In vivo MR Measurement of Refractive Index, Relative Water Content and T2 Relaxation time of Various Brain lesions With Clinical Application to Discriminate Brain Lesions

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Citation

Abstract
PURPOSE: Refractive index (RI) is unique in discriminating various tissues. It is dependent on water and solid components such as protein and phospholipid of brain tissue resembling hydrogel. Our purpose was to determine RI of normal and pathological tissues of brain from the MR T2 relaxation time (T2 value) to determine relative water and solid contents of a tissue and discriminate various pathological lesions by the help of RI in T2 weighted MR images.METHODS: In a 0.3 Tesla permanent and 3 Tesla superconductor magnets T2 maps were created along with T2 shades to get T2 value of various brain tissue homogenates prepared from biopsy samples of known RI using multi-echo datasets by a read out train. T2 value of CSF, gray/white matter and various pathological lesions were determined. From the relationship of RI and T2, a RI map of brain and RI shade were produced. Computer generated RI and color coded map of T2 weighted images of various lesions were created. RESULTS: Analyzing various RI of pathological lesions it was noted that, RI of malignant lesions were more than 1.421 whereas RI of benign lesions were less than 1.395 CONCLUSION: RI and color coded map may discriminate between benign and malignant lesions of brain.

INTRODUCTION
Gray matter and white matter of Brain have Refractive indices (RI) of 1.395 and 1.4102 respectively. Any deviation or shift from the normal values of gray matter and white matter is considered abnormal (1).

Refractive index (RI) is one of the important parameters in biomedical diagnostics. The in situ RI of living tissue can serve as an important indicator of tissue state. Its Image quality depends on the refractive index differences of its constituents (2). RI is related to the physical structure of the medium through which light or electromagnetic photon is passing. For this reason, RI is a characteristic of substances that can be employed to identify unknown (2). Index of refraction can be calculated if the structure of a compound is known, or the refractive index can be experimentally determined.

The RI of tissue is directly related to its water content and percentage of solid component. This is based on Gladstone-Dale law (3) of a compound like hydrogel which can predict final RI (N) of a medium based on the RI of water content (N1) and solid portion (N2) and their relative proportions as follows :N=N1. X1 + N2.X2 ---- [1]

Where X1 is the relative proportion of water present in the medium as a percentage ( a) and X2 is the relative proportion of solid present in the medium (100-a) (3).

Or we can write N= N1.a +N2. (100-a) --- [2]

This equation also holds good in brain tissue as it resembles hydrogel containing water and protein/phospholipids in different percentages.

It was noted that RI values of various benign and malignant tumors including metastasis determined by an Abbe refractometer in the laboratory were found to be unique (4). They are different and dependent on water and protein/phospholipid percentages. Magnetic resonance is highly sensitive to water and water changes. Accurate and quantitative water and solid components like protein and phospholipids of a brain lesions can be extracted in the
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clinical setting using T1 or T2 relaxation time (5-12).

To determine RI of gray matter, white matter, brain tumors and other brain masses of living human beings from MRI, water content of the various tissues were determined in protein solution. 1H relaxation is influenced by exchange of protons between water, amide or hydroxyl group on macromolecules, leading to proton relaxation rates (R1=1/T1 and R2=1/T2) that are linearly related to protein/metabolites concentration (6).

In the brain, relaxation is dominated by exchange of protons between intracellular free and bound water and various tissue and tumor related proteins (7) so that proton relaxation particularly the T2 value will be strongly dependent on various protein/metabolite concentrations. It has been shown that a very strong correlation between T2 and water content in brain exists and that a reduction of the water content from 80 to 40 wt.% decreases the measured T2 by a factor of about 4 whereas the longitudinal relaxation time T1 decreases by a factor of 0.66 (13). Unlike T1, T2 relaxation time changes slightly by the strength of the magnet. It has been reported that RI of intra ocular lens is related to various lens protein concentration (14). Similarly a relationship between T2 relaxation of the normal gray/white matter and brain lesions is expected and can be obtained in combination of standard MRI methods to map the RI distribution in various brain lesions (RI=1/T2)(9,14).

