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Original Article

Dentistry Section

Evaluation of Changes in Serum Insulin like Growth Factor-1 Levels with Growth Modulation via Twin Block Therapy- A Prospective Clinical Study

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ABSTRACT

Introduction: Serum insulin like growth factor is a pertinent growth regulator in bone and cartilage, therefore assessment of changes in serum Insulin like Growth Factor (IGF) levels in patients undergoing functional appliance therapy can act as valuable tool to understand the mode of action of functional appliances.

Aim: To evaluate the changes in serum Insulin like Growth Factor-1 (IGF-1) levels in the patients undergoing twin block therapy and to establish IGF-1 as a biochemical marker produced in response to mechanical disturbance in the condylar region by twin block appliance.

Materials and Methods: This prospective clinical study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Postgraduate Institute of Dental Sciences, Rohtak, Haryana, India from May 2014 to November 2015. Total 27 patients included in study, divided into two groups, the test group which comprised of 15 patients treated with standard twin block appliance therapy and the control group which comprised of 12 patients not treated with the same. Serum samples were collected from test group and control group patients at four time intervals {pretreatment (T0), 1 month (T1), 3 months (T3) and 6 months (T6)} and subjected to Enzyme-Linked Immunosorbent Assay (ELISA) to measure IGF-1 levels. The data thus obtained was subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) for windows version 20.0. Intragroup differences were evaluated using paired t-test, while intergroup differences were evaluated using Independent sample t-test. The p-value <0.05 was considered as statistically significant.

Results: The data was obtained from 11 patients (6 males and 5 females) in the test group and 10 patients (6 males and 4 females) in the control group after elimination of dropouts. Mean age of the test group subjects was 12.8±0.9 years and that of control group subjects was 13.1±0.9 years. The mean IGF-1 levels in the test group were 124.5 ng/mL (T0), 126.27 ng/mL (T1), 130.90 ng/mL (T3) and 135.54 ng/mL (T6). In the control group, 151.5 ng/mL (T0), 140.8 ng/mL (T1), 157.8 ng/mL (T3) and 120.2 ng/mL (T6). In the test group, mean levels of serum IGF-1 did not change significantly when baseline levels were compared with the serum IGF-1 levels at T1 (0.467), T3 (0.729) and T6 (0.62) of functional appliance therapy, although an increase in IGF-1 levels was noted at each interval. Comparison of serum IGF-1 levels of test and control group showed no statistically significant difference between the groups at T0, T1, T3 and T6.

Conclusion: Functional appliance therapy was not found to be associated with concomitant increase in serum IGF-1 levels.

Keywords: Condyle, Functional appliances, Growth modulation, Serum marker

INRODUCTION

Condyle is an important growth site in the developing mandible and plays a vital role in the development of orofacial complex. The growth of the condyle is highly adaptable to functional factors. This property has therefore received special attention and is exploited in orthodontics for the treatment of Class II malocclusion during growth period using functional appliances [1].

There are several varieties of removable and fixed functional appliances which are designed to alter the position of mandible to induce supplementary lengthening of the mandible by stimulating increased growth at the condylar cartilage [2-5]. In 1982, Clark introduced Twin Block (TB) appliance, which by virtue of its configuration, has gained wide spread popularity due to its patient friendly nature [6].

It is known that condylar growth is partly genetically determined but is strongly influenced by epigenetic factors. The latter include systemic factors and local factors such as growth factors and mechanical stimuli [7]. There is ample evidence in literature which states that mechanical perturbation of the condyle with functional appliances leads to metabolic changes within the tissue, causing expression of several growth (Vascular endothelial growth factor, Insulin like Growth Factors (IGF), Fibroblast growth factor, Transforming growth factor-ß, Platelet derived growth factor etc.,) and transcription factors which modulate cell proliferation and differentiation in condyle [1,8].

During recent years, many studies have been conducted to find out the relation between IGF levels in blood and skeletal maturity [9-11]. A recent study also correlated blood IGF-1 levels with increments in mandibular growth and spurts in IGF-1 levels were found to be a promising tool for prediction of timing and intensity of mandibular growth [11]. IGF or somatomedins, are a family of low molecular weight peptides which resembles insulin both in their structure and in their effects. There are two main types IGF-I and IGF-II [12]. IGF-1 was first discovered by Salmon W and Daughaday WH in 1957 [13]. It is mainly produced in liver as an endocrine hormone as well as in the target tissues in paracrine or autocrine fashion. It functions as a local and systemic growth regulator especially in bone and cartilage. In orofacial area, IGF system is involved in growth and development of teeth, mandible, maxilla and tongue [14,15].

