Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Retrospective Study to Establish Incidence of Peri-Implantitis in Elderly Patients

Namita Khandelwal

khandelwal@uchc.edu

University of Connecticut Health Center

Kruttika Bhuse

University of Connecticut Health Center

Article

Keywords:

Posted Date: June 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-5112623/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Implant placement is on the rise among the elderly population as the best replacement for natural teeth. With advances in augmentation and various implant placement techniques in severely atrophied jaws, medically stable patient can be a candidate for implant placement. As the number of implant placement increases, so does peri-implant disease. Undiagnosed periimplant disease can progress and lead to implant failure. There is insufficient data regarding the incidence of periimplantitis among the elderly hence the rate of incidence is currently unknown. Observing this lacuna in the literature, our study attempted to answer the question.

Our hypothesis is that peri-implantitis incidence in patients over 65 years of age is higher than the documented incidence in a mixed age population. The primary aim of the study is to establish incidence of peri-implantitis in elderly patients (> 65 years of age) enrolled in a dental school maintenance program. The secondary aim is to longitudinally evaluate peri-implant clinical and radiographic parameters.

DATA AVAILABILITY STATEMENT:
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Introduction

The elderly population has been growing in numbers globally due to increased life expectancy. The estimated rate of increase in the elderly population is predicted to be around 50 million annually. The elderly population, as defined by age > 65 years, is also estimated to double by the year 2050 [10]. In medically compromised patients there is an increased risk of implant loss or compromised peri-implant health [3]. However, multiple studies have shown successful implant restoration in elderly patients if their systemic health is under control [4, 11]. There are limited studies which focused on patients 65 years or older. Studies specifically designed for geriatric groups [1, 6, 9] have included younger participants which makes it difficult to deduce an age-related treatment effect of the implant placement.

The role of biofilm in etiology and progression of peri-implant disease is well-established. Maintenance visits help in keeping disease in check. In aging populations, reduced bone metabolism, weakened immune defenses, poly pharmacy, chronic systemic diseases, and reduced dexterity can play a role in compromising peri-implant health [14]. Implant maintenance every 6 months helps to maintain stable peri-implant health [12]. Studies reviewing compliance rates showed female and older patients reported having better compliance than male and younger patients [2]. Prevalence rate of peri-implant disease is estimated between 1–47% [4]. The range varies widely among studies in part due to various disease case definitions, different parameters evaluated, and variability in local and systemic factors.

Gender-related changes have been studied in limited capacity. A prospective study evaluated marginal bone changes in post-menopausal women [8]. The study concluded that women on hormone replacement therapy had 25% more peri-implant bone loss compared to 15% who were not on hormone replacement therapy. Whether systemic bone loss or use of anti-resorptive therapy has an influence on marginal bone levels changes is not yet determined. A retrospective cross-sectional study has identified implants to be successful in osteoporosis female patients [15]. No clear reference to age grouping was mentioned in either of the gender-related studies. There is limited research evaluating combination of these variables on success and survival of dental implants.

A cross-sectional study examined oral health disparities in elderly patients of low socioeconomic status and minority racial or ethnic groups [7]. The study demonstrated existence of oral health disparities in this group. Peri-implantitis management can place a financial burden on patients, and insurance coverage influences frequency of maintenance visits in patients from different socioeconomic levels. On evaluating the role of socioeconomic status in incidence of peri-implantitis, access to care in this vulnerable population should be addressed.

Our hypothesis is that peri-implantitis incidence in patients over 65 years of age is higher than the documented incidence in a mixed age population. The aim of the study was to establish incidence of peri-implantitis in elderly patients (> 65 years of age) enrolled in a dental school maintenance program. Multiple studies have evaluated bone loss in this population, but this is the first one which will attempt to establish a role of gender and maintenance compliance on incidence of peri-implantitis. The seconday aim was to longitudinally evaluate peri-implant clinical and radiographic parameters.

Methods

The retrospective study was carried out in accordance to the guidelines and regulations established by the Institutional Review Board of University of Connecticut School of Dental Medicine (UConn Health protocol number 20X-182-1from June 3, 2020). All patients consent to data collection as part of their treatment in UConn Health. Ethics approval was deemed un necessary according to national legislature .

