A BOLD appraisal of cancer
M. L. Asha, Laboni Ghorai, Basetty Neelakantam Rajarathnam, H.M. Mahesh Kumar, Srilakshmi Jasti, P. Deepak
Department of Oral Medicine & Radiology, Dr. Syamala Reddy Dental College, Hospital & Research Centre, Bengaluru, Karnataka, India

Abstract
Tissue hypoxia is a biological condition characterized by oxygen deficiency at the tissue level. Hypoxia has been seen to play a crucial role in tumor recurrence in head and neck cancer patients. The detection and assessment of tumor hypoxia plays a critical role in both validation and development of hypoxia modification therapies. Hypoxic cancers being more resistant to radiotherapy and chemotherapy, attempts have been made to improve the response of hypoxic cancers to radiotherapy through the use of radiosensitizers such as carbogen and others. Several techniques to assess the status of tumor oxygenation have been developed in the past, among which functional imaging techniques remain the most validated. Blood-oxygen-level-dependent magnetic resonance imaging (BOLD MRI) is a non-invasive functional imaging technique that can recognize hypoxic cancers which will respond to accelerated radiotherapeutic treatment with radiosensitizers. BOLD MRI employs a T-sensitive sequence which can detect a transient rise in signal during oxygen inhalation. This increase in signal intensity is caused by the reduced paramagnetic effect due to a decrease in the blood deoxyhaemoglobin level within cancer. Hence, BOLD MRI can detect reduced hypoxia in the malignant solid tumors and may pave the path for more conservative treatment approach for hypoxic head and neck cancers in future.

Keywords
Blood-oxygen-level-dependent magnetic resonance imaging, functional imaging, radiosensitizers

Correspondence
Dr. Laboni Ghorai,
Department of Oral Medicine & Radiology,
Dr. Syamala Reddy Dental College, Hospital & Research Centre, #111/1, SGR College Main Road, Munnekolala, Marathahalli (Post), Bengaluru - 560 037, Karnataka, India.
Phone: +91-9035709695.
Email: dr.labonidey@gmail.com

Received 01 Jun 2015; Accepted 25 Jul 2015
doi: 10.15713/ins.jmrps.26

Introduction
Tissue hypoxia is a biological condition that is characterized by oxygen deficiency at the tissue level. Hypoxic microenvironment is frequently found in solid tumors and is a well-known factor responsible for poor prognosis in head and neck squamous cell carcinoma (HNSCC). It indicates an imbalance between the increased oxygen demand of the rapidly proliferating cancer cells and the decreased oxygen delivery due to poor vascularization and blood supply. The resultant compensatory mechanisms utilized by tumors in response to hypoxia has an unfavorable impact on the therapeutic response, irrespective of the treatment modality employed. Hence, tumor hypoxia has become a central issue in cancer treatment. The detection and assessment of tumor hypoxia now plays a critical role in both the validation and development of hypoxia modification therapies. Functional imaging techniques being non-invasive are most common used in the field of cancer research.

Pathophysiology of Hypoxia
Hypoxia is a pathophysiological state that may be classified as generalized or localized. Localized tissue hypoxia, as it relates to tumors, can be “acute” which may be a result of temporary reduction in blood supply or “chronic” which may be caused by insufficient vascularization impairing the metabolic needs of the growing tumor. Thomlinson and Gray were the first to report the presence of tissue hypoxia in the cancerous tissue. All solid tumors, especially malignant solid tumors, are subject to hypoxia and often exhibit a decreased oxygen tension than their tissue of origin. Recurring tumors most often exhibit a higher hypoxic fraction than primary tumors.

Oxygen diminution in hypoxic tumor brings about physiological and biochemical changes that aid in tumor growth and propagation. Increased anaerobic glycolysis causes increased uptake of glucose, decreased tissue pH and acquisition of more malignant characteristics. A permanent alteration in the cellular
composition of tumor cell is brought about by the hypoxia-inducible factors (HIFs).\(^1\)

The role of hypoxia in tumor progression is explained in Flowchart 1.\(^1\)

**Effect of Tumor Hypoxia on the Effectiveness of Curative Treatment of Cancer**

1. Accumulation and propagation of cancer stem cells
2. Reduction of the effectiveness of radiotherapy
3. Increased risk of metastasis and reduction of the effectiveness of surgery
4. Resistance to chemotherapy and chemo-radiation.\(^1\)

**Diagnosis of Tissue Hypoxia**

Hypoxia or oxygen deprivation is a well-established phenomenon that plays an important role in tumor progression and is a prime cause of tumor resistance to therapy.\(^5\) Hence, there has been a positive incentive to develop various methods to assess the tissue hypoxia which include.

**Direct methods**

1. Oxygen electrode
2. Phosphorescence quenching
3. 19F-magnetic resonance spectroscopy
4. Electron paramagnetic resonance
5. Overhauser-enhanced magnetic resonance imaging (MRI).

**Endogenous markers of hypoxia**

1. HIFs 1α
2. Carbonic anhydrase IX
3. Osteopontin
4. Glucose transporter 1
5. A combined immunohistochemistry panel of protein markers for hypoxia

**Physiologic methods**

1. Near-infrared spectroscopy/tomography
2. Photoacoustic tomography
3. Contrast-enhanced color duplex sonography
4. Blood-oxygen-level-dependent MRI (BOLD MRI)
5. EF5 (pentafluorinated etanidazole)
6. Pimonidazole
7. Hypoxia positron emission tomography imaging
   a. 18F fluoromisonidazole
   b. 18F fluoroazomycinarabinofuranoside
   c. 18FEF5 (pentafluorinated etanidazole)
   d. 18F flortanidazole
   e. Copper (II) (diacetylbis [N4methylthiosemicarbazone])
   f. 18F Fludeoxyglucose imaging of hypoxia.\(^1\)

**Imaging of Tissue Hypoxia**

Imaging is an important diagnostic modality that has a valuable contribution in staging, framing treatment plan and post-treatment follow-up of the patients with head and neck cancer.\(^6\) Functional imaging is a method in medical imaging that can detect or measure changes in metabolism, blood flow, regional chemical composition, and absorption. Hence, the functional imaging techniques can predict cancer behavior and treatment response, can evaluate new anti-angiogenetic agents and can identify residual or recurrent tumor. With such advanced hypoxia imaging techniques, spatiotemporal characteristics of tumor hypoxia and the changes in the tumor microenvironment can be analyzed.\(^7\)

**BOLD MRI**

BOLD effect was first presented by Ogawa et al. in 1990. They found that the magnetic resonance signal reduces when the concentration of oxyhemoglobin (HbO) decreases and also showed that the reduction of signal not only occurs in the blood but also outside the blood vessels.\(^8\)

BOLD MRI is an MRI technique that can examine the physiology of tumors without exogenous contrast. BOLD imaging takes the advantages of subtle differences in magnetic susceptibility between HbO and deoxy-Hb to assess tissue blood oxygen levels.\(^9\)

BOLD imaging has been a well-known technique in performing functional MRI studies of brain activation in
neuroimaging and also been used to evaluate renal perfusion at high temporal resolution. Now-a-days, BOLD MRI is widely used for oncologic investigations and has further been extended for the assessment of the oxygenation status of head and neck tumors.[8-11]

BOLD contrast principle

The relative decrease in the concentration of paramagnetic deoxy-Hb can be detected by MRI as a weak transient rise in the $T_2$ weighted signal. This is known as the BOLD contrast principle.[12]

Normally, blood contains a mixture of deoxy-Hb and HbO. Deoxy-Hb is paramagnetic, and HbO is diamagnetic.[10] The paramagnetic nature of deoxy-Hb (Pauling and Coryell, 1936)[13] and its influence on the MR signal (Brooks et al. 1975)[14] were well known before the development of MRI.

BOLD-MRI is sensitive to perivascular bulk microscopic magnetic field alterations caused by paramagnetic deoxygenated Hb (Ogawa et al. 1990, Ogawa 2012). The effective transversal MR relaxation time $T_2$ has been found to be affected by the changes in the amount of deoxy-Hb per tissue volume element (voxel).[15] Since BOLD MRI utilizes this paramagnetic deoxy-Hb as an endogenous contrast agent, this method is sensitive to the changes in partial pressure of oxygen in and around the blood vessels.[11] Deoxygenated blood appears darker on $T_2$-weighted images compared with oxygenated blood. Any increase in Hb oxygen saturation would therefore be expected to cause an increase in the signal and conversely, $T_2$ decreases and signal attenuation in $T_2$-weighted MR images occur with an increase in the volume fraction of deoxy-Hb in blood within a tumor. Thus, the contrast is said to be blood-oxygen-level-dependent (BOLD).[10]

Henceforth, BOLD MRI may provide complementary information related to tissue oxygenation that aids in defining optimal treatment strategies for patients with hypoxic tumors.[11]

The oxygenation status of cancer cells was shown by Gray et al. in 1953 to affect the outcome of radiotherapy, with more oxygenated cells being more radiosensitive. Since then, considerable research has been carried out on techniques that aim to increase radiosensitivity with the aim of improving the efficacy of radiation treatment. Increasing the oxygen available to the cells can be accomplished by inhaling 100% oxygen,[16] hyperbaric oxygen[17,18] and other high-oxygen gas mixtures such as carbogen (conventionally 95% O$_2$ and 5% CO$_2$).[16,19-23] Breathing high concentrations of oxygen should increase blood oxygen saturation and the measured signal from tissues on $T_2$-weighted images; however, the inhalation of 100% oxygen can cause a degree of vasoconstriction within tumor vessels. The addition of the vasodilator gas carbon dioxide to the oxygen (forming carbogen) is intended to counter this vasoconstriction. An additional effect of adding carbon dioxide to oxygen is to shift the HbO dissociation curve to the right.[10] Compared with air, both the increased percentage of oxygen in the carbogen and the shift in the HbO curve will make more oxygen available in the capillaries. Radiosensitizing agents such as ARCON (Accelerated Radiotherapy with Carbogen and Nicotinamide)[20,24] which alleviates acute hypoxia by reducing the intermittent closure of blood vessels, have also been investigated for their ability to improve the results of radiotherapy in patients with advanced HNSCC.[2,10]

The identification of patients who might benefit from the radiosensitizers is a difficult task, as the hypoxic fraction inside tumors needs to be assessed. $T_2$-weighted MR techniques show promise in being able to identify such regions within tumors non-invasively. Imaging human tumors before and during the administration of a radiosensitizer and vasodilator such as carbogen may permit identification of regions with oxygenation and flow changes, which may in turn indicate regions of reversible hypoxia.[10] BOLD MRI can successfully detect these regions of reversible hypoxia within the cancerous tissue through changes in blood deoxy-Hb level during administration of radiosensitizers and hence, may pave a path for a more conservative treatment approach for hypoxic cancers in the future.[6]

MRI protocol

Patient preparation is an important step before the MRI procedure. The patient should be positioned supine in the body coil with anesthetic face mask comfortably but securely strapped in place. The patient’s blood oxygen saturation and pulse rates should then be continually monitored by a pulse oximeter attached to a finