

# EVALUATION OF PAIN PERCEPTION ON PERIODONTAL PROBING IN PATIENTS WITH DENTAL PLAQUE INDUCED GINGIVITIS AND CHRONIC PERIODONTITIS - A CROSS SECTIONAL STUDY, PART I.

Evaluación de la percepción del dolor al sondaje periodontal en pacientes con gingivitis inducida por placa dental y periodontitis crónica: un estudio transversal, Parte I.

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#### ABSTRACT:

**Background:** Probing of periodontal pockets is an essential part in the diagnosis of periodontal disease. Fifteen to seventy seven percent of untreated periodontal patients experience pain during probing. Hence the aim of this study is to evaluate the pain perceived by patients with gingivitis and periodontitis during periodontal probing. The goals of this study were to compare the patients' pain perception when using a conventional UNC15 probe and a manual pressure sensitive periodontal probe, and to relate the clinical features of gingivitis and periodontitis to the discomfort associated with periodontal probing.

**Material and Methods:** A total of 475 subjects were recruited into the study. The subjects were initially divided into two groups – Group – A (Gingivitis group - 275 patients) and Group – B (Chronic Periodontitis group -200 patients) according to the AAP 1999 Classification. These two groups were further subdivided into two groups each (Gingivitis – Conventional Probe – GCP, Gingivitis – Manual Pressure Sensitive Probe – GMPS, Periodontitis - Conventional Probe – PCP, Periodontitis – Manual Pressure Sensitive Probe – PMPS) using a computer generated program of random numbers.

**Results:** A significant difference was noted in pain perception when pressure sensitive probe was used compared to conventional UNC-15 probe. Reduced Bleeding on Probing and Pain scores were noted in Chronic periodontitis subjects with use of pressure sensitive probe, which was statistically significant ( $p < 0.001$ ).

**Conclusion:** Dentistry has changed its focus towards painless dentistry. In this context, the present study presents data towards use of manual pressure sensitive probes, which offers an advantage of low cost when compared to more advanced computerized systems with reduced pain during periodontal examination. It could result in a positive attitude of the patients towards continuous supportive periodontal therapy thereby monitoring periodontal health.

#### KEYWORDS:

*Periodontitis; gingivitis; pain; chronic periodontitis; pain perception; periodontics.*

## RESUMEN:

**Antecedentes:** El sondaje de los sacos periodontales es una parte esencial en el diagnóstico de la enfermedad periodontal. Del quince al setenta y siete por ciento de los pacientes periodontales no tratados experimentan dolor durante el sondaje. De ahí que el objetivo de este estudio fué evaluar el dolor percibido por pacientes con gingivitis y periodontitis durante el sondaje periodontal. Los objetivos de este estudio fueron comparar la percepción del dolor de los pacientes al usar una sonda UNC15 convencional y una sonda periodontal sensible a la presión manual, y relacionar las características clínicas de la gingivitis y la periodontitis con la incomodidad asociada con el sondaje periodontal.

**Material y Métodos:** Un total de 475 sujetos fueron reclutados en el estudio. Los sujetos se dividieron inicialmente en dos grupos - Grupo - A (grupo de Gingivitis - 275 pacientes) y Grupo - B (grupo de Periodontitis Crónica - 200 pacientes) de acuerdo con la Clasificación AAP 1999. Estos dos grupos se subdividieron en dos grupos cada uno (Gingivitis - Sonda convencional - GCP, Gingivitis - Sonda

manual sensible a la presión - GMPS, Periodontitis - Sonda convencional - PCP, Periodontitis - Sonda manual sensible a la presión - PMPS) usando un programa generado por computadora de datos aleatorios. números.

**Resultados:** Se notó una diferencia significativa en la percepción del dolor cuando se usó una sonda sensible a la presión en comparación con la sonda UNC-15 convencional ( $p < 0,001$ ).