Study Approval was exempted since the retrospective nature of the study .

Due to retrospective nature of the study Informed consent was exempted by 45 CFR 46.116(f) by the IRB.

Study Population

This was a single cohort electronic dental record analysis. Patients who were registered in University of Connecticut School of Dental Medicine's maintenance program were included in this longitudinal evaluation. Electronic Dental Records from AxiUm® and radiographs from MiPacs® were accessed for data collection. The data was collected for up to two years post-restoration.

Inclusion criteria

- Age 65yrs and above at the time of implant placement.
- Final restoration in function for a year.
- Implants restored between 2013–2016.
- Maintenance visit scheduled for at least a year until 2019.
- Clinical measurements completed every follow-up visit.
- Radiographs taken at baseline.

Exclusion criteria

- Missing clinical measurements
- Missing baseline radiograph
- Implants restored between 2017–2019

Data Collection

List of patients aged over 65 years and had implant restored between 2013–2016 was assimilated by authorized personnel. One investigator (KB) sorted through the records to ensure inclusion criteria was satisfied. Records were stored in locked room in an encrypted hard drive. Clinical measurements and radiographs at baseline and maintenance visits were compared. The type of radiographic exam was dictated by clinician preference and the number of implants

placed at the time. Panorex, bitewings, and periapical were acceptable forms of 2D radiographs. For standardization purpose only periapical and bitewings were utilized for crestal level bone changes.

Utilizing clinical and radiographic measurements, diagnosis of peri-implant health or peri-implantitis was established. The case definition from the Proceedings of 2017 World Workshop on Classification of Periodontal and Peri-implant disease was used. *Peri-implant health*: Clinically, lack of bleeding on probing, suppuration, probing pocket depths of ≤ 5.0 mm but can differ depending on height of the soft tissue. An increase in probing depth over time conflicts with health. Radiographically, absence of bone loss following remodeling not to be more than 2mm. *Peri-implantitis*: Increase in probing depth, profuse bleeding, suppuration, radiographs showing bone loss of at least 3mm. Due to heterogeneity in data collection by individuals at different levels of training, only 215 cases satisfied inclusion criteria out of 1250 charts reviewed. Missing data was attributed to limited understanding of periimplant diseases prior to 2017 Workshop and absence of standardized case definition for periimplant health and disease (Table 1).

Statistical Analysis

Power Analysis:

This study was powered to detect the association between gender and incidence of peri-implantitis. Assuming that the incidence in men is 10%, the expectation was to include 200 men and 200 women, which would allow detection of mean odds ratio of 1.33 using a two-sided test for 80% power at the 5% significance level. The mean detectable odds ratio ranges from 1.80 to 2.25 when the male incidence is in the range of 0.1 to 0.4. This power analysis was performed in R using the epiR package.

Statistical Methods:

The purpose of this study was to estimate the incidence of peri-implantitis and link it to the predictors including gender, insurance type and number of follow-up visits. The incidence of peri-implantitis was estimated during the study period and a shorter period. A descriptive analysis was conducted to summarize patient-level or implant-level variables and the survival outcome (yes/no). The implants that did not survive were compared with surviving implants for the variables above using Fisher's tests for exploratory purpose. p-values smaller than 5% were considered statistically significant.

Results

A total of 1250 charts were reviewed of which 125 patients satisfied the inclusion criteria. In total 215 implants were evaluated (Table 1). The small number of patients that qualified for the analysis does suggest possible bias. The results for the patient gender showed about 65% of implants were placed in males and about 35% in females. The highest percentage of implants were placed in Whites followed by Blacks. A higher number of implants were placed in patients with a history of poly pharmacy and patients in the ASA II category. Augmented sites were sites with previous or simultaneous bone grafting procedures done at implant site, or history of sinus augmentation. About 77% of implants were placed in non-augmented sites. Fifty percent of implants were placed to restore single sites with missing teeth. In the study, the highest number of implants were placed by residents (85%). Clinically the deepest were recorded to be 7mm (1%) and the shallow PD of 2mm was recorded for 45%. Six percent of the study population showed presence of BOP and suppuration. Radiographic evidence of crestal bone loss was recorded for 4% of the study population. 9 implants showed signs of peri-implantitis out of the 215 implants evaluated.