The relaxation of both T1 and T2 of brain tissue are influenced by the dynamic structure and amount of water present. The free water is associated with long relaxation time and motion restricted ‘bound water’ which is about 20% of the total tissue (of brain parenchyma) that has a much shorter relaxation time. Both free and bound water are important as they reflect the patho-physiological alteration of tissue (15). Previous studies have shown that multi-component T2 relaxation decay curves are an indicator of compartmentation. The individual T2 compartments have been interpreted in terms of different water compartments within the heterogeneous tissue (5). However signal intensity interpretation in

has a major problem, often there is no intuitive approach to signal behavior as signal intensity is a very complicated function of the contrast-determining tissue parameter, and the machine parameters TR and TE.

The signal intensity measured is related to the square of the xy magnetization, which in a SE

\[ M_{xy} = M_{xy} \left(1 - e^{-TR/T1}\right) e^{-TE/T2} \] (16-18) where Mxy is the initial intensity.

So, a T2 decay has been measured by using multi echo datasets acquired during spin echo readout train,

By using special mathematical software T2 maps were generated to determine the T2 values in the image. The objective of the present study is to characterize rain lesion after obtaining an accurate measure of water content of various tissue, tumor and lesions on observed proton relaxation time and determination of RI value on normal and pathological brain tissue indirectly from the 1/T2 value and agree with the RI value determined directly from the tissue in the laboratory.

MATERIALS AND METHODS

After getting proper institutional ethics and consent biopsy specimen of the pathological tissues were collected in normal saline solution for determination of 1) RI 2) water content and 3) solid component fraction of the tissue.

Pathological tissues included low grade glioma, glioblastoma, oligodendrogloma (grade II), solid brain abscess, tumefactive multiple sclerosis, lymphoma, metastasis from breast and lung cancer (Table1).

Figure 1

TABLE 1: Type and number of pathological cases

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>NUMBER OF CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLIOMA</td>
<td>17</td>
</tr>
<tr>
<td>Low grade Glioma</td>
<td>6</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>7</td>
</tr>
<tr>
<td>Oligodendrogloma (G-II)</td>
<td>2</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>2</td>
</tr>
<tr>
<td>INFLAMMATORY</td>
<td>3</td>
</tr>
<tr>
<td>Tumefactive MS</td>
<td>2</td>
</tr>
<tr>
<td>Solid brain abscess</td>
<td>1</td>
</tr>
<tr>
<td>OTHERS</td>
<td></td>
</tr>
<tr>
<td>Metastasis</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>Arteriovenous malformation</td>
<td>1</td>
</tr>
</tbody>
</table>

PREPARATION OF TISSUE HOMOGENATES OF
DIFFERENT RI

RI of the biopsy tissues were determined with an Abbe refractometer (Suprashes Model AAR-33, India). Various tissue homogenates with different RI were prepared by mixing 80,70,60,40 and 25 percent of water respectively. To make a uniform solution tissue homogenates were stirred for twenty minutes in a tissue homogenizer (BD144 homogenizer). RI of the homogenates were also determined with an Abbe refractometer (Table 2). The solutions were prepared carefully so that RI and percentage of water maintained a definite relationship as shown in Table 2.

Figure 2

TABLE 2 Various tissues with their physical densities, water percent and RI

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>Physical density g/cm³</th>
<th>WATER%</th>
<th>RI Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>-</td>
<td>-</td>
<td>1.333</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-</td>
<td>-</td>
<td>1.098</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>-</td>
<td>-</td>
<td>1.027</td>
</tr>
<tr>
<td>Middle lobe</td>
<td>-</td>
<td>-</td>
<td>1.024</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>-</td>
<td>-</td>
<td>1.034</td>
</tr>
</tbody>
</table>

A solution of 100% distilled water (with RI value of 1.333) was included as an external reference for comparison with CSF (RI-1.333) as the internal reference. Physical densities of the tissue homogenates were determined as well from the specific gravity of the solution. Approximate RI of the solid components were determined as well after drying out the water (Table 3).