**Conclusion:** La odontología ha cambiado su enfoque hacia una odontología sin dolor. En este contexto, el presente estudio presenta datos hacia el uso de sondas manuales sensibles a la presión, que ofrece una ventaja de bajo costo en comparación con sistemas computarizados más avanzados con reducción del dolor durante el examen periodontal. Podría resultar en una actitud positiva de los pacientes hacia la terapia periodontal de apoyo continuo, monitoreando así la salud periodontal.

## PALABRAS CLAVE:

*Periodontitis; gingivitis; dolor; periodontitis crónica; percepción del dolor; periodoncia.*

## INTRODUCTION.

Fear of pain is one of the factors for patients that prevents them from approaching the dentist. Periodontal examination and records are a mandatory prerequisite for all patients who come to the dental office to assess the status of periodontal disease.

Pain during probing for periodontal examination has become a concern for both the patient and the dentist. Methods to reduce pain in order to create a positive attitude among patients are of greatest concern among the dental professional community. Periodontal diseases can be classified as the afflictions of the gingiva, (*i.e.*, gingivitis) and the underlying tissues of the periodontium, (*i.e.*, periodontitis).<sup>1</sup>

Dental plaque induced gingivitis is an inflammatory response of the gingival tissues resulting from bacterial plaque accumulation located at and below the gingival margin.<sup>2</sup> Patients may notice symptoms that include bleeding while tooth brushing, blood in saliva, gingival swelling, redness and halitosis in the case of established forms of gingival and periodontal diseases.<sup>3</sup>

The intensity of the clinical signs and symptoms will vary among individuals<sup>4</sup> as well as among sites within a dentition. The common clinical signs of Dental plaque-induced gingivitis include erythema, edema, bleeding, tenderness, and enlargement.<sup>5,6</sup> Gingival inflammation is associated with progression to periodontitis.<sup>7-12</sup> However, the presence of gingival

inflammation does not mean that all affected sites are destined to progress to destructive forms of periodontal disease.<sup>10,11</sup>

Monitoring health or inflammation of gingival tissues is best documented by the clinical parameter of bleeding on probing (BOP).<sup>13</sup> Moreover BOP is considered as the earliest clinical sign of gingival inflammation.<sup>14</sup>

Bleeding on probing can be measured as bleeding provoked by applying a probe to the bottom of a gingival sulcus/pocket.<sup>14</sup> Various factors, such as probe dimension, angulation of probe and applied pressure, affect the probing based assessment of gingival inflammation. This led to the standardization of probing force to preferably not exceed 0.25 N.<sup>14</sup> Periodontal disease is the host response, an inflammatory response to the plaque and bacteria inside the pocket. Periodontitis is defined as an inflammatory disease of the supporting tissues around the teeth, which can cause irreversible loss of periodontal ligament, alveolar bone, and ultimately, if left untreated, tooth exfoliation.<sup>15</sup>

The periodontal pocket is the cardinal sign of periodontitis.<sup>1</sup> It is a pathologic fissure between tooth and sulcular or pocket epithelium, limited at its base by the junctional epithelium. It is an abnormal apical extension of the gingival sulcus caused by an extension of the junctional epithelium along the root surface and formation of a pocket epithelium as the periodontal ligament is detached and destroyed by the disease process.<sup>16</sup>

The accurate measurement of periodontal pockets is important in the diagnosis of periodontal conditions, assigning a prognosis, and evaluating response following periodontal treatments.<sup>17-19</sup> Diagnosis of periodontal diseases requires recording of clinical, periodontal variables: probing depth (PD), attachment loss (AL), furcation involvement and bleeding on probing.<sup>20</sup> A periodontal probe is the commonly used instrument to assess periodontal conditions and the severity of periodontal lesions through recording of the above parameters.<sup>18,19</sup>

Possible measurement errors in recording the periodontal findings are dependent on the mea-

surement method, precision of reading, angulations of the probing tip, fluctuation of inflamed gingiva, and inflammatory status of the tissue and documentation errors in the transfer of data.<sup>21-24</sup> Further imprecision is introduced when findings are taken by two different users.