Table 1
Demographic data and characteristics of study population

		n=	%
Gender	**Male**	81	64.8
	Female	44	35.2
Race	**White**	163	75.8
	American Indian or Alaska Native	10	4.7
	Asian	9	4.2
	Black or African American	32	14.9
	Native Hawaiian and other Pacific Islander	1	.5
Medications/Medical History	**Single pharmacy**	32	14.9
	Polypharmacy	177	82.3
ASA	**I**	20	9.3
	II	179	83.3
	III	16	7.4
Augmentation	**No**	166	77.2
	Yes	49	22.8
Site placed	**Single**	107	49.8
	Partial	74	34.4
	Complete	34	15.8
Placed By	**Resident**	182	84.7
	Faculty	33	15.3
Current Probing Depth Average	**2mm**	97	45.1
	3mm	56	26.0
	4mm	36	16.7
	5mm	19	8.8
	6mm	5	2.3
	7mm	2	0.9
Current BOP/Suppuration	**Absent**	202	94.0
	Present	13	6.0
Current Bone loss	**Loss**	9	4.2
	Stable	206	95.8
Survival	**No**	9	4.2

	n=	%
Yes	206	95.8

Table 2
Correlation between various factors and implant survival

		Survival : No		Survival : Yes		P value
Variable		n	%	n	%	
Gender	**Male**	**7**	**4.7**	**143**	**95.3**	**0.593; NS**
	Female	**2**	**3.1**	**63**	**96.9**	
Race	**White**	**2**	**1.2**	**161**	**98.8**	**< 0.001****
	American Indian/ Alaska Native	**4**	**40.0**	**6**	**60.0**	
	Asian	**0**	**0.0**	**9**	**100.0**	
	Black/African American	**3**	**9.4**	**29**	**90.6**	
	Native Hawaiian/ other Pacific Islanders	**0**	**0.0**	**1**	**100.0**	
Medications	**Single**	**0**	**0.0**	**32**	**100.0**	**0.200; NS**
	Poly	**9**	**4.9**	**174**	**95.1**	
ASA Status	**Grade I**	**2**	**10.0**	**18**	**90.0**	**0.298; NS**
	Grade II	**7**	**3.9**	**172**	**96.1**	
	Grade III	**0**	**0.0**	**16**	**100.0**	
Augmentation	**No**	**7**	**4.2**	**159**	**95.8**	**0.967; NS**
	Yes	**2**	**4.1**	**47**	**95.9**	
Site	**Single**	**2**	**1.9**	**105**	**98.1**	**0.018***
	Partial	**7**	**9.5**	**67**	**90.5**	
	Complete	**0**	**0.0**	**34**	**100.0**	
Placed by	**Resident**	**4**	**2.2**	**178**	**97.8**	**0.001***
	Faculty	**5**	**15.2**	**28**	**84.8**	
Probing Depth (PD) (Baseline)	**2 mm**	**2**	**2.1**	**94**	**97.9**	**0.024***
	3 mm	**2**	**3.2**	**61**	**96.8**	
	4 mm	**1**	**3.0**	**32**	**97.0**	
	5 mm	**4**	**19.0**	**17**	**81.0**	
	6 mm	**0**	**0.0**	**1**	**100.0**	
	7 mm	**0**	**0.0**	**1**	**100.0**	
Current PD	**2 mm**	**0**	**0.0**	**68**	**100.0**	**< 0.001****
	3 mm	**0**	**0.0**	**57**	**100.0**	
	4 mm	**0**	**0.0**	**49**	**100.0**	
	5 mm	**0**	**0.0**	**30**	**100.0**	

	6 mm	**0**	**0.0**	**1**	**100.0**	
	7 mm	**2**	**66.7**	**1**	**33.3**	
	9 mm	**7**	**100.0**	**0**	**0.0**	
Current BOP/ Suppuration	**Absent**	**1**	**0.5**	**201**	**99.5**	**< 0.001****
	Present	**8**	**61.5**	**5**	**38.5**	
Baseline Bone Loss	**Loss**	**1**	**100.0**	**0**	**0.0**	**< 0.001****
	Stable	**8**	**3.7**	**206**	**96.3**	

Chi-Square Test: NS: p > 0.05; Not Significant; *p < 0.05; Significant; **p < 0.001; Highly significant

Based on the AAP 2017 classification, we know that clinical parameter of increase in the probing depths in conjunction with profuse bleeding represents peri-implant disease. As presented, the deeper the probing depth, the lower the survival, with depths of 7mm and 9mm showing the lowest survival ($p < 0.001$). Implants that presented with BOP and suppuration showed lower survival than those without ($p < 0.001$). Bone loss was present at 9 implants, and 206 implants had stable crestal bone levels. Of the 215 included 9 implants that presented with crestal bone loss were the implants that did not survive, showing a significant correlation between crestal bone loss and implant survival ($p < 0.001$) (Table 2).

Among the races, the American Indian/Alaska Native group showed lowest implant survival followed by the Black/African American group, with higher survival in the White and Asian groups ($p < 0.001$) (Table 2).

Number of implants placed ($p = 0.018$) and surgeon experience ($p = 0.001$) showed statistical significance for implant survival. Based on the sites at which the implants were placed, implants placed to rehabilitate a partial site showed lower survival than complete and single replacements ($p = 0.018$). The implants placed by residents had higher survival rates than the implants placed by faculty ($p = 0.001$) (Table 2).

Survival amongst gender, ASA status, single versus poly pharmacy, and augmentation at the site of implant placement did not show any statistical significance.

Discussion

Implant placement is on the rise among the elderly population as the best replacement for natural teeth. Implant supported dentures have given the finest experience of fixed or removal dentures to the elderly population [9]. Due to the recent advancement in implant procedures, such as various supporting treatment methods including sinus lift, ridge augmentation, bone grafting, platelet rich plasma, and bone morphogenetic protein, there is now better placement and success of placed implants. Peri-implantitis as one of the significant reasons for implant failure is a matter of concern. There is insufficient data regarding the incidence of peri-implantitis, especially among the elderly population. Observing this lacuna in the literature, our study was the first planned to analyze the incidence of peri-implantitis among individuals above 65 years of age.

The survival rate in our study was 95.81%. Similarly, in a study by Becker et.al, 15 males with mean age 84 years and 16 females with mean age 83 years who received dental implants had excellent implant survival rates and low periodontal disease index scores with minimal changes in interproximal bone levels. Results from this study indicate that older

patients in reasonably good health may have high implant survival rates and may maintain good oral health and quality of life scores [1].

In our study, American Indians/Alaska Natives had the highest implant failures in the total study population, followed by Black or African Americans, which were significantly higher than in the other races ($p < 0.001$). Oral health disparities persist in older adults living in poverty and among those from minority racial/ethnic groups, with Black older adults found to have lower self-rated oral health and fewer teeth [7]. Our findings are consistent with this prior study. Our study contributes new findings on the American Indian/Alaska Native population over 65 years of age; few studies have described oral health disparities in this group.

Provider experience has been shown to be a significant factor in implant survival in some cases. A meta-analysis found that surgeon experience did not significantly affect implant failure, but when considering surgeons who have placed over 50 implants, there was significantly higher survival than with less experienced surgeons [13]. In our findings residents had higher implant survival but did not include data on specific experience with implants and level of training in the program at the time of placement. It will be helpful to evaluate implant survival to the type of restoration placed and the experience of restorative dentist.

A systematic review by Derks and Tomasi in 2015 found the prevalence of peri-implantitis ranged from 1 to 47%, [4] and peri-implantitis was 38.2% (Mean age of 59.5 years) [16]. Studies have reported that 41.8% and 37.2% of the implants of the affected patients presented with peri-implantitis [17, 18]. Similarly, a prospective study reported that the extent of moderate/severe peri-implantitis (bone loss > 2 mm) as 40.1% [4]. Three studies used assessments of bone levels including different thresholds reported a prevalence of peri-implantitis ranging from 12 to 37% [17, 19, 20]. There was a negative relationship between prevalence of peri-implantitis and threshold for bone loss. Implant loss was reported in five of the included studies and ranged from 0 to 13.6% at the patient level and from 0 to 8.3% at the implant level [4].

Patient centered risk assessment included smoking, number of dental implants ≥ 4, >25% FMBS and ≤ 2 frequency of maintenance was significantly associated with peri-implantitis [3, 16]. The most important risk indicators for peri-implantitis were plaque, PPD ≥ 4 mm, absence of at least 1 mm of KT at the implant level, and number of implants and BOP > 25% at the patient level [16]. Peri-implantitis is associated with younger ages and diabetes at the time of placement and with periodontal status at the time of follow-up [3].

A study by Koszuta group concluded that the hormone replacement therapy contributes to a greater peri-implant bone loss due to greater risk of osseointegration failure, whereas patients' age, gender and menopausal status did not significantly affect the implantation procedure success rate or bone loss [8]. Our study also found no association between gender and implantation success rates. The older 50% of patients in a study showed a decreased mortality as compared to normal population. The few complications in relation to implant failure and/or obvious bone loss could be associated with both local impact factors (surface, implant site, surgical protocol) as well as to host response factors and systemic health [5].

The strength of our study was that it was limited to the population age group above 65 years old and required maintenance visits for study inclusion. The limitation of our study was that it was conducted in a single institution within a limited demographic population. Though there is a potential risk of selection bias, this is the first study utilizing standardized case definition in an age group that can potentially present with predisposing conditions for periimplantitis. The data collection methods are also a potential limitation as it could have created discrepancies in our findings. Future studies should have a longitudinal assessment to establish incidence rate of periimplantitis in this cohort.

Conclusions

This study is the first one to use the new 2017 AAP guidelines to standardize the criteria for implant survival. The American Indian/Alaska Natives had the lowest implant survival percentage, followed by the Black/African American population. Clinical parameters such as increased probing depth, presence of bleeding on probing, and suppuration were shown to have highly significant associations with lower implant survival. The study showed an overall 95.8% implant survival rate for population aged 65 years and older.

Declarations

Author & Contributions.

The authors contributed.
tothestudydesign,dataacquisition,statisticalanalysesandinterpretation,anddraftedandrevisedthemanuscript.

Declaration ofConflictingInterests.

The authors declared. no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding.

The authors received. nofinancialsupportfortheresearch,authorship,and/orpublicationofthisarticle.

Author Contribution

N.K and K.B wrote the main manuscript text and prepared all figures. N.K is the primary advisor for the research.K.B worked on data collection and statistical analysis.All authors reviewed the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Becker, W., Hujoel, P., Becker, B. E. & Wohrle, P. Dental Implants in an Aged Population: Evaluation of Periodontal Health, Bone Loss, Implant Survival, and Quality of Life. *Clin. Implant Dent. Relat. Res.* **18** (3), 473–479. 10.1111/CID.12340 (2016).
2. de Carvalho, V. F. et al. Compliance improvement in periodontal maintenance. *J. Appl. Oral Sci.* **18** (3), 215. 10.1590/S1678-77572010000300003 (2010). /pmc/articles/PMC5349053/
3. Curtis, D. et al. Patient-Centered Risk Assessment in Implant Treatment Planning. *Int. J. Oral Maxillofac. Implants*. **34** (2), 506–520. 10.11607/JOMI.7025 (2019).
4. Derks, J. & Tomasi, C. Peri-implant health and disease. A systematic review of current epidemiology. *J. Clin. Periodontol*. **42** (S16), S158–S171. 10.1111/JCPE.12334 (2015).
5. Friberg, B. & Jemt, T. Rehabilitation of edentulous mandibles by means of osseointegrated implants: a 5-year follow-up study on one or two-stage surgery, number of implants, implant surfaces, and age at surgery. *Clin. Implant Dent. Relat. Res.* **17** (3), 413–424. 10.1111/CID.12116 (2015).
6. Hoeksema, A. R., Visser, A., Raghoebar, G. M., Vissink, A. & Meijer, H. J. A. Influence of Age on Clinical Performance of Mandibular Two-Implant Overdentures: A 10-Year Prospective Comparative Study. *Clin. Implant Dent. Relat. Res.*

18 (4), 745–751. 10.1111/CID.12351 (2016).