Figure 3

TABLE 3: Individual contribution of water fraction and solid fraction toward final RI of tissue along with T2 value

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>WATER%</th>
<th>RI due to Water fraction</th>
<th>T2 value ms</th>
<th>Solid component (100-w)</th>
<th>RI due to Protein/lipid fraction (spec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.335</td>
<td>100</td>
<td>100</td>
<td>40</td>
<td>0</td>
<td>0.00000</td>
</tr>
<tr>
<td>1.341</td>
<td>90</td>
<td>30</td>
<td>240</td>
<td>10</td>
<td>0.02310</td>
</tr>
<tr>
<td>1.395</td>
<td>80</td>
<td>20</td>
<td>1367</td>
<td>20</td>
<td>0.02893</td>
</tr>
<tr>
<td>1.4128</td>
<td>70</td>
<td>30</td>
<td>75</td>
<td>32</td>
<td>0.02884</td>
</tr>
<tr>
<td>1.4186</td>
<td>60</td>
<td>20</td>
<td>91</td>
<td>40</td>
<td>0.02881</td>
</tr>
<tr>
<td>1.4284</td>
<td>50</td>
<td>25</td>
<td>21</td>
<td>75</td>
<td>0.02820</td>
</tr>
</tbody>
</table>

DETERMINATION OF WATER CONTENT AND RI FROM T2 DECAY:

Calibration of the relationship between the refractive index and the water transverse relaxation rates (1/T2 values) were obtained by placing the previously mentioned tissue homogenates of gray matter, white matter and pathological brain masses of different known RI in small plastic tubes (small syringes). All efforts were taken to eliminate air bubble from the tube.

Five such tubes of various solutions having different RI along with a tube of distilled water (as an external reference) were placed around the head of a volunteer, three on each side and place in the head coil (Figure 1A).

Figure 4

FIGURE 1. A. Six tubes of external references are around the head B. T2 map in a 3T C. T2 map in a 0.3T magnet D. Pixel value for T2 of internal references. E and F T2 shade preparation from T2 map

A quadrature head coil was used and a T2 weighted scan was performed in a 0.3 T Hitachi (Japan) AIRIS II and 3T Siemens (Germany) Magnetom Trio Tim syngo MR B15 scanner. Two different magnets of different field strength were used to note effect of T2 decay and its relationship with water content. In order to obtain T2, 5 images of different echo times (TE) of 60,81,90,100,120 ms respectively were obtained in the 0.3T magnet and six different echo times 30,60,90,120,150,180ms respectively in a 3.0T magnet. In the 0.3T magnet the following parameters were selected – FOV- 320mm, TR-4000ms, thickness-7mm, Interval-8 and phase-224.

In the 3.0T magnet the selected parameters were slice thickness - 8mm at the ventricular levels with a matrix 256 X 256 (FOV 220mm), To measure the mean T2 intensity (S) regions of interests (ROI) of 5mm² were placed in the body and frontal horns of the lateral ventricle.
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for CSF, cortex of parietal lobe for gray matter and centrum semiovale for the white matter as internal reference, distilled water

and another 5 external tubes of tissue homogenates as external references. A T2 map was generated (Figure1 B and C) using dedicated software installed within the magnets by fitting the image signal intensities (S) acquired at different echo times to a single exponential decay, \( S = S_0 e^{-\frac{TE}{T2}} \) on a pixel by pixel basis.

To avoid fitting noise, pixels having intensity values below a global threshold in the TE=30ms for 3T and 69ms for 0.3T image, and those for which the fit failed, were assigned a T2 value of zero. T2 values of tissue homogenates and brain were determined from the T2 map directly (Figure1D and E). A T2 shade was also prepared to get the tissue value easily (Figure1 E and F).

In the T2 map T2 values of various tissues and masses were easily obtained and tabulated(19) (Table 3).

