Telomerase Activity in Diabetic Patients with Angiopathy

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Abstract: Background: Diabetes mellitus (DM) is associated with damage to target organs and premature aging and telomeres serve as a mitotic clock and biological marker of senescence. Aim of study: was to A) evaluate telomerase activity in both type 1 and type 2 diabetic patients with microangiopathy and macroangiopathy, B) to study the possible factors that affect the activity of this enzyme in these patients. Subjects and Methods: Study was carried out on 40 patients from those attending diabetes clinics of Banha University Hospital, they were divided into three groups: Group I: 10 type 1 diabetic patients with angiopathy (4males & 6 females). Group II: 10 type 2 diabetic patients with angiopathy (3males & 7females). Group III: 20 apparently healthy age & sex matched volunteers serving as a control group. The following laboratory investigations were performed to all patients: Plasma glucose (fasting and post prandial), Glycated hemoglobin, Lipogram (Total cholesterol, LDLc, HDLc and Triglycerides), Serum creatinine, Urinary microalbumin and Study of telomerase activity in whole blood. Results: Our results showed: that (HBA1C, microalbumin, creatinine, LDL, TG, TCH, PPBS and FBS) are significantly higher in diabetic patients compared to control group where (telomerase and HDL) are significantly lower in diabetic patients compared to control group. Also, Telomerase activity was significantly low in diabetic patients with HbA1C (≥7%), LDL-c (>100mg/dl), HDL-c (<45mg/dl), TG (>150mg/dl), TCH (>200mg/dl), microalbuminuria (>30mg/ml) respectively compared to diabetic patients with HbA1C (<7%), LDL-c (<100mg/dl), HDL-c (<45mg/dl), TG (<150mg/dl), TCH (<200mg/dl), and normoalbuminuria (<30mg/ml). There was significant relation between telomerase activity and macroangiopathic complications, There was no significant correlation between telomerase activity and each of creatinine level and age in the case group. Statistical analysis showed that 80% of diabetic patients (16 patients) were telomerase negative (lower than cut off value ≤49.20), whereas 20% of diabetic patients (4 patients) were telomerase positive (higher than cut off value > 49.20). Conclusion: it was concluded that telomerase enzyme activity decreased in both type 1 and type 2 diabetic patients with angiopathy, There was a relation between telomerase activity and both micro & macroangiopathic complications. [Ayman El Badawy, Mohamed Shawky, AmrM. ElHammady, Mohamed El-Assal, Ashraf Talaat, Ahmed M. Hussein and Khalid Belal. Telomerase Activity in Diabetic Patients with Angiopathy. Life Sci J 2014;11(4):348-357]. (ISSN:1097-8135). http://www.lifesciencesite.com.

Key words: telomerase, diabetic patients, angiopathy (micro- or macroangiopathy).

1. Introduction:

Diabetes mellitus (D.M) is a syndrome of disordered metabolism, associated with damage to target organs and premature aging1. Usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia)2.

Telomerase enzyme is a ribonucleoprotein maintaining the length of the telomeres (telomeres serve as a mitotic clock and biological marker of senescence) by adding G-rich repeats to the end of the eukaryotic chromosomes. Normal human somatic cells, cultured in vitro, have astirictly limited proliferative potential undergoing senescence after about (50-70) population doublings. In contrast, most of the tumor cells have unlimited replicative potential3.

Telomeres are composed of telomeric DNA and multiple binding proteins that together act as a protective cap on the end of chromosomes. The length of telomeres is maintained by a dynamic equilibrium between processes that shorten and lengthen telomeric DNA. Telomere shortening serves as a checkpoint for the initiation of cell cycle arrest, which leads to cellular senescence (aging) and apoptosis (death). In healthy human somatic cells that lack telomere-lengthening by telomerase, the size of telomeres decreases with each cell division and thus the cells have a finite capacity for replication1. Telomere shortness in humans is emerging as a prognostic marker of disease risk, progression, and premature mortality as in diabetes4. It was found that telomerase activity decreases in both type 1 and type 2 diabetic patients and, since both types of diabetediffer in several aspects, including pathogenesis, severity, participation of immunological system and other biological parameters, the difference in telomerase activity between them may be of interest5. The exact mechanism of low telomerase activity in diabetic patients is still...
controversial and may be explained by hyperglycemia, the proliferative stress imposed on the relevant cells or by the effect of the oxidative and metabolic stress 