7. Huang, D. L. & Park, M. Socioeconomic and racial/ethnic oral health disparities among US older adults: oral health quality of life and dentition. *J. Public. Health Dent.* **75** (2), 85–92. 10.1111/JPHD.12072 (2015).
8. Koszuta, P., Grafka, A., Koszuta, A., Łopucki, M. & Szymańska, J. Effects of selected factors on the osseointegration of dental implants. *Prz Menopauzalny*. **14** (3), 184–187. 10.5114/pm.2015.54343 (2015). Epub 2015 Sep 30. PMID: 26528107; PMCID: PMC4612555.
9. Mueller, F. et al. Implant- supported mandibular overdentures in very old adults: a randomized controlled trial. *J. Dent. Res.* **92**, 154S–160S (2013).
10. Ortman, J. M., Velkoff, V. A. & Hogan, H. AnAgingNation:TheOlderPopulationintheUnitedStatesPopulationEstimatesandProjectionsCurrentPopulationReports. (2014).
11. Pang, J. K. et al. Predicting peri-implant disease: Chi-square automatic interaction detection (CHAID) decision tree analysis of risk indicators. *J. Periodontol*. **90** (8), 834–846. 10.1002/JPER.17-0501 (2019).
12. Renvert, S., Hirooka, H., Polyzois, I., Kelekis-Cholakis, A. & Wang, H. L. Diagnosis and non-surgical treatment of peri-implant diseases and maintenance care of patients with dental implants - Consensus report of working group 3. *Int. Dent. J. 69 Suppl.* (2(Suppl 2), 12–17. 10.1111/IDJ.12490 (2019).
13. Sendyk, D. I., Chrcanovic, B. R., Albrektsson, T., Wennerberg, A. & Zindel Deboni, M. C. Does surgical experience influence implant survival rate? A systematic review and meta-analysis. *Int. J. Prosthodont.* **30** (30), 341–347. 10.11607/ijp.5211 (2019). https://pubmed.ncbi.nlm.nih.gov/28697203/
14. Srinivasan, M., Meyer, S., Mombelli, A. & Müller, F. Dental implants in the elderly population: a systematic review and meta-analysis. *Clin. Oral Implants Res.* **28** (8), 920–930. 10.1111/CLR.12898 (2017). https://pubmed.ncbi.nlm.nih.gov/27273468/
15. Wagner, F. et al. Does osteoporosis influence the marginal peri-implant bone level in female patients? A cross-sectional study in a matched collective. *Clin. Implant Dent. Relat. Res.* **19** (4), 616–623. 10.1111/CID.12493 (2017).
16. Vignoletti, F., Di Domenico, G. L., Di Martino, M., Montero, E. & de Sanctis, M. Prevalenceandriskindicatorsofperi-implantitisinasampleofuniversity-baseddentalpatientsinItaly:Across-sectionalstudy.JClinPeriodontol.;46(5):597–605. (2019). 10.1111/jcpe.13111.PMID:30980410.
17. Mir-Mari, J., Mir-Orfila, P., Figueiredo, R., Valmaseda-Castellón, E. & Gay-Escoda, C. Prevalenceofperi-implantdiseases.Across-sectionalstudybasedonaprivatepracticeenvironment.JClinPeriodontol.;39(5):490-4. (2012). 10.1111/j.1600-051X.2012.01872.x.PMID:22486273.
18. Fransson, C., Wennström, J. & Berglundh, T. Clinicalcharacteristicsatimplantswithahistoryofprogressiveboneloss.ClinOralImplantsRes.;19(2):142-7. (2008). 10.1111/j.1600-0501.2007.01448.x.PMID:18184340.
19. Máximo, M. B. et al. Peri-implant diseases may be associated with increased time loading and generalized periodontal bone loss: preliminary results. *J. Oral Implantol*. **34** (5), 268–273. 10.1563/1548-1336(2008)34[(2008). 269:PDMBAW]2.0.CO;2. PMID: 19170293.
20. Marrone, A., Lasserre, J., Bercy, P. & Brecx, M. C. Prevalenceandriskfactorsforperi-implantdiseaseinBelgianadults.ClinOralImplantsRes.;24(8):934 – 40. (2013). 10.1111/j.1600-0501.2012.02476.x.Epub2012May3.PMID:22551347.