From the T2 map, a relationship of fraction of water content of tissue \((1/fw)\) and \(1/T2\) value was derived determining the following equation depending on free and bound state of water(20)(Figure2A).

\[
\frac{1}{fw} = A + \frac{B}{T2} \\
\text{Or } \frac{1}{fw} = 0.9707 -15.82 \times \frac{1}{T2} --- [3].
\]

Here, \(A=0.9707\) and \(B=-15.82\)

The constants \(A\) and \(B\) are dependent on the hydration fraction \(k\) (the ratio of bound water fraction to solid tissue components as well as the relaxation properties of free and bound water fractions). Measurement of \(k\) for white matter was 0.37 at 37 ° C (21).

RI values were also plotted against \(1/T2\) values and a relationship was obtained (Figur2B)

The subsequent T2 map was converted to a RI map (Figure2C and D) using the above equation ascertained from the refractive index versus T2 calibration and fraction of water and T2 relationship (equation 3 and 4). In the RI map thus generated, intensity of each pixel directly measured the RI at that location of brain tissue or lesion (14, 22). A RI shade was also prepared from the RI map (Figure2E). Color coded RI map and color shade (Figure2 F) were also generated viewable as a false-color overlay on a background of the T2 weighted image. The nature of the tumor or lesion and associated vesogenic edema can be easily appreciated for tissue discrimination.

**Figure 5**

Figure2 A. Relationship of \(1/T2\) and \(1/water\) in 3T magnet

B. RI and \(1/T2\) relationship C. Normal T2 weighted image

D. RI map of normal brain E. RI shade F. Color coded shade

**Figure 6**

Figure 3A.Curve of T2 value of brain (intrinsic reference) versus tissue homogenates of the samples (extrinsic reference). B. Relationship of T2 value of brain tissue (intrinsic reference) and tissue homogenates (extrinsic reference).

**ESTIMATION OF TISSUE WATER CONTENT**

Desiccation method was used for the estimation of water content of biopsy materials. In this method, the tissue samples were first weighed in a digital
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balance, then cut into small pieces and subsequently dried at 40°C to constant weight.

Concentration of water: Weight / Weight percent (w/w %) (Table 3) was calculated as follows:

Weight of the tissue before heating - weight of the dry tissue X100

Weight of the tissue before heating

RESULTS

REFRACTIVE INDEX OF VARIOUS TISSUE AND WATER CONTENT AND PHYSICAL DENSITY

In Figure 3A and B relationship of T2 value between brain tissue and CSF as internal reference and tissue homogenates as external reference was also established:

T2 value of brain = 1.174 X 1/T2 value of tissue homogenates - 0.4393 --- [4]

In Table 2 we get the RI values of CSF, gray and white matter and various pathological tissues. CSF with physical density 1, water content 100(W/W%) and RI value of 1.333 was taken as the internal reference (23,24) RI values of normal gray matter (water content 80%, density-1.186) and white matter (water content 70%, density 1.237) were found to be 1.395 and 1.412 respectively. Distilled water with RI, 1.333 was also taken as the external reference for the calibration of tissue solution.

RI values of various grades of glioma, lymphoma and metastasis vary from 1.432 to 1.483 with increasing physical density and decreasing water content. RI value

of solid brain abscess, multiple sclerosis were closer to RI of water with 96 to 97% (W/ W%) of water content and physical density slightly more than water. Figure 4A and B depict the linear relationship between water content and RI value and physical density and RI values respectively. In the figure 4C relationship of T2 and solid components like protein and phospholipids of tissues and in the figure 4D relationship of RI and water percentages were also depicted.

Figure 7

Figure 4A. Relationship between water content and RI values
B. Relationship between RI values and physical density
C. T2 value and protein percent relationship. D. Relationship of water content, refractive index and protein/ phospholipids

T2 VALUE OF BRAIN, TISSUE HOMOGENATES AND REFRACTIVE INDEX

In the figure 2B linear relationship of RI value and 1/T2 value of tissue is depicted.

RI = 4.338 X1/T2 + 1.3338 --- [5]

Linear relationship is also observed between 1/water content (1/f) and 1/T2 value of brain tissue (Figure 2A).