Therefore, it is assumed that the force generated by a mandibular propulsor appliance is transmitted to chondrocytes via surface receptors, which transduce this signal into a biological response, such as gene expression and regulation. This ultimately leads to expression of various growth factors like IGF-1 which play role in local growth stimulation [16].

Therefore, the present study was conducted with the aim to evaluate the changes in serum IGF-1 levels in the patients undergoing twin block therapy in order to establish IGF-1 as one of the biomarkers produced in response to the mechanical disturbance in the condylar region.

MATERIALS AND METHODS

This prospective clinical study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Postgraduate Institute of Dental Sciences, Rohtak, Haryana, India from May 2014 to November 2015. The study sample consisted of subjects who reported to the regular Outpatient Department (OPD) and were indicated for functional appliance therapy. Ethical clearance from Institutional Review Board was obtained (PGIDS/IEC/2014/113), and informed consent from the patients and their parents was also taken before the study.

Inclusion criteria: Patients with Class II division 1 malocclusion on account of retrognathic mandible with a positive Visual Treatment Objective (VTO) were included in the study. Growth status was assessed using lateral cephalogram by Cervical Vertebrae staging method given by Baccetti T et al., [17]. Patients in prepubertal growth spurt phase (CS2 and CS3) and with average to horizontal growth pattern assessed by Sella Nasion (SN)-Mandibular Plane (MP) Gonion and Gnation (Go-Gn) angle were included in the study.

Exclusion criteria: Patients contraindicated for functional appliance therapy, patients with systemic illness, growth abnormalities, bleeding disorders and facial asymmetry were excluded from the study.

Sample size calculation:

α=0.05

β=0.20

r=0.70

Standard normal deviate for alpha=Z_α=1.96

Standard normal deviate for beta=Z_β=0.8416

C=0.5×ln{(1+r)/(1-r)}=0.8673

Sample size N=$\{(Z_\alpha+Z_\beta)/C\}^2$=13

20% attrition rate=13×0.2=2.6 (approximately 2)

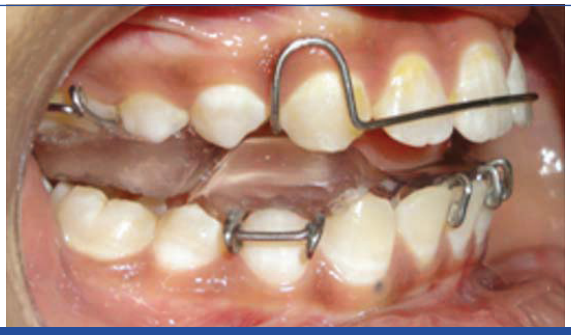
Total sample size in each group=13+2=15

The sample consisted of total 27 patients divided into two groups,

- The test group which comprised of 15 patients treated with standard twin block appliance therapy and
- The control group which comprised of 12 untreated patients. Control group patients were kept in the waiting list and were treated after the follow-up period in accordance with some previous studies where control group patients were untreated till the follow-up period [18,19].