The first generation probes were designed with a focus on pressure audit<sup>25</sup> and resolution. Conventional probes have a production-linked inaccuracy of  $\pm 1\text{mm}$ .<sup>26</sup> The second generation of periodontal probes brought about a constant generation of force during periodontal probing. This allowed for reduction of errors during measurement and providing an improved standard of probing. These probes were called the pressure sensitive probes.<sup>1</sup>

Patient discomfort associated with the insertion of a periodontal probe into the periodontal pocket is a common clinical event. The intensity of the pain or discomfort has been perceived by practitioners to differ dramatically between sites and patients. In untreated cases, the pain associated with full mouth periodontal probing is mainly due to the persisting inflammation of the periodontal tissues.

The pain experienced during this baseline examination procedure has always been a matter of concern but overlooked by the examiner. In an untreated periodontal site, probing leads to penetration of the periodontal probe into the surrounding connective tissue, which is heavily infiltrated with chronic inflammatory cells. The higher the degree of periodontal inflammation the higher the discomfort or pain elicited by periodontal probing.<sup>28</sup>

The aim of this study is to evaluate the pain perceived by patients with gingivitis and periodontitis during periodontal probing and to compare their pain perception when using a conventional UNC<sup>15</sup> probe and a manual pressure sensitive periodontal probe. Additionally, how the clinical features of gingivitis and periodontitis relate to the painfulness associated with periodontal probing is assessed.

Comparison between the intensity of the pain

to the inflammatory status of the periodontium as assessed by the presence of bleeding on probing and measurement of the clinical attachment levels and the probing pocket depths is conducted.

## MATERIALS AND METHODS.

### Subject population and selection

The present study was carried out as a prospective study on patients who reported to the Out-Patient Department, Department of Periodontology, Chettinad Dental College and Research Institute, Kelambakkam.

This study was approved by the Institutional Human Ethical Committee, Chettinad Health City (IHEC No: 470). All of the participants were informed about the study protocol and gave verbal