Figures

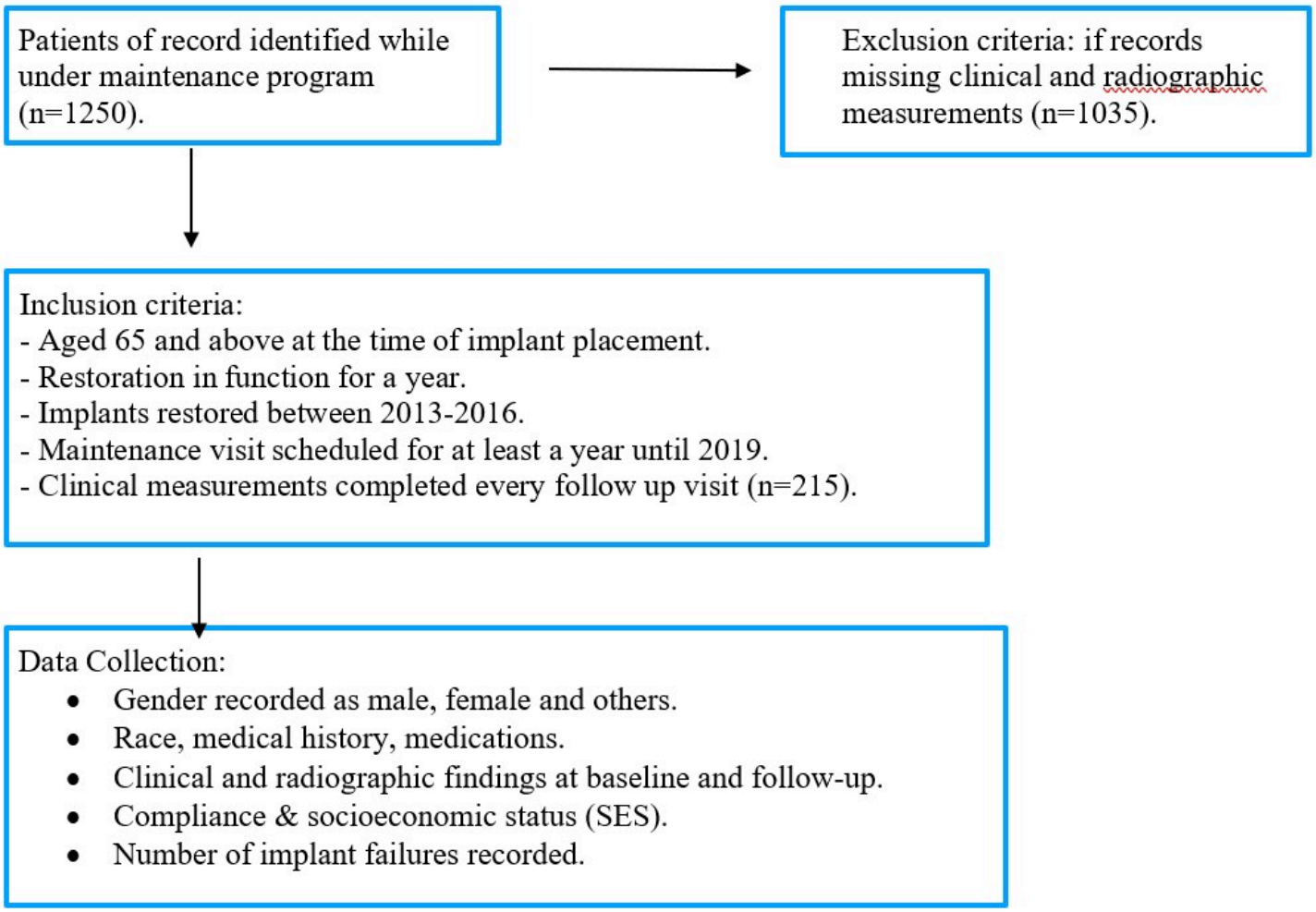


Figure 1

Unnumbered image in the **Methods** section.