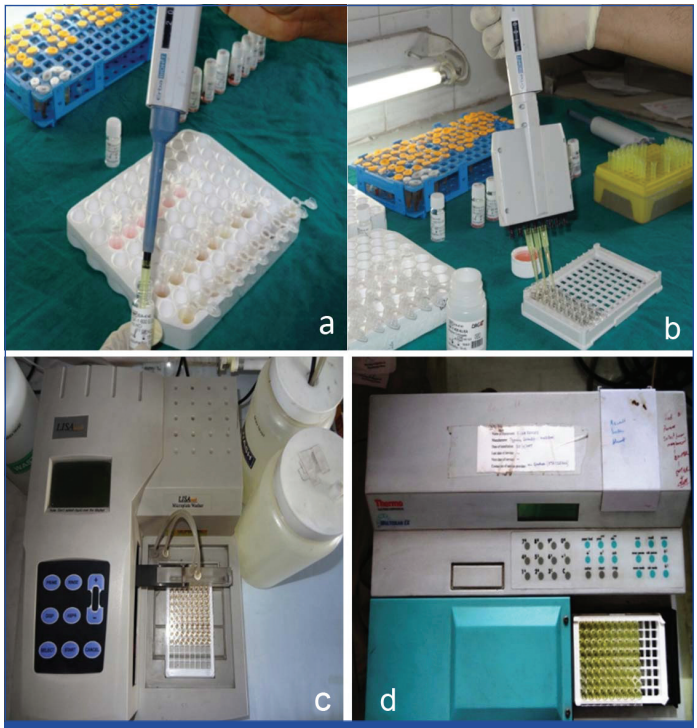
Procedure

The subjects were asked to wear the appliance for 24 hours, even while eating after they got adjusted to it, which took 7-10 days time. Total duration of treatment was 6-8 months. Standard twin block appliance [Table/Fig-1] was constructed for test group subjects.



[Table/Fig-1]: Twin block design.

A 5 mL blood was collected from antecubital vein in plain vacutainer tubes for the investigation of serum IGF-1. The samples were centrifuged at 3000 rpm for 4 minutes for separation of serum. The serum was then placed in plastic Eppendorf disposable centrifuge tubes. Serum samples were collected from subjects at T0 (pre-treatment), T1 (after 1 month), T3 (after 3 months), T6 (after 6 months). The serum samples were numbered and stored at -20°C in deep refrigerator. Estimation of serum IGF-1 levels was done using ELISA kit (IGF-1 600 ELISA, DRG international, Germany) according to manufacturer's instructions [Table/Fig-2a-d]. The absorbance (optical density) of each sample obtained from ELISA reader was calibrated on a standard curve of mean absorbance value and corresponding IGF-1 concentration for each sample was determined. IGF-1 levels were obtained in ng/mL [10].



[Table/Fig-2]: Assay procedure; a) and b) Acidification of the samples followed by addition of enzyme substrate and enzyme complex; c) Washing procedure; d) Determination of optical density in ELISA reader.

STATISTICAL ANALYSIS

All data were analysed using the SPSS for windows, version 20.0. Normality of the sample was checked using Kolmogorov-Smirnov test and sample was found to be normally distributed, so parametric tests were used to check the statistical significance of the results. Independent sample t-test was used to compare mean IGF-1 levels between test group and control group. Intragroup comparisons were made using paired t-test to determine changes in IGF-1 levels over time in both test group and control group. The p-value <0.05 was considered as statistically significant.

RESULTS

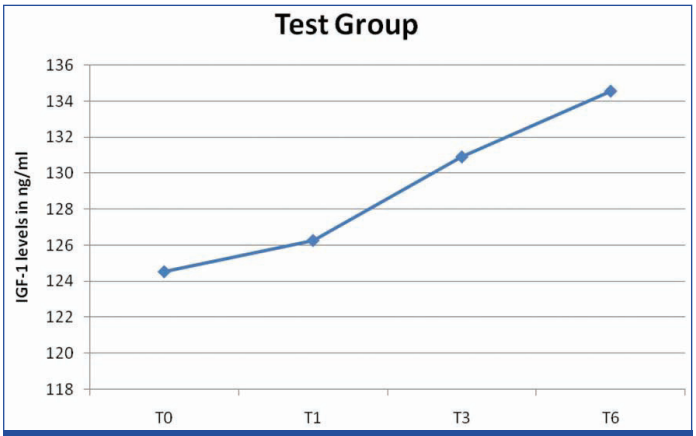
Four subjects from test group and two subjects from control group failed to complete the follow-up. After elimination of dropouts, total number of subjects in the test group were 11 (6 males and 5 females) and in the control group were 10 (6 males and 4 females). Mean age of the test group subjects was 12.8±0.9 years and that of control group subjects was 13.1±0.9 years. Both the groups were comparable at baseline with respect to age. Pairwise comparison of mean change in IGF-1 levels at different time intervals was done with the help of paired t-test.

In the test group, mean change in IGF-1 levels between T0-T1, T0-T3, T0-T6, T1-T3, T1-T6 and T3-T6 was not found to be statistically significant [Table/Fig-3]. Although an increase in IGF-1 levels at each interval was noted [Table/Fig-4].