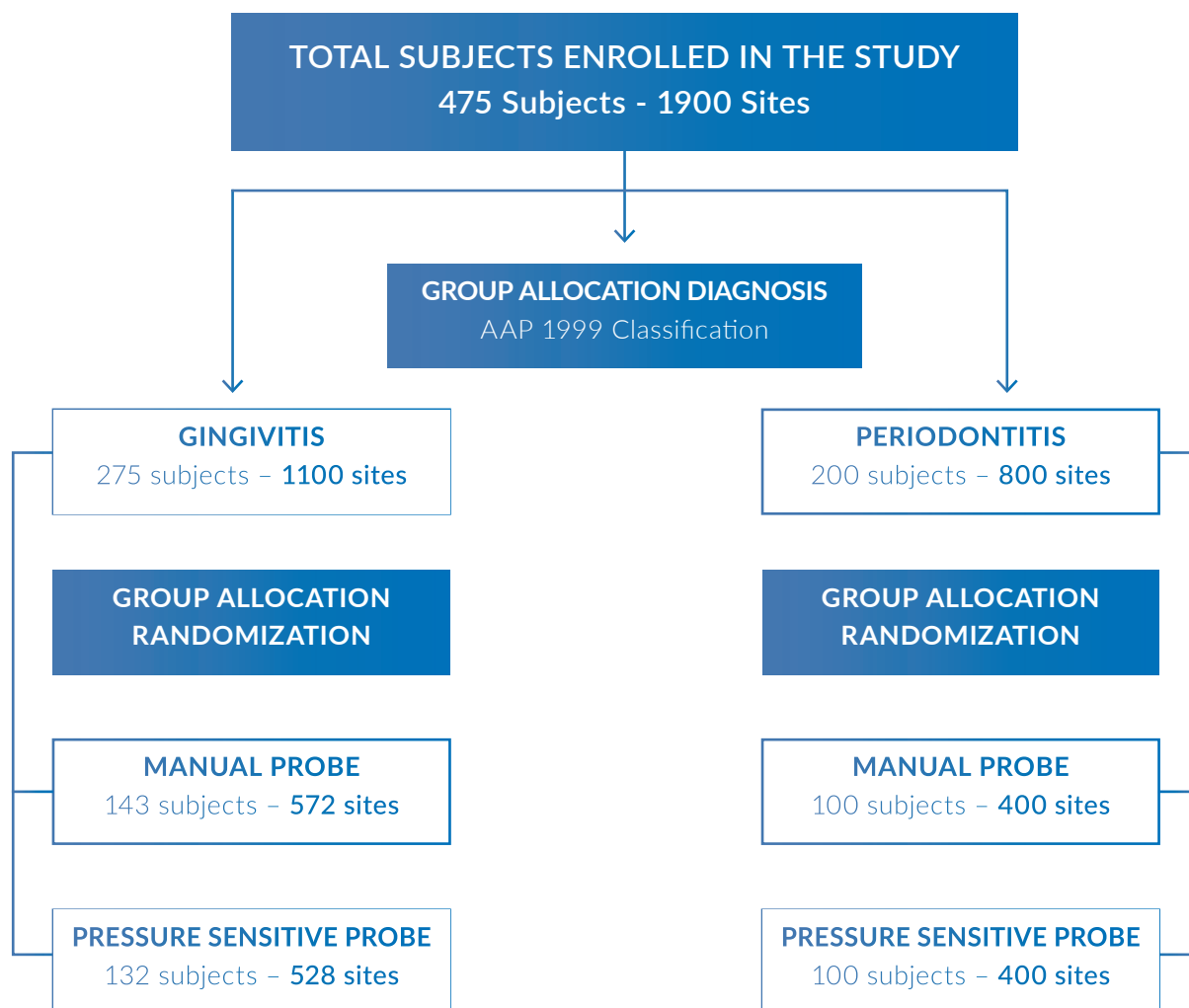
and signed informed consent. The study was conducted between April and September 2019. A total of 475 patients were enrolled in the study.

The subjects were enrolled in the study after fulfilling the inclusion and exclusion criteria. Inclusion criteria included: systemically healthy individuals, presence of a minimum of 24 fully erupted teeth including third molars. Exclusion criteria included: patients who had undergone previous Phase-I periodontal therapy in the previous six months, patients undergoing orthodontic therapy, patients presenting with pulpitis, acute periodontal pain or any other acute infections.

