Computed Tomography for Evaluation and Follow-up of Hepatocellular Carcinoma after Microwave Ablation

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: When surgical procedures are not possible, image-guided HCC tumor ablation provides curative treatment or acceptable therapeutic choices in appropriately selected patients. Post-ablation tumor response evaluation is critical for assessing treatment effectiveness and further therapy. Ultrasound (US) and Computed Tomography (CT) are critical in the follow-up of patients who have undergone liver thermal ablation therapy. The purpose of this study was to evaluate the role of computed tomography for evaluation and follow-up of hepatocellular carcinoma following Microwave ablation.

Methods: This prospective study was carried out on 30 patients radiologically proved with hepatocellular carcinoma.

Results: Triphasic CT imaging arterial phase revealed homogenous enhancement lesions in 46.7% of patients and heterogeneous enhancement in 53.35% of patients. All the lesions showed early washout in Porto venous and delayed phases with a patent portal vein in all patients. The diameter of the ablated area was different according to the duration of ablation. 60 W was applied for 8-10 minutes, resulting in ablation zones of 3.5-4 x 4.7-5.2 cm whereas applications of 60 W for 10-15 minutes resulted in ablation zones with a size of 4.5 - 5.5 x 5.6-6.5 cm. Triphasic CT was
performed after 1 month and revealed that the success rate was 93.8% for tumors measuring ≤ 3 cm, 92.9% for tumors measuring 3-5 cm. Local tumor progression was shown in 2 patients (6%) after 1 month, in 3 patients (10%) after 3 months, and in 5 patients (16.7%) after 6 months of follow-up. There was an intrahepatic distant recurrence in 9 patients (30%) after 1 month, in 15 patients (50%) after 3 months, and in 18 patients (60%) after 6 months of follow-up.

Conclusions: Percutaneous microwave ablation guided by ultrasound for the treatment of challenging HCC tumors up to 5 cm in diameter, including exophytic or subcapsular targets as well as those located in the hepatic dome or close to the diaphragm / hepatic hilum / heart, shown satisfactory efficacy and safety rates. For both technical and clinical success, selecting the proper approach is critical.

Keywords: Computed tomography; hepatocellular carcinoma; microwave ablation.

1. INTRODUCTION

The standard therapeutic options of hepatocellular carcinoma (HCC) consist of surgical resection, ablation, trans-arterial therapy (chemoembolization or radiotherapy). Hepatectomy, liver transplantation, and percutaneous thermal ablation are all potentially curative therapies. The remaining therapeutic options are mostly palliative with a positive impact on survival [1].

Microwave Ablation (MWA) utilizes electromagnetic energy (up to 2 cm surrounding the antenna); in the absence of current flow, the electromagnetic field rapidly and uniformly heats the tissue, resulting in coagulation necrosis. Tissue with a high water content has the highest heating effect whereas fat has the worst. Ionic polarization is considered another mechanism of MWA, which results in the conversion of kinetic energy into heat. MWA can be CT or US-guided [2]. MWA is effective in the treatment of 5-8 cm HCC. In addition, MWA permits simultaneous tumor ablation or even combination resection and ablation.

CT plays an important role before and after the MWA of HCC. CT imaging is done before ablation to determine the size, number, location and relation to vascular structures and immediately after ablation to detect any potential complications. Follow up by CT at 1, 3, 6 and 12 months post-ablation and annually ever since are done for assessment of tumor response to prove the efficacy of ablation or detect residual activity [3]. The modified Response Evaluation Criteria in Solid Tumors (mRECIST) is used nowadays to assist tumor response after MWA. It includes evaluation of response in both target lesions and non-target lesions as well as potential new lesions. It defines the diameter of viable tissue of the target lesion by contrast enhancement in the arterial phase [4]. The purpose of this work was to evaluate the role of computed tomography in the evaluation and follow-up of HCC after microwave ablation.