T2 values of brain tissue and tissue homogenates in relation to water content are tabulated in table 3. The difference in the value is due to the difference between the room (28°C) and the body temperature (37°C)

T2 VALUE AND PROTEIN PHOSPHOLIPID RELATIONSHIP

We obtain the protein and phospholipid fraction of the tissue with their T2 values and contribution toward final RI, tabulated in Table 3.

DISCUSSION

RI WITH PATHOLOGICAL NATURE OF THE TISSUE

Brain lesions with an average RI value less than 1.395 were found to be benign, such as multiple sclerosis (1.3425), or solid brain abscess near the wall (1.341). Thus, a RI value closer to that of water (1.333) signifies a benign lesion.

Alternatively, a lesion with a RI value greater than 1.412 was found to be malignant, such as low grade glioma (1.432), medulloblastoma (1.444), glioblastoma (1.447),
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primary lymphoma (1.452 to 1.465) and metastasis (1.471 to 1.486). The degree of malignancy varies as directly with RI (Figure 4D). Figure 4C also depicts the relationship between protein/lipid and lipid fraction of tissue and their T2 values.

RI AND WATER CONTENT
A striking observation was the percentage of water content in a specimen and its relationship to RI value. A linear relationship between RI value and water content (Figure 4A) was noted. Water content is 100% (w/w) at a RI of 1.333 (e.g. CSF).

A low grade glioma has less than 58% water content in the tissue. The lower the water content, the greater the chance of malignancy (Figure 4D). Water content is high as 98 to 99% (w/w) in benign conditions such as multiple sclerosis (9,25) and 97% in solid brain abscess (Table 2). RI of tissue maintains a linear relationship with density of tissue (Figure 4B).

ROLE OF PROTEIN, PHOSPHOLIPID CONTENT IN THE TISSUE
The protein/lipid fraction also influences the final RI of the tissue such as in gray matter (RI of 1.395), 80% of water component of the tissue contributes 1.3667 and 20% of protein or lipid component contributes RI to 0.0285 and final RI becomes 1.395. We can corroborate the above fact with the equation 2:

\[ N = N_1 + N_2 \times (100 - a) \]

Or 1.395 = 1.3667 (contributed by 80% of water) + 0.0285 (contributed by 100-80, or 20% of protein etc), Where \( a = 80 \)

The higher the protein/lipid content (40% to 75%) and lower water fraction, the greater the malignancy of the tissue such as metastasis and lymphoma. Presence of increased mobile lipids due to necrosis and membrane breakdown (2, 3) appears to be responsible for low T2 value in glioblastoma (Astrocytoma III and IV), metastasis and lymphoma (Figure 4D).

T2 VALUE AND RI
A linear relationship is noted between the RI and 1/T2. As per equation 4,

\[ RI = 4.3384 \times \frac{1}{T2} + 1.3338. \]

RI is inversely proportional to T2 value. Greater the malignancy lower is the T2 value. It is low in lymphoma and minimum in metastasis. It appears that low water content in the malignant tissue is responsible for low T2 value.

CLINICAL APPLICATION OF RI SHADE AND COLOR MAPPING
A RI shade was prepared from the T2 shade with the help of equation 4 and a computer generated brain RI map was created from the T2 weighted image (Figure 5B,F,I,L) of various lesions. Figure 5A and 5E represent T2 map of a metastasis and glioblastoma respectively. A dedicated software (color converter©) was used to prepare the RI Map (Figure 5C,G,J,M) and color coded map as overlay on a background of T2 weighted image (5 D,H,K,N). The software responsible for producing RI map has a limitation of producing a white demarcation between tissue of two different RI like a chemical shift artifact.

Depending on the regional RI value, color mapping of the brain lesions was also generated to display the region of malignancy. As a color coded shade, low RI closer to water and CSF was depicted as a bluish gray/grayish white color.
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**Figure 8**

Figure.5 A Metastasis from ca lung in right occipital lobe in A. T2 map. B. T2 weighted image C. RI Map (selected portion) D Color coded map. E. Glioblastoma in T2 map. F. T2 weighted image G. RI map (selected portion) H. Color coded map overlay on background of T2 weighted image. I. Low grade Glioma – T2 weighted image J. RI Map and K. Color map of the of lesion, a false-color overlay on a background of the T2 weighted image. Highly malignant component is depicted in red and edema in blue. L. Primary Lymphoma (biopsy proven) - A. T2 weighted image. M. RI Map and N. Color map of the lesion and surroundings colour and bright red to dark red as the RI increased indicating higher grade of malignancy. This RI mapping technique produces precise and highly reproducible images of brain lesions which easily discriminates between benign and malignant components of the lesions. Standardization of the images was performed prior to running the program, correcting mid tone and contrast manually particularly in a screen saved images.

In the metastasis from lung (Figure5A, B, C, D) irregular and nodular wall of the lesion, depicted as bright red to dark red as a false-color overlay on a background of the T2 weighted image indicating high RI value (RI >1.4602).

Surrounding normal gray matter looked brownish (RI=1.395 to1.412) and edema was discerned as white to bluish white color (RI=1.341 to1.362).

In a T2 weighted image, a selected RI and color mapping of a low grade Glioma and Glioblastoma of cerebral cortex were depicted (Figure.5I,J,K and 5E,F,G,H respectively). Malignant portion was depicted as red to bright red (RI=1.4320 to 1.4412). Surrounding edema was discerned as white to bluish white color.

RI map of primary Lymphoma of CNS (Figure5 L,M,N) also shows regions of increased RI of more than 1.4591 indicating its high malignant nature. High lipid content was noted in RI – phospholipid / protein relationship (58 to 60% Table3).

Diffusion weighted images can distinguish between epidermoid tumor and arachnoid cyst by depicting the restriction of water movement. RI map can also differentiate these lesions. Screen saved images of Arachnoid cyst (Figure6A) and epidermoid cyst (6C) were also tried with this software to create RI mapping. Arachnoid cyst has a mean RI value between 1.332 to 1.345(6B) where epidermoid cyst have RI value between 1.35 to 1.36(6D).

**Figure 9**

Figure 6 Arachnoid cyst in A.T2 weighted image B.RI Map C. Epidermoid cyst – T2 weighted image in D. RI Map Multiple cysticercosis in E T2 weighted and F. RI map Solid abscess in G T2 weighted and H. RI map. Regular and markedly thin wall (1mm) of the pyogenic membrane of abscess and wall of cysticercosis compared to wall of metastasis of figure 5C. Focal demyelinating lesion I.T2 weighted image J. RI mapping Arteriovenous malformation K.T2 weighted L. RI map containing fluid with RI of 1.34 to 1.35. suggesting benign nature.

Neurocysticercosis and solid brain abscess/cysts (Figure6E,G) can some times cause diagnostic dilemmas. RI maps generated by this software (F,H) clearly depict the RI of fluid within the central component of these lesions. Fluid of neurocysticersosis and abscess has a RI of 1.341 to1.357. Another observation is the wall of the cysts and pyogenic membrane of abscess is very thin and regular (1mm) unlike the irregular, thick and nodular wall (>7mm) of metastasis (Figure5C,D).

A focal demyelinating white matter disease (Figure6 I,J) such as tumefactive MS has a RI value of 1.342 and is well
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appreciated where as spectroscopy may be deceptive at times as it shows high choline to creatine and Choline to NAA ratio mimicking malignancy. Lipid and lactate peaks are present only in acute stages and may disappear in chronic MS and controlled cases(27).

An arteriovenous malformation(Figure6K,L) resembling a low grade glioma clearly depicts low RI value closer to 1.333 and well less than 1.395(normal value of brain) in RI map.

We can conclude that RI map and color coded map of the T2 weighted image of various pathological lesions clearly demarcate the pathology and the potential tissue signature to discriminate benign from malignant characteristics of lesions. This noninvasive method can complement spectroscopy to increase diagnostic accuracy.

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