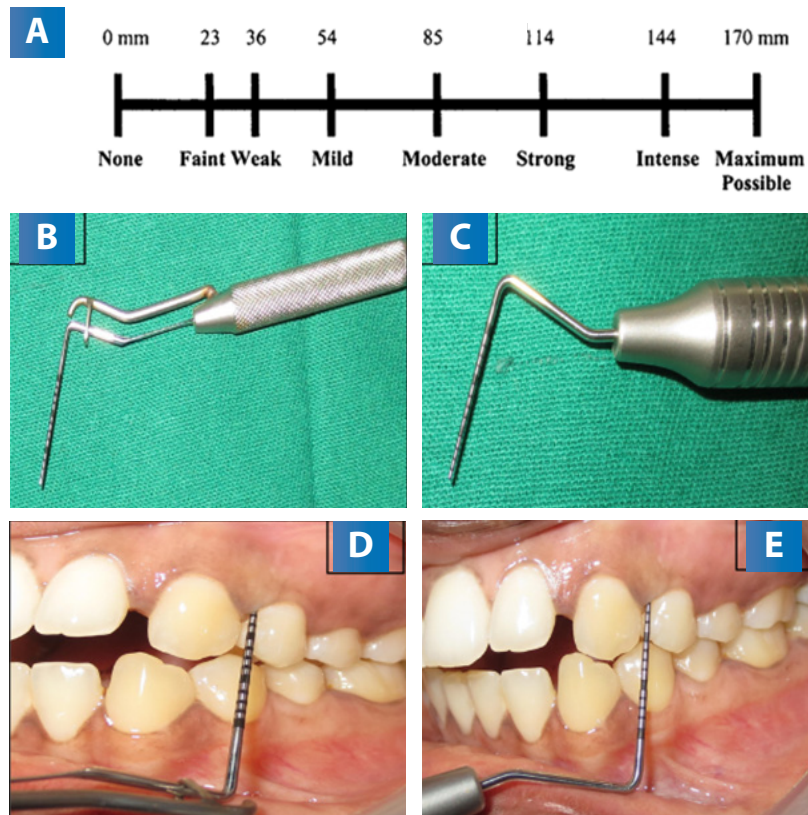
### Study design

This study was planned as a randomized, single-

**Figure 1.** Representation of the study design.



**Figure 2.** Materials and methods used in this study.



**A:** Heft Parker Visual Analog Scale. **B:** Manual Pressure Sensitive Probe. **Figure 2-c:** UNC-15 Probe. **C:** Probing the sulcus using UNC-15 Probe **D:** Probing the sulcus using Manual Pressure Sensitive Probe. **E:** Probing the sulcus using UNC-15 Probe

blinded clinical study. The subjects were initially divided into 2 groups –

Group A (Dental Plaque Induced Gingivitis group consisting of 275 patients) and;

Group B (Chronic Periodontitis group consisting of 200 patients) based on the Classification of Periodontal Diseases and Conditions – AAP 1999.<sup>16</sup>

For the gingivitis (Dental Plaque Induced Gingivitis) group, patients presenting with either of the following clinical signs:

Redness and sponginess of gingival tissue, bleeding on provocation, changes in contour and presence of calculus or plaque with no radiographic changes of crestal bone loss, were considered.<sup>16</sup> For the chronic periodontitis group patients presenting with either of the following clinical signs: gingival inflammation, pocket formation with a pocket depth

ranging from 4mm and above, clinical attachment loss ranging from 1-5mm with radiographic changes in bone loss up to middle 1<sup>st</sup>/3<sup>rd</sup> of the roots with furcation involvement were considered.<sup>16</sup>

These two groups were randomly further subdivided into two groups each (Gingivitis – Conventional Probe – GCP, Gingivitis – Manual Pressure Sensitive Probe – GMPS, Periodontitis – Conventional Probe – PCP, Periodontitis – Manual Pressure Sensitive Probe – PMPS) using a computer generated program of random numbers (Figure 2).

A conventional UNC-15 (Hu-freidy, Germany) probe (Figure 2C) was used in the GCP and PCP groups and a Manual pressure sensitive (AXE pressure-sensitive probe, Bludent, India) probe (Figure 2B) was used in the GMPS and PMPS groups to record the clinical parameters.

### Clinical examination and measurements

The clinical examination of gingiva was performed to include pain perception at the probing site; two parameters of inflammation were reported: a modification of gingival index (Loe and Silness 1963) and a bleeding on probing score, assessment of periodontal probing depth and clinical attachment levels.

The clinical examination and data recording were performed by a single trained examiner - a dental graduate intern of the Department of Periodontology under the supervision of a senior faculty, in order to reduce inter examiner variability.

The clinical examination was performed using the periodontal probes (conventional probe and manual pressure sensitive probe) which was placed parallel to the long axis of the tooth at six sites per tooth examined (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) with same depth and direction.

### Gingival index

The gingival index (recorded at mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual aspects) provides a non-invasive (observational) measure of gingival inflammation. The response obtained was graded as follows:

**Table 1.** Demographic data of study participants.

		N	GROUP 1	GROUP 2
<b>Mean age (years)</b>			<b>32.4</b>	<b>44.4</b>
<b>GENDER</b>	Male	272	139	133
	Female	203	93	110
<b>POCKET PROBING DEPTH</b>	0-3mm (n)	359	184	175
	4-5mm (n)	368	175	193
	>5mm (n)	73	41	32
<b>CLINICAL ATTACHMENT LOSS</b>	0-3mm (n)	606	326	280
	4-5mm (n)	89	36	53
	>5mm (n)	105	38	67