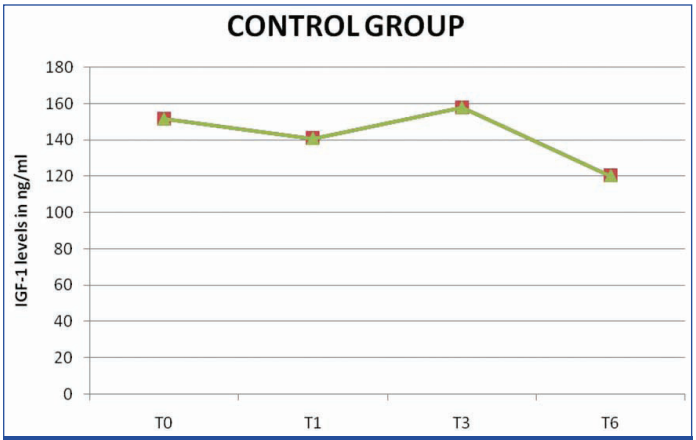
Variations of serum IGF-1 levels (ng/mL) were seen in test as well as control group as seen in [Table/Fig-4,5].

IGF-1 levels	Mean difference	Standard deviation	p-value
T0-T1	+1.7273	50.1500	0.911
T0-T3	+5.6000	84.6249	0.839
T0-T6	+11.000	77.6428	0.649
T1-T3	+6.7000	82.6372	0.803
T1-T6	+9.2727	76.3729	0.696
T3-T6	+3.6400	40.6399	0.904

[Table/Fig-3]: Intragroup comparison of mean change in IGF-1 levels at different time intervals in test group (paired t-tests).
Change in IGF-1 levels in ng/mL; Plus sign denotes increase in levels; T0-IGF-1 levels at 0 month (baseline); T1-IGF-1 levels at 1st month; T3- IGF-1 levels at 3rd month; T6-IGF-1 levels at 6th month; The p-value <0.05 was considered as statistically significant



[Table/Fig-4]: Showing mean IGF-1 levels of test group at T0, T1, T3 and T6.
The mean IGF-1 levels in the test group were 124.54 ng/mL (T0), 126.27 ng/mL (T1), 130.90 ng/mL (T3) and 135.54 ng/mL (T6)



[Table/Fig-5]: Showing mean IGF-1 levels of control group at T0, T1, T3 and T6.
In the control group 151.5 ng/mL (T0), 140.8 ng/mL (T1), 157.8 ng/mL (T3) and 120.2 ng/mL (T6).

In the control group, mean change in IGF-1 levels between T0-T1, T0-T3, T0- T6, T1-T3, T1-T6 was also not statistically significant [Table/Fig-6]. But there was a decline in IGF-1 levels from T3 to T6 with a mean difference of 37.6 ng/mL and it was found to be statistically significant with p-value of 0.041.

Mean change in IGF-1 levels	Mean difference	Standard deviation	p-value
T0-T1	-10.7000	44.5173	0.467
T0-T3	+6.3000	55.6338	0.729
T0-T6	-31.3000	46.4592	0.062
T1-T3	+17.0000	62.0537	0.409
T1-T6	-20.6000	48.5391	0.212
T3-T6	-37.6000	49.7487	0.041

[Table/Fig-6]: Intragroup comparison of mean change in IGF-1 levels at different time intervals in control group (paired t-tests).
Change in IGF-1 levels in ng/mL; Plus sign denotes increase in levels; Minus sign denotes decrease in levels; T0-IGF-1 levels at 0 month (baseline); T1-IGF-1 levels at 1st month; T3-IGF-1 levels at 3rd month; T6-IGF-1 levels at 6th month; p-value <0.05 was considered statistically significant

Further, as shown by Independent samples t-test, there was no difference of statistical significance between mean IGF-1 levels of test and control group at T0, T1, T3 and T6 [Table/Fig-7].

Mean IGF 1 levels (ng/mL)	Test group	Control group	p-value
T0	124.54	151.50	0.250
T1	126.27	140.80	0.573
T3	130.90	157.80	0.327
T6	135.54	120.20	0.413

[Table/Fig-7]: Comparison of mean IGF-1 levels between the test and control groups at T0, T1, T3 and T6 (Independent sample t-test).
IGF-1 levels in ng/mL; T0-IGF-1 levels at 0 month (baseline); T1-IGF-1 levels at 1st month; T3-IGF-1 levels at 3rd month; T6-IGF-1 levels at 6th month; p-value <0.05 was considered statistically significant

DISCUSSION

Functional appliance therapy is an important treatment modality adopted for correction of skeletal class II malocclusion by harnessing the growth potential of the patient. Literature is replete with work concerning the modus operandi of functional appliances [20]. Although acceleration of rate of growth during the therapy has been reported with evidence, it is still a controversial issue whether overall growth of mandible is increased due to functional appliance therapy or not [21,22].

Enhanced condylar growth has been demonstrated in animals with the use of functional appliances. Graber T et al., explained the mechanism of action of functional appliances using the servosystem theory [23]. Mandibular growth was proposed to be a controlled variable which changed according to the constantly changing reference input i.e., maxillary position via occlusal contacts, which is facilitated by regional extrinsic factors (blood supply, nerve signals, growth factors) and general factors {Growth Hormone (GH), somatomedin, thyroxine etc.,} [23].

It has been established through various studies that biomarkers such as GH, IGF-1, Parathyroid Hormone related Protein (PTHrP), osteocalcin, Alkaline Phosphatase (ALP), etc., play an explicit role in growth phenomenon [24-26]. Growth factors and osteocytes, which act as mechanosensors, play a key role during the bone formation after mechanical stimulation [27]. Estimation of these hormones and bone remodeling biomarkers may provide an early indication of the response to growth promoting treatment such as functional appliance therapy.

Growth hormones appears to be the most important factor regulating condylar growth but measurement of GH is difficult because of its short half life, pulsatile secretion, diurnal variation and effects of environmental stimuli on its secretion [25]. According to a study done by Hussain M et al., serum PTHrP levels do not correlate with early pubertal stages and hence, its validity to predict growth is questionable [27]. Serum osteocalcin and ALP levels correlate with pubertal stages in boys, but not in girls [28]. Out of all the suggested biomarkers, IGF-1 has shown to be the most promising marker for growth assessment [12,13].

Studies have shown importance of IGF-1 in cartilaginous growth [24]. IGF-1 is an important regulator of bone turnover at tissue level. It has been shown to enhance osteoblast proliferation, to stimulate type I collagen production and Blood Alkaline Phosphatase (BAP) activity and to modulate osteoblast-osteoclast interactions. Circulating IGF-1 levels also directly regulate bone growth and density, and studies have suggested a causal relationship between serum IGF-1 levels and bone density [29]. IGF-1 also stimulates bone resorption by promoting osteoclastogenesis, thus important for bone remodeling [30].

Hajjar D et al., in his study found an increase in IGF-1 mRNA expression in the proliferating cells of condylar cartilage of animals fitted with mandibular propulsor appliance [31]. So, the present study was based on the assumption that the IGF-1 produced locally may enter the circulation and might be detectable in serum.

In humans, IGF-1 is measurable in serum, urine and saliva. Salivary IGF-1 levels reflect its levels in the plasma. However, salivary IGF-1 levels are less than 1% of serum levels. This makes accurate measurements difficult. In addition, contamination with gingival fluid or blood can result in inaccurate measurement [32]. IGF-1 levels have also been monitored in GCF during fixed orthodontic treatment and it was found that IGF levels are altered during fixed mechanotherapy. But, this increase is attributed to the alveolar bone remodeling induced as a consequence of force application [33]. Serum IGF-1 levels generally reflect GH status and were reported to be high in patients with acromegaly and low in those with GH deficiency [34].

There is a paucity of studies regarding the systemic changes associated with growth modulation via functional appliance therapy. A study conducted by Reijnders C et al., had evaluated the serum changes associated with localised experimental mechanical loading [25]. There was a twofold upregulation of IGF-1 mRNA synthesis in the osteocytes present in mechanically stimulated rat tibia. But no difference was found in the serum concentration of IGF-1 between experimental and control group. This may be explained on the basis that this animal study evaluated the acute effect by exposing the bone to a single session of mechanical loading. The effect of prolonged stimulation like that occurs with functional appliance therapy was not evaluated [27].

Hence, authors estimated the changes in serum IGF-1 levels in the subjects undergoing treatment by a functional appliance i.e., twin block and compared them with subjects of similar demographic profile not undergoing any functional appliance therapy. IGF-1 levels were evaluated at 1 month because normally initial neuromuscular response to functional appliances appears at this time. IGF-1 levels at 3rd month denote progress of active therapy and 6th month levels represent end of active therapy in most patients [35].

The IGF-1 assay was performed with ELISA technique. In previous studies different techniques including ELISA, radio-immunoassays, and immunoradiometric assays used in IGF-1 analysis have been found comparable in terms of accuracy [9,10,28]. The ELISA technique was used in this research because of the availability of the kit, and the applicability and accuracy of the technique [10].

Reference ranges of serum IGF-1 levels have been established by various studies according to Cervical vertebral maturation index (CVMI) staging. Mean IGF-1 levels at baseline (T0) found in the present study in test group as well as control group were comparable to the IGF-1 levels of subjects with CS2 (135±33 ng/mL) and CS3 (139±69 ng/mL) in the studies done by Masoud MI et al., (CS2-212±79 ng/mL and CS3-208±78 ng/mL) and Sinha P et al., (114±21 ng/mL) [34,36]. However, in other studies done by done by Ishaq RA et al., and Gupta S et al., range of serum IGF-1 of the subjects in stage CS3 were much higher (519.7±175 ng/mL) [10,26]. This discordance may be attributed to the difference in ethnic background.

Also, relatively high standard deviation was observed in the present study sample. This could be a reflection of great inter individual variation of serum IGF-1 levels in the study groups.

In the present study, comparison of serum IGF-1 levels of test and control group showed no statistically significant difference between the groups at T0, T1, T3 and T6. Serum IGF-1 levels of the test group patients showed a steady increase from T0 to T6 but this increase was very slight and was not statistically significant. And in the control group, fluctuation of IGF-1 levels was seen during the 6 months follow-up period. A sharp decline in serum IGF-1 levels was seen from T3 to T6 in the control group. These observations point out that in the test group, with the functional appliance therapy the IGF-1 levels were maintained at a certain level, whereas in the control group, a significant decline in IGF-1 levels occurred from 3rd to 6th month.

Several studies previously conducted on IGF-I have reported that its serum levels in children and adolescents followed a pattern that was closely related to the pubertal growth curve: low in the prepubertal stages followed by sharp increase at puberty and, returning to lower baseline values after pubertal growth had ceased [10,32]. However, in the present study, fall in serum IGF-1 levels was seen in the control group while the patients have still not crossed puberty. This difference could be due to the fact that most of the human studies for evaluating serum IGF-1 levels done earlier are cross sectional in design. Only the study done by Masoud MI et al., is longitudinal in which serum IGF-1 level measurements had been taken annually [11]. These measurements represented the yearly snapshots of the patients showing increase in circulating IGF-1 levels as the patient matures till puberty. But in the present study, it was seen that there was intermittent fall in IGF-1 levels while the patients had still not crossed puberty. This suggests that patients experience several peaks in levels of circulating IGF-1 instead of continuously rising serum levels till puberty. These variations need to be further investigated for confirmation with longitudinal studies.

Limitation(s)

The sample size was small for the study so, the results could not be generalised. The study was underpowered due to insufficient number of individuals in the study and high attrition rate. It seems logical that the areas in the vicinity of the site of proliferative activity i.e., the condylar cartilage and synovial fluid should have been examined for the changes in IGF-1 levels, as is done in most animal studies. But the same is not possible in humans because of invasive nature of the procedure.

CONCLUSION(S)

Functional appliance therapy was not found to be associated with concomitant increase in serum IGF-1 levels. However, the factors like small sample size, sexual dimorphism of serum IGF-1 levels, stage of skeletal maturation had a significant effect on outcome of the present study. Thus, in order to generate a substantive evidence for association between functional appliance therapy and serum IGF-1 levels, further long-term studies with greater sample size and more uniformity in sample selection with regard to stage of maturation of subjects are needed.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 19, 2021
- Manual Googling: Nov 13, 2021
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- Was informed consent obtained from the subjects involved in the study? Yes
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