our study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the institutional review boards of our hospital.

Informed consent Informed consent was obtained from all individual participants included in the study.

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