**Table 2.** Pain score and probe group comparison according to chi-square test.

		PROBE		TOTAL (N=1900)	ASYMP. SIG. (2-SIDED)
		PRESSURE (N=928, %)	MANUAL (N=972, %)		
<b>PainScore/Teeth</b>	None	58.2 (89)	41.8 (64)	153	$p < 0.001$
	Faint	58.4 (194)	41.6 (138)	332	
	Weak	40.1 (244)	59.9 (365)	609	
	Mild	47.2 (220)	52.8 (246)	466	
	Moderate	54.4 (143)	45.6 (120)	263	
	Strong	62.5 (35)	37.5 (21)	56	
	Intense	0.0 (0)	100.0 (16)	16	
	Maximum Possible	60.0 (3)	40.0 (2)	5	



**Table 3.** Pain score and probe group comparison within gingivitis and periodontitis, according to chi-square test.

GROUP			PROBE		TOTAL (N=1100)	ASYMP. SIG. (2-SIDED)
			PRESSURE (N=528, %)	MANUAL (N=572, %)		
GINGIVITIS	Pain Score/Teeth	None	61.0 (47)	39.0 (30)	77	$p < 0.001$
		Faint	87.5 (63)	12.5 (9)	72	
		Weak	37.4 (155)	259(62.6%)	414	
		Mild	44.7 (136)	55.3 (168)	304	
		Moderate	55.7 (102)	44.3 (81)	183	
		Strong	68.6 (24)	31.4 (11)	35	
		Intense	0.0 (0)	100.0 (13)	13	
		Maximum Possible	50.0 (1)	50.0 (1)	2	
PERIODONTITIS	Pain Score/Teeth	None	55.3 (42)	44.7 (34)	76	$p < 0.541$
		Faint	50.4 (131)	49.6 (129)	260	
		Weak	45.6 (89)	54.4 (106)	195	
		Mild	51.9 (84)	48.1 (78)	162	
		Moderate	51.2 (41)	48.8 (39)	80	
		Strong	52.4 (11)	47.6 (10)	21	
		Intense	0.0 (0)	100.0 (3)	3	
		Maximum Possible	66.7 (2)	33.3 (1)	3	

**Table 4.** Pain score and probe group comparison with bleeding on probing (BOP) according to chi-square test.

PROBE			BOP		TOTAL (N=928)	ASYMP. SIG. (2-SIDED)
			PRESENT (N=444, %)	ABSENT (N=484, %)		
PRESSURE	Pain Score/Teeth	None	28.1 (25)	71.9 (64)	89	$p < 0.001$
		Faint	43.3 (84)	56.7 (110)	194	
		Weak	46.3 (113)	53.7 (131)	244	
		Mild	46.4 (102)	53.6 (118)	220	
		Moderate	65.0 (93)	35.0 (50)	143	
		Strong	71.4 (25)	28.6 (10)	35	
		Intense	0.0 (0)	100.0 (2)	2	
		Maximum Possible	66.7 (2)	33.3 (1)	3	
MANUAL	Pain Score/Teeth	None	28.1 (18)	71.9 (46)	64	$p < 0.05$
		Faint	44.9 (62)	55.1 (76)	138	
		Weak	37.5 (137)	62.5 (228)	365	
		Mild	43.1 (106)	56.9 (140)	246	
		Moderate	49.2 (59)	50.8 (61)	120	
		Strong	52.4 (11)	47.6 (10)	21	
		Intense	68.8 (11)	31.2 (5)	16	
		Maximum Possible	0.0 (0)	100.0 (2)	2	

0. Normal gingiva

1. Mild inflammation – slight change in colour and slight edema but no bleeding on probing

2. Moderate inflammation – redness , edema and glazing , bleeding on probing

3. Severe inflammation – marked redness and edema, ulceration with a tendency to spontaneous bleeding.

### **Bleeding on probing**

The periodontal probe was inserted into the space between the tooth surface and the marginal gingival tissue. Measurements were taken in the gingival sulcus or the periodontal pocket at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiopalatal, distopalatal and midpalatal).