2. PATIENTS AND METHODS

30 patients were enrolled in this prospective study who were radiologically proved with hepatocellular carcinoma, who were referred to Diagnostic Radiology and Medical Imaging Department at Tanta University Hospitals and Liver Institute at Kafr-Elsheikh. Abdominal ultrasonography & triphasic CT scan were performed for all patients. The exclusion criteria for the percutaneous US-guided MWA: Age, patients above the age of 80, presence of vascular involvement, more than three intrahepatic nodules, evidence of extrahepatic metastases, patients with severe hemodynamic instability, patients with serous coagulation disorder or acute sepsis or cardiac patients, severely debilitated patients and patients with renal insufficiency. The patients were subjected to the following: Complete history taking: Personal history that includes sex, age and special habits like smoking, alcoholism, current illness's history with special concern on right hypochondrial pain or swelling and history of viral hepatitis and cardiac or renal trouble. The patients were asked about any previous imaging procedures (abdominopelvic ultrasound and abdominopelvic CT). All the patients were subjected to clinical abdominal and surgical examinations. Vital signs as Blood Pressure (BP), Respiratory Rate (RR) and Pulse Rate (PR). Airway, breathing, circulation and disability are all evaluated according to the role of A B C D. Laboratory investigations were conducted such as Liver functions: Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), serum albumin and total bilirubin level, complete blood
picture, coagulation profile including International Normalized Ratio (INR), Prothrombin time and concentration and Alpha-fetoprotein level: It was done before and after 1, 3 and 6 months after ablation. Moreover, imaging techniques were used such as Pelvi-abdominal US for evaluation of: (Focal lesions (size, shape, size, number and echogenicity), biliary or vascular invasion and the percutaneous approach possibility - Exclusion of Splenomegaly- extra-hepatic metastases especially porta hepatitis or paraaortic lymph node enlargement- Presence of ascites and if present evaluation of its amount- Color Doppler the US is used to determine the patency of the portal vein. For all patients, a triphasic CT scan of the liver is performed: The aim was to study the enhancement pattern of the focal lesion. The satellite's presence and vascular or biliary invasion was also evaluated, exclusion of extrahepatic metastases. It was done before ablation and repeated after 1, 3 and 6 months from the ablation. Evaluation of previous triphasic CT, abdominal ultrasound and colored doppler imaging. Furthermore, Multi-slice CT was performed by using multi-slice 16 detectors CT Toshiba.

**Patient preparation:** No specific patient preparation was requested except fasting for 6 hours before performing the procedure, filling the stomach and bowel with water to help proper subtraction techniques and visualization of the target vessels. All instructions were given to the patients about table movement, voice messages, timing and manner of breath-holding.

**Patient position:** The patients were laid down on the couch in the supine position, headfirst with the arms elevated above the head from the level of the tracheal carina down to the level of the symphysis pubis.

Gonadal shielding was used in all patients. Using a detector collimation of 1.2 mm and voltage 120 kV, thin-slice images were obtained with slice thickness 2 mm, an increase of 1 mm then transferred to an independent workstation.

In anteroposterior view, one scout was acquired. Pre and post-contrast sequences were planned on these scouts from the level of the top of the right diaphragmatic copula (hepatic dome) to about 20 cm caudally or the iliac crest with a slice thickness of around 6-8 mm.

Interventional procedure US-guided microwave ablation (one session was done for twenty-eight patients & two sessions for two patients with incomplete ablation in our study).