Apart from replicative senescence, other exogenous factors e.g., oxidative stress, hypertension, hypercholesterolemia, and hyperglycemia, may be associated with low telomerase activity and telomere attrition. Reduced replicative capacity has been observed in cells harvested from diabetic subjects and this finding has been correlated with some of the degenerative complications of poor glucose control in diabeticsubjects 

2. Subjects and Methods:
This study was carried out on forty patients selected from those attending diabetes clinics of Banha University Hospital. Patients were divided into three groups: Group I: included 10 type 1 diabetic patients with angiopathy(4males & 6females), Group II: included 10 type 2 diabetic patients with angiopathy (3males & 7females), Group III: Included 20 apparently healthy age & sex matched volunteers serving as a control group. Any patient without micro- or macroangiopathic complications was excluded from the study.

I- All patients were subjected to a thorough history and clinical examination with stress on Mode of therapy, Microangiopathy (nephropathy, retinopathy, and neuropathy), Macroangiopathy (coronary, cerebral, and peripheral atherosclerosis) and Duration of diabetes.

II- Specimen collection: After 12 hours fasting, 10 ml venous blood sample was collected from all subjects by veni-puncture and divided as follow:
1. 1.5 ml of blood was delivered into a tube containing 1.2 mg/mlK3EDTA used for glycosylated hemoglobin (HbA1C).
2. The remainder was transferred into a plain tube, allowed to clot at 37°C followed by centrifugation at 3000 r.p.m for 15 minutes. The serum was collected and used for determination of lipogarm, fasting serum glucose level and determination of telomerase activity (white blood cells are the most readily available source of normal human cells in which to measure telomerase activity directly).
3. Another blood sample was withdrawn postprandial for determination of post prandial serum glucose level.

III- The following laboratory investigations were performed to every subject:
A) Fasting and two hours post prandial serum glucose (glucose oxidase) and lipogarm(total cholesterol, triglyceride, HDLcholesterol) and serum creatinine (jaffé method) by Dimension RXL MAX auto analyser (Siemens Medical Solution Diagnostics, UL, USA) with estimation of LDL cholesterol by Fridwald equation LDLc= TCH – (TG/5 + HDLc).

B) Glycated hemoglobin (HbA1C): was assessed by BioSystems reagent kit provided by BioSystems, S.A. Costa Brava 30, Barcelona (SPAIN).

C) Microalbumin: was measured by BioSystems reagent kit provided by BioSystems, S.A. Costa Brava 30, Barcelona (SPAIN).

D) Specific study (Telomerase activity): was assayed in mononuclear cells extract using (Telomerase PCR ELISA PLUS) kit by Photometric Enzyme Immunoassay for Quantitative Determination of Telomerase Activity, Utilizing the Telomeric Repeat Amplification Protocol (TRAP), (developed by Roche Applied Science), (Cat. No. 12 013 789 001).

Statistical analysis:
Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. Data were presented as range, mean ±SD and number (%). An explanation of logistic regression begins with an explanation of the logistic function, which, like probabilities, always takes on values between zero and one.