The presence of bleeding after the removal of the probe from the site was noted. The bleeding on probing score was documented for each quadrant as present or absent.

### **Periodontal probing**

Probing was performed along the long axis of the tooth in the mid-facial and mid-lingual/palatal locations (Figure 2D and Figure 2E). On the proximal surfaces, the probe was angled to get under the middle of the contact point.

Where signs of inflammation on inspection along with the indexed teeth were found according to the modification of the gingival index, they were noted.

### **Perception of pain**

Following examination of each quadrant, the participant used the Heft Parker Visual Analog Scale (Figure 2A) to document the subjective pain perception experienced during the procedure.

A copy of the scale was provided to each patient at the time of probing and the patient was asked to point to the score they felt was accurate in depicting the pain perceived. Four pain scores (1 per quadrant) were obtained from each participant enrolled in the study. A total of 1900 sites were analysed for pain scores from all the subjects and submitted for statistical analysis.

### **Clinical periodontal parameters**

Probing depth (PD) was measured at six sites per

tooth examined (mesiobuccal, midbuccal, distobuccal, mesiopalatal, distopalatal and midpalatal). The measurement was made from the free gingival margin to the bottom of the sulcus (Figure 2D and Figure 2E).

A time gap of ten minutes was maintained between each site for probing. Clinical attachment loss (CAL) was measured at the same reference points used for PD. The measurement was made from the cemento- enamel junction (CEJ) to the bottom of the sulcus and six readings per tooth were recorded.

### **Statistical analysis**

The statistical analysis was performed using IBM.SPSS statistical software, Version 23.0. The measurement data was evaluated in terms of normal distribution by application of the Kolmogorov-Smirnov test. The chi-square test was performed to analyze the significance between the groups (Pain score with BOP, gingival index and probes, classified into groups-pressure-sensitive and manual groups).

Significance was analyzed for all tests performed, whereby a  $p$ -value<0.05 was considered to be statistically significant for all tests.

## **RESULTS.**

Four hundred seventy five subjects within the age range of 18-45 years were recruited into the study. Gingival index/teeth, BOP, pocket probing depth (PPD), clinical attachment loss (CAL) and pain score during probing (HPVAS pain scale) were collected from a total of 1900 sites. All patients were characterized as having gingivitis (Group-A) or chronic periodontitis (Group B) based on AAP classification 1999. The demographic data of which is shown in Table 1.

The comparison of pain elicited when probing using a pressure-sensitive probe and manual probe group was analyzed using the chi-Square test. A lower pain perception with a statistical difference ( $p < 0.001$ ) was noted in the pressure sensitive probe compared to the manual probe group. In the present study, 58.2% of the patients showed a pain score of none (HPVAS pain scale) with use of a pressure sensitive probe (Table 2) indicating the significance of controlled force during probing.



Patients were assessed for gingivitis score/teeth using two probes (1100 sites in the gingivitis group and 800 sites in the periodontitis group). There appeared to be a statistical difference ( $p < 0.001$ ) in pain perception when pressure-sensitive probe was used in group A compared to group B.

However no statistical difference was noted between the two probes ( $p = 0.541$ ) regarding pain elicited in the patients in the chronic periodontitis group (Table 3) indicating the influence of inflammation in pain perception in untreated periodontitis patients, who had less resistant gingival tissue with use of manual probing in comparison with a controlled pressure sensitive probe.

Table 4 shows the presence of BOP using two probes (928 sites in the pressure sensitive probe and 927 sites in the manual probe). Decreased BOP with a statistical difference ( $p < 0.001$ ) was noted when the pressure sensitive probe was used (Table 4). This illustrates the role of standardization of force during probing. A strong possibility exists for the traumatization of clinically healthy gingival tissues if a probing force applied exceeds 0.25N.