**Patient preparation:** Patients fast overnight and were admitted on the day of the procedure in the morning.

On the other hand, the Ablation technique used; Sedation and anesthesia: Microwave ablation were performed with the use of anesthesia for all patients (short-acting anesthesia). The equipment included microwave coagulator and thermal monitoring system. The technique before the beginning of treatment, a detailed plan describing the electrode placement. On a tumor-by-tumor basis, the emission time and power output set were determined. The tumor and the 0.5–1.0 cm of normal-appearing liver tissue that surrounding it were to be eradicated during therapy. Microwave ablation for lesions located in the right lobe was conducted under real-time US guidance utilizing a 3.5 MHz probe by freehand technique, Intercostal approaches with the patient in the left lateral decubitus posture were more frequently employed, whereas subcostal approaches were most frequently used for lesions found in the left lobe. To establish local anesthetic, 10 ml of 2% xylocaine is used to anesthetize the skin, subcutaneous tissue, muscles, and the liver capsule along the assumed track of entry, after povidone-iodine has been used as a contact medium, using sonographic guidance, a 16-gauge 15-cm guide needle with an antenna was inserted and placed at the targeted tumor location. The active tip was entered into the deepest portion of the tumor and attached to the microwave generator. The energy application was then initiated. To avoid damage to surrounding structures such as the gastrointestinal tract, the diaphragm, or gallbladder, the microwave ablation electrode was inserted into the tumor perpendicular to the surrounding structure. Power output and time emission were variable according to the site and the size of the tumor. The applications of 60 W for 8-10 minutes were effective for the treatment of tumors <3 cm and applications of 60 W for 10-15 minutes were effective for the treatment of tumors 3-5 cm. The needle track was cauterized for 10 seconds before removing the antenna, to avoid seeding of tumors. Throughout the procedure, vital signs as heart rate, blood pressure, rate of respiration, and the levels of oxygen saturation were regularly monitored.

**Aftercare:** Complete bed rest for 12 hours. Observation of blood pressure and pulse for
every 30 minutes for 2 hours and then every 2 hours for 12 hours. Analgesic (Panadol 500 mg 3 times/day for 5 days) to control any pain experienced after the procedure. Intravenous administration of antibiotics (Ciprofloxacan 200mg /12h) was routinely used for 3 days. Patients were discharged following a six-hour hospitalization with stable vital signs.

**Evaluation of therapeutic efficacy and follow-up**: To determine the responsiveness of tumors to microwave ablation therapy; Triphasic CT where before ablation to determine the location and size of HCC (concerning vascular structures and hepatic segments) and post-contrast enhancement criteria. After ablation at 1, 3, and 6 months to determine the response to the ablation and any complications.

Following treatment, patients who achieved complete ablation had a follow-up, whereas those who did not achieve complete ablation were scheduled for further therapy. Regrowth of tumor inside or adjacent to the nodule that had previously been ablated was defined as Local Tumor Progression (LTP) whereas the emergence of additional lesions in the liver parenchyma or elsewhere was described as Distant Tumor Progression (DTP). Serum alpha-fetoprotein level was checked at 1, 3, and 6 months after MWA.

**2.1 Statistical Analysis**

The data were collected and entered into the computer using SPSS (Statistical Package for Social Science) program for statistical analysis, version 21 (SPSS Inc, Chicago, IL, USA). As appropriate, data were input numerically or categorically. Two different types of statistical analysis were conducted: Descriptive statistics: Qualitative data were expressed as frequency and percent at 95% confidence interval (95% CI) and quantitative data were shown as a range, SD and mean.

**3. RESULTS**

Demographic data of the studied groups, risk factors for HCC, and clinical presentation of the study participants were that the total number of studied participants was 30; just 8 (26.7%) of which were between 40 to 50 years old; where 6 of them were males and 2 were females. While 13 participants (43.3%) aged between 50 and 60 years old; divided to 10 males and 3 females. Moreover, 7 (23.3%) participants aged between 60 and 70; where 6 were males and 1 female. Lastly, 2 (6.7%) male participants were between 70 and 80 years old. The risk factors included HCV 25 (83.3%), HBV 4 (13.3%), Diabetes mellitus 18 (60%), and smoking 9 (30%). While regarding clinical presentation; pain was faced by 15 (50%) participants, while Jaundice 10 (33.3%), loss of weight and appetite 10 (33.3%), and fever 8 (26.6%).

Concerning the liver function tests of the study participants: Albumin level (g/dL) of >3.5 were 18 (60%) participants, while for the 2.8 - 3.5 level they were 12 participants (40%).