3. Results:
Table(1): shows that (HBA1C, microalbumin, creatinine, LDL, TG, TCH, PPBS and FBS) are significantly higher in diabetic patients compared to control group where (telomerase and HDL) are significantly higher in diabetic patients compared to control group. Fig (1): Statistical analysis showed that 80% of diabetic patients (16 patients) were telomerase negative (lower than cut off value ≤49.20), whereas 20% of diabetic patients (4 patients) were telomerase positive (higher than cut off value >49.20). Fig (2) shows the value of telomerase in case group & control group. Table (2) and Figs (3,4,5,6,7,8,9,10) shows that there was significant negative correlation between telomerase activity and each of HBA1C level, microalbumin level, LDL level, TG level and TCH level, FBS level, PPBS level, while there was significant positive correlation between telomerase activity and HDL. There was nonsignificant correlation between telomerase activity and each of creatinine level and age in the case group. Table(3) shows that telomerase activity was significantly low in diabetic patients with HbA1C (≥7%) compared to diabetic patients with HbA1C (<7%). Table(4) shows that telomerase activity was significantly low in diabetic patients
with microalbuminuria (>30mg/ml) compared to diabetic patients with normoalbuminuria (<30mg/ml). **Table(5)** shows that telomerase activity was significantly low in diabetic patients with LDL-c (>100mg/dl) compared to diabetic patients with LDL-c (<100mg/dl). **Table(6)** shows that telomerase activity was significantly low in diabetic patients with HDL-c (<45mg/dl) compared to diabetic patients with HDL-c (>45mg/dl). **Table (7)** shows that telomerase activity was significantly low in diabetic patients with TG (>150mg/dl) compared to diabetic patients with TG (<150mg/dl). **Table(8)** shows that telomerase activity was significantly low in diabetic patients with TCH (>200mg/dl) compared to diabetic patients with TCH (<200mg/dl). **Table (9)** shows that there is no significant difference between telomerase activity and creatinine level. **Table (10)** shows that there was no significant correlation between telomerase activity and duration of diabetes. **Table (11):** Logistic Order Regression analysis table, it provided the order of values of patient parameters (HBA1C, lipid profile, microalbumin, Creatinine) for telomerase activity in which HBA1C was the most important parameter (score=21.3 & \( P < 0.001 \)), followed by LDL (score=20 & \( P < 0.001 \)), followed by TCH (score=8.23 & \( P < 0.001 \)), followed by HDL (score=6.55 & \( P < 0.001 \)), then microalbumin (score=5.3 & \( P < 0.05 \)) but creatinine was non significant. **Table(12)** shows that there was significant relation between telomerase activity and macroangiopathic complications. Mean ± SD of telomerase activity in patients with macroangiopathic complications (7 coronary atherosclerosis & 1 cerebral atherosclerosis & 3 peripheral atherosclerosis) (N=11, 55%) Vs patients without macroangiopathic complications (N=9, 45%) was (35±1.6) Vs (75.8±41.4)
Table (2): Correlation of Telomerase Activity with HBA1c, Microalbumin, Creatinine, lipidprofile, FBS, PPS and Age in Case Group:

<table>
<thead>
<tr>
<th>CASE</th>
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<tr>
<td>HBA1C (%)</td>
<td>r</td>
<td>-0.58</td>
<td>&lt; 0.05</td>
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<tr>
<td>Microalbumin (Mg/ml)</td>
<td>r</td>
<td>-0.51</td>
<td>&lt; 0.05</td>
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<td></td>
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<tr>
<td>Creatinine (Mg/dl)</td>
<td>r</td>
<td>-0.34</td>
<td>NS</td>
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<tr>
<td>LDL-c (Mg/dl)</td>
<td>r</td>
<td>-0.72</td>
<td>&lt; 0.001</td>
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<tr>
<td>HDL-c (Mg/dl)</td>
<td>r</td>
<td>0.51</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>TG (Mg/dl)</td>
<td>r</td>
<td>-0.67</td>
<td>&lt; 0.001</td>
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<tr>
<td>TCH (Mg/dl)</td>
<td>r</td>
<td>-0.81</td>
<td>&lt; 0.001</td>
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<tr>
<td>PPBS (Mg/dl)</td>
<td>r</td>
<td>-0.47</td>
<td>&lt; 0.05</td>
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<tr>
<td>FBS (Mg/dl)</td>
<td>r</td>
<td>-0.45</td>
<td>&lt; 0.05</td>
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<tr>
<td>AGE / y</td>
<td>r</td>
<td>-0.1</td>
<td>NS</td>
<td></td>
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</table>

Fig(1) ROC Curve

Fig(2): Histogram for Telomerase Value in Control and Case group.
Fig(3): Scatter diagram for correlation between Telomerase activity and HBA1c in case group.

Fig(4): Scatter diagram for correlation between Telomerase activity and Microalbumin in case group.

Fig(5): Scatter diagram for correlation between Telomerase activity and LDL-C in case group.

Fig(6): Scatter diagram for correlation between Telomerase activity and Triglycerides in case group.

Fig(7): Scatter diagram for correlation between Telomerase activity and Total Cholesterol in case group.

Fig(8): Scatter diagram for correlation between Telomerase activity and FBS in case group.

Fig(9): Scatter diagram for correlation between Telomerase activity and PPS in case group.

Fig(10): Scatter diagram for correlation between Telomerase activity and HDL-C in case group.
Table(3): Comparison of telomerase activity in patients with HBA1C(<7%) versus patients with HBA1C(>7%).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>Hba1c</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;7%</td>
<td>89.2±47.7</td>
<td>43.1±26</td>
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</table>

Table(4): Comparison of telomerase activity in patients with Microalbumin(<30mg/ml) versus patients with Microalbumin(>30mg/ml).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>Microalbumin</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;30mg/ml</td>
<td>58.3±39.3</td>
<td>34.5±1.1</td>
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</tbody>
</table>

Table(5): Comparison of telomerase activity in patients with LDL-C(<100mg/dl) versus patients with LDL-C(>100 mg/dl).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>LDL-c</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;100mg/dl</td>
<td>106.2±58.4</td>
<td>42.9±20.5</td>
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</tbody>
</table>

Table(6): Comparison of telomerase activity in patients with HDL-C(<45 mg/dl) versus patients with HDL-C(>45 mg/dl).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>HDL-c</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&gt;45mg/dl</td>
<td>81.3±41.8</td>
<td>36.8±4.4</td>
</tr>
</tbody>
</table>

Table(7): Comparison of telomerase activity in patients with Triglycerides(<150mg/dl) versus patients with TG(>150mg/dl).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>TG</th>
<th>t</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;150mg/dl</td>
<td>87.9±73.6</td>
<td>37.1±4.4</td>
</tr>
</tbody>
</table>

Table(8): Comparison of telomerase activity in patients with Total Cholesterol(<200 mg/dl) versus patients with TC(>200 mg/dl).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>TCH</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;200mg/dl</td>
<td>77.7±45.4</td>
<td>42.9±22.5</td>
</tr>
</tbody>
</table>

Table(9): Comparison of telomerase activity in patients with Creatinine(<1.5mg/dl) versus patients with Creatinine(>1.5mg/dl).

<table>
<thead>
<tr>
<th>Telomerase activity</th>
<th>Creatinine</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>&lt;1.5mg/dl</td>
<td>52.8±36.3</td>
<td>47.9±0.00</td>
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</table>
4. Discussion

Telomerase enzyme is a ribonucleoprotein maintaining the length of the telomeres (telomeres serve as a mitotic clock and biological marker of senescence) by adding G-rich repeats to the end of the eukaryotic chromosomes. Activation of telomerase by extracellular factors may have significant implications in activating and mobilizing stem cells, for tissue repair, organ regeneration and anti-aging regimes. Inactivation of telomerase has been demonstrated to be critical to stem cell renewal and potentially to incur aging diseases e.g. type 2 D.M.

There are several reasons why we studied telomerase activity in diabetic patients: firstly, to gain an objective measurement of disease activity as regard angiopathy in type 1 & type 2 patients; and secondly, trying to find a tool for prediction and management of CVD.

Our study was conducted on forty persons: 10 type 1 diabetic patients with angiopathy, 10 type 2 diabetic patients with angiopathy& 20 age and sex matched apparently healthy volunteers serving as control.

Our study showed that 80% of diabetic patients (16 patients) were telomerase negative, whereas 20% of diabetic patients (4 patients) were telomerase positive, fig.(1). Among 16 telomerase negative diabetic patients, 9 patients (56.25%) were type 1 D.M and 7 patients (43.75%) were type 2 D.M.

Our work showed that in comparison to control group, telomerase activity was significantly low in diabetic patients. This is in agreement with other studies who reported that telomeres serve as a mitotic clock and biological marker of senescence.

Our results showed that the state of glycemic control had a significant impact on the telomerase activity in diabetic patients. This could be inferred from the following:

1. Telomerase activity was significantly low in diabetic patients with HbA1C (≥7) compared to diabetic patients with HbA1C (<7), table(3).
2. A significant negative correlation was found between telomerase activity and each of HBA1C level, FBS and PPBS in diabetic patients, table (2), figs. (3, 8, 9).
3. In Logistic Order Regression analysis: HbA1C greatly affected telomerase activity in which HbA1C
value indicative of a poor glycemic control in this study show HbA1C levels higher than 7%, about 70% of elderly Type2DM patients enrolled. About the 70% of elderly Type2DM patients enrolled in this study show HbA1C levels higher than 7%, a value indicative of a poor glycemic control.

Previous studies have shown that Type2DM patients with well controlled diabetes did not have statistically significant shorter telomeres than controls, denoting the protective effect of efficient glycemic control on damage and premature senescence of these cells.

In our study all cases had microangiopathic complications and most of them had decreased telomerase activity. So, we can conclude that there is a relation between telomerase activity and microangiopathic complications. Also, there was 11 cases (55%) had macroangiopathic complications (7 coronary atherosclerosis & 1 cerebro-arteriosclerosis & 3 peripheral atherosclerosis). Our results showed that macroangiopathic complications had a significant impact on telomerase activity in diabetic patients. This could be inferred from that telomerase activity was significantly low in diabetic patients with macroangiopathic complications (N=11, 55%) compared to diabetic patients without macroangiopathic complications (N=9, 45%), table (12). This is consistent with other studies who reported that patients with diabetes are at high risk of macro- and microangiopathic complications development. Macroangiopathic complications in diabetes are considered to be as symptoms of premature aging.

Our results showed that microalbuminuria had a significant impact on the telomerase activity in diabetic patients. This could be inferred from the following:

1. Telomerase activity was significantly low in diabetic patients with microalbuminuria (≥30) compared to diabetic patients with no microalbuminuria (<30), table (4).
2. A significant negative correlation was found between telomerase activity and microalbumin in diabetic patients, table (2), fig.(4).
3. In Logistic Order Regression analysis: microalbumin also, affected telomerase activity in which microalbumin Score = (5.3), P= (<0.05), table (11).

This is in agreement with other studies who reported that some Type2DM complications, such as microalbuminuria (a urinary albumin excretion rate >30 mg/24 h.) and MI, are associated with lower telomerase activity & shorter telomere length.

Also, in our results we found that there was no relation between telomerase activity and age, tables (1 & 2).

This may be due to narrow age range in our study with (Mean ± SD) = (39.6±5.2) years. Although there is confirmed relation between telomerase and aging as reported in researches in the past two decades that points to a link between organismal aging and aging-related diseases and cellular senescence caused by telomere shortening. Several lines of evidence strongly suggest that the resulting telomere dysfunction could have a causal role in some aging and aging-related diseases.

Also, in our results we found that there was no relation between telomerase activity and creatinine, tables (2 & 9 & 11). This is in agreement with that plasma creatinine concentration inversely related to GFR but GFR can decrease by 50% before plasma creatinine concentration rises beyond the normal range. Plasma creatinine concentration doubles for each further 50% fall in GFR. So, normal plasma creatinine doesn't necessarily imply normal renal function, and also, raised plasma creatinine doesn't usually indicate impaired renal function. This may be due to that changes in plasma creatinine can occur independently of renal function, due to changes in muscle bulk, thus a decrease can occur in starvation, wasting diseases, immediately after surgery and in patients treated with corticosteroids. Our results showed that lipid profile (HDL-c, LDL-c, T.G, and T.C.H) had a significant impact on the telomerase activity in diabetic patients. This could be inferred from the following:

1. Telomerase activity was significantly low in diabetic patients with LDL-c (≥100), TG (≥150), TCH (>200), HDL-c (<45) compared to diabetic patients with LDL-c (<100), TG (<150), TCH (<200), HDL-c (>45), tables (5, 6, 7, 8).
2. A significant negative correlation was found between telomerase activity and (LDL-c, T.G, T.C.H) in diabetic patients, table (2), figs.(5, 7, 8).
3. A significant positive correlation was found between telomerase activity and HDL-c in diabetic patients, table (2), fig.(6).
4. In Logistic Order Regression analysis: LDL-c affected telomerase activity in which Score = (20), P= (<0.001), HDL-c affected telomerase activity in T.C.H.
which Score = \((6.55)\), \(P= (<0.001)\), T.G affected telomerase activity in which Score = \((6.56)\), \(P= (<0.001)\), and also, T.CH affected telomerase activity in which Score = \((8.23)\), \(P= (<0.001)\); table (11).

This is in agreement with other studies who reported that diminished levels of high-density lipoprotein cholesterol (HDL-C) are associated with an increased risk for atherosclerosis\(^{21,22}\), a disease that is marked by chronic, low grade, inflammation and increased burden of oxidative stress\(^{23,24}\).

It was found that there is positive correlation between HDL-C and telomerase activity in many\(^{25,26}\). Low levels of HDL cholesterol are considered as one of the components of, atherogenic dyslipidemia\(^{27}\) and low HDL cholesterol phenotypes have been recently shown to display elevated oxidative stress, attenuated antioxidative activity\(^{28}\) and accelerated senescence\(^{29}\).

Conclusions:

From the previous results it was concluded that telomerase enzyme activity decreased in both type 1 and type 2 diabetic patients with angiopathy. There was a strong and significant relation between telomerase activity and the occurrence and presence of both micro & macroangiopathic complications. Telomerase activity and telomere length may be a possible new marker in the prediction of cvd and diabetes representing the contribution of tissue aging, atherosclerosis and their linked pathology, also we recommend that this trial must be done on a large number in diabetic patients to assess, compare and confirm these results.

References


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