## DISCUSSION.

Stedman's Medical Dictionary defines pain as "an unpleasant sensation associated with actual or potential tissue damage and mediated by specific nerve fibres to the brain, where its conscious appreciation may be modified by various factors".<sup>29</sup>

Dental anxiety and dental fear are strong negative feelings associated with dental treatments and are often used interchangeably in the dental literature.<sup>27,28</sup> Hence the present study provides information on the pain perceived by the patients diagnosed with gingivitis and periodontitis on probing and compared the pain perception on using conventional and pressure-sensitive probe.

The study also correlates the pain perceived by subjects and the intensity of pain with the clinical features of the disease, in both the groups. The intensity of pain was analyzed using Heft Parker Visual Analogue Scale with horizontal line end points marked "none" to "maximum possible"

(Figure 2A). This scale has been previously been shown to be simple, reliable and valid for analyzing dental pain.<sup>28</sup>

Previous reports indicate that the discomfort during probing seems to apply to both conventional and automated probes.<sup>30,31</sup> In the present study the incidence of pain and discomfort was moderate to severe during periodontal probing, as shown in Table 3 and Table 4.

It has also been shown that untreated patients had a less resistant gingival tissue where higher probing forces by manual probing in comparison with a controlled manual pressure sensitive probe, could lead to distorted measurements. Further studies taking periodontal variables before and after active periodontal therapy would be necessary. However, reduced pain/discomfort was experienced with the use of a pressure sensitive probe compared to a conventional probe.

The result of the present study and previous studies<sup>31</sup> showed that the degree of inflammation is directly proportional to the pain and discomfort during periodontal probing. It was shown that the conventional probe showed deeper measurements due to lack of controlled probing force<sup>31</sup> and previous studies have shown that while using a conventional probe, the probing pressure was close to 100 grams, hence a pressure sensitive probe was used and compared for clinical features and pain correlation. Table 4 shows lower pain perception in gingivitis patients compared to patients with periodontitis.

Bleeding on probing is an important objective clinical parameter to assess the level of periodontal tissue inflammation. Proye *et al.*,<sup>32</sup> demonstrated increased bleeding when probing forces of 15, 20, 50 grams and manual force were used, which is consistent with the findings of the present study (Table 4). Therefore, the use of a standardized probing force provided by a pressure-sensitive probe would facilitate reducing subjectivity for bleeding determinations.

The study findings are conclusive that reduced pain perception along with BOP was noted with a

manual pressure-sensitive probe.

The present findings, based on a large sample of 475 patients and 1900 sites, indicate clearly that discomfort during periodontal probing is a significant factor when patients are questioned about it. These experiences are remembered by patients and may influence their pain perception during the next periodontal treatment.

These subjective perceptions may make the patient hesitant about seeking further periodontal diagnostics and/or care. The advantage the manual pressure sensitive probe offers the general dental professionals and even periodontists is the low cost when compared to the more advanced computerized systems. This may encourage the general dental professionals to comprehensively evaluate the periodontium, thereby enabling more efficient diagnosis of underlying periodontal diseases.

## CONCLUSION.

Dentistry has changed its focus towards painless dentistry. In this context, the present study presents data supporting the use of manual pressure sensitive probes, which offers an advantage of low cost with reduced pain during periodontal examination, which could contribute to a positive attitude of the patients towards continuous supportive periodontal therapy thereby monitoring periodontal health.

**Conflict of interests:**

The authors declare that this study had no external source of funding and/or conflict of interest.

**Ethics approval:**

This study was approved by the Institutional Human Ethical Committee, Chettinad Health City (IHEC No: 470).

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**Authors' contributions:**

Ashwath B: Conceptualization, Data interpretation, Writing – original draft, Writing – review & editing.

Aishwarya D and Agila.E: Sample collection, Statistical analysis and Writing – original draft.

Shanmugam M and Anitha V: Formal Analysis and Writing – original draft.

Meenakshi M and Aanish Za: Sample collection, Data entry and Writing – original draft.

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