For the Total Bilirubin level (mg/dL) of level ≤ 1 they were 20 participants (66.7%), while level <1-2 was 10 participants (33.3%).

Serum Glutamic-Oxaloacetic Transaminase (SGOT) Aspartate Transaminase [AST] (IU/L) at level ≤ 40 had 14 participants (46.7%), while for level > 40 they were 16 participants (53.3%). Also, the (Serum Glutamic-Pyruvic Transaminase) SGPT [Alanine Aminotransferase ALT] (IU/L) at level ≤ 40 where 14 participants (46.7%), while level > 40 were 16 participants (53.3%). For Prothrombin concentration (%) at level > 80 they were 13 participants (43.3%), while for 50-80 they included 17 participants (56.7%).

For the International Normalized Ratio (INR) at level ≤1.4 this included 14 participants (46.7%), while level >1.4-2 had 16 participants (53.3%).

US findings of the study participants are shown in Table 1.

US and CT criteria of hepatic focal lesions and segmental distribution of hepatic focal lesions (hepatocellular carcinoma) of the study participants are shown in Table 2.

HCC can be treated locally utilizing microwave ablation with cooled shaft antennas.

Recurrence of HCC in the study participants. The distribution of the studied sample according to AFP, after 1 month, showed a significant decline.

Regarding the different complications occurred after microwave ablation in the studied thirty patients, major complications included: Ablation lesion infection (liver abscess) 1 (3.3%), Pneumothorax 1 (3.3%), Hydropneumothorax 2 (6.7%), and Hematoma 4 (13.3%).
Table 1. US findings of the study participants

<table>
<thead>
<tr>
<th>US examination</th>
<th>Study participants (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotic liver</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Liver size</td>
<td></td>
</tr>
<tr>
<td>Enlarged</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>Average</td>
<td>20 (66.7%)</td>
</tr>
<tr>
<td>Shrunken</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Patent portal vein</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>2 (6.67%)</td>
</tr>
<tr>
<td>Mild</td>
<td>11 (36.67%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Marked</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>8 (26.67%)</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
</tr>
<tr>
<td>Minimal ascites</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>No ascites</td>
<td>24 (80%)</td>
</tr>
</tbody>
</table>

**US**: Ultrasound

Table 2. US and CT criteria of hepatic focal lesions and segmental distribution of hepatic focal lesions (Hepatocellular carcinoma) of the study participants

<table>
<thead>
<tr>
<th>Criteria of the lesion</th>
<th>Study participants (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US criteria</td>
<td></td>
</tr>
<tr>
<td>Echogenicity</td>
<td></td>
</tr>
<tr>
<td>Hypoechoic</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Hyperechoic</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Heterogenous</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>Size</td>
<td></td>
</tr>
<tr>
<td>≤3cm</td>
<td>16 (53.3%)</td>
</tr>
<tr>
<td>3-5cm</td>
<td>14 (46.7%)</td>
</tr>
<tr>
<td>Lesion location</td>
<td></td>
</tr>
<tr>
<td>Right lobe</td>
<td>17 (56.6%)</td>
</tr>
<tr>
<td>Left lobe</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>Right and left lobes</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>CT criteria</td>
<td></td>
</tr>
<tr>
<td>Arterial enhancement</td>
<td></td>
</tr>
<tr>
<td>Homogenous</td>
<td>14 (46.7%)</td>
</tr>
<tr>
<td>Heterogeneous</td>
<td>16 (53.35%)</td>
</tr>
<tr>
<td>Hepatic segment</td>
<td></td>
</tr>
<tr>
<td>Study participants</td>
<td></td>
</tr>
<tr>
<td>(n = 30)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>III</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>IV</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>V</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>VI</td>
<td>5 (16.7%)</td>
</tr>
<tr>
<td>VII</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>VIII</td>
<td>8 (26.7%)</td>
</tr>
</tbody>
</table>

**US**: Ultrasound, **CT**: Computed Tomography

4. DISCUSSION

This study showed that overall, males had a more than 4-fold increased incidence of HCC compared to females. Consistent with previous data from Liu et al. [5] and Zang et al. [6] studies [6,7]. For those patients with an age over 70 years, the magnitude of male predominance was the lowest. According to these findings, sex hormones and reproductive factors may have a vital role in HCC development. in HCC risk. Elevated estrogens levels are believed to have a role in protecting against the development of HCC, while elevated androgen levels may promote tumorigenesis [7]. However, even among those over 70 years, males had a nearly 2-fold increased risk of developing HCC compared to females. Males have higher rates of HCV infection and heavy alcohol use than females, which may explain the male prevalence in HCC, although hormonal and reproductive factors may explain some of the sex effects in HCC [6].

Our results were supported by what was reported by Puoti, 2018 [8], that the age between 50 and 69 showed the highest incidence of HCC. Our findings are in agreement with [9], who found that
the age range of HCC patients was 42 to 70 years, and also with Shaker et al., [10] who found that in Egypt the most frequently affected age category by HCC was between 51 and 60 years. The age of onset of HCC is variable according to different locations of the world. In North America, Japan and Europe, where the onset average age is more than 60 years, HCC is more likely to develop later in life. By contrast, in the majority of African countries and parts of Asia, according to Yang et al., 2019 [11] HCC is most frequently diagnosed in participants between the ages of 30 and 60. Burnot et al., 2016 [12] explained HCC onset in middle-aged and elderly populations in their study. They reported that the increasing prevalence of noninfectious cirrhosis liver, which is mostly associated with HCC, is a result of the disease developing later in life. Additionally, Antiviral medications and vaccination have improved long-term management of chronic HBV or HCV infection, but have also delayed the onset of liver cirrhosis and hepatocellular carcinoma development. Furthermore, HCV infection occurs more often in adults and has more severe consequences in the elderly, including severe histological damage and increased liver cirrhosis. The latter is a significant contributor to the development of HCC in the elderly. Yang et al., 2019 [11] documented that chronic HBV and HCV infection is the primary cause of HCC, accounting for approximately 80% of patients of HCC worldwide. In Eastern Asia and the majority of Africa, chronic HBV infection is thought to be the primary cause of HCC, except in northern Africa, where HCV is more prevalent. This is supporting our findings, which are in agreement also with what has been established by Blachier et al., 2013 [13] that HCV-infected patients have a 15-20-fold risk of developing HCC compared with HCV negative patients.

Concerning diabetes mellitus, Rashed et al., 2020 [14] mentioned that several Genome-Wide Association Studies (GWAS) have revealed various locations associated with an increased risk of type 2 diabetes. The link between type 1 diabetes and an increased risk of developing (HCC) is currently unclear. There are several probable explanations for the association between diabetes and an increased risk of Hepatocellular Carcinoma (HCC). One of the metabolic syndrome components which may cause NASH and HCC after it is diabetes. Also, Yang et al., 2017 [15] mentioned that persistently elevated insulin levels in type 2 diabetes patients result in both increases in insulin-like growth factor-1 (IGF-1) levels and Insulin Resistance (IR) in the majority of tissues, including the liver, which may improve the development of carcinogenesis. Moreover, hepatocyte damage and oxidative stress might be caused by prolonged hyperglycemia. In aligning with Fenoglio et al., 2013 and Aljumah et al., 2016 [16,17] studies. They reported that most patients in their studies were of Child-Pugh class A. Evaluation of liver function is particularly important in clinical trials because it is perceived that cirrhosis is a competing cause of mortality. Many HCC therapy trials are confined to patients with C-P grade A to isolate the effect of a particular HCC medication on survival. In a match with these findings, Holah et al., 2015 [18] study also revealed that HCC cases were virtually equal in the left and right lobes (50.0 and 44.6%, respectively) while only 5.4% of HCC cases affected both lobes. This may be explained by that their study population lesions were mainly of the fibrolamellar type, with its documented left lobe predominance. In accordance, Zhou et al., 2013 [19] reported that microwave ablations with ≥ 5 minutes time duration can induce coagulation zones with clinically desirable ablation shape at the power from 40 W to 80 W. Guan, 2015 [20] found that with tumor sizes ranging from 3 to 5 cm in diameter, microwave power for ablation is set at 50-60 W for 5-15 min. Nevertheless, our figures are relatively distant from those of Liu et al. 2013 and Poggi et al. 2013 [21,22] which may be attributed to the use of different microwave machines.

Our data are comparable to previous studies. Dawoud et al., 2019 [23] revealed that total complete response was achieved in 92.3% of patients, Alexander et al., 2015 [24] reported technical success rates of 95.3%. Xu et al., 2017 [25] reported primary local efficiency of 95.2% and also Hetta et al., 2011 [26] reported complete ablation in 96% of nodules. In the study of Dawoud et al., 2019 [23], tumor size was the single significant predictor of initial complete ablation.

Our results emphasized the previously concluded association between the HCC status and AFP levels such as in the studies of Toro et al., 2014 and Wang et al., 2016 [27,28]. Yolk sac cells, Fetal hepatocytes, and gastrointestinal cells produce α-Fetoprotein, which is considered an oncopetal protein, AFP is generated by dedifferentiated HCC cells in comparison to normal hepatocytes. Thus, even in the absence
of evident cancer, dedifferentiation and carcinogenesis in hepatocytes can be predicted by an increase in AFP serum levels. In addition to the persistence of HCC, elevated AFP levels following RFA show that the non-cancerous liver is in a highly carcinogenic condition [29].

Pain and fever that occurred in the present study are expected and mostly resulted from an inflammatory response to the necrotic tissue with cytokines production [30]. The presence of pleural effusion following thermal ablation was previously attributed to temporary pleurisy caused by the thermal effect. Direct thermal damage to the pleural membranes may cause an increase in pleural capillary filtration and interference with the evacuation of parietal pleural fluid, resulting in the creation of a pleural effusion [31].

For the treatment of malignant liver tumors, MW ablation is a well-tolerated therapy with an acceptable risk of significant complications [32]. In variable distances from our findings, over 13 years, a large-scale study conducted by Liang et al. 2009 found that the rate of significant complications is approximately 2.6 % (152). Fever was the most often reported adverse effect, occurring in 83.4 % of participants in the study of Liang et al. [33]. Livraghi et al., 2012 [34] stated the safety of MWA as reported 0% mortality, 7.2% minor complications and 2.9% major complications. Mobarak et al., 2020 [35] reported no major complications or mortality were related to the MWA procedure, and only minor complications in the form of right hypochondrial pain (12%) were reported. In the study of Li et al., 2012 [36] moderate to massive pleural effusion occurred in 3.1% of patients, and Huang et al., 2014 [37] reported one case of portal vein thrombosis (0.7%) and two cases of tumor seedling (1.4%) out of 139 perivascular lesions.

In a multicenter effort that collect data for patients treated with MWA for tumors of any origin, Microwave ablation has many advantages over RF ablation, including a shorter total period of microwave application for each lesion (median: 4 minutes/lesion), the use of fewer microwave applications for each ablated lesion, and the capability to coagulate blood vessels more efficiently and an important advantage of MWA over RF ablation is a less severe heat sink effect [34,38].

5. CONCLUSION
Percutaneous microwave ablation guided by ultrasound for the treatment of challenging HCC tumors up to 5 cm in diameter, including exophytic or subcapsular targets as well as those located in the hepatic dome or close to the diaphragm / hepatic hilum / heart, shown satisfactory safety and efficacy rates. For both technical and clinical success, selecting the proper approach is critical.

DISCLAIMER
The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT
Informed written consent before participation in the study was collected from all patients.

ETHICAL APPROVAL
This study was approved by our local research ethical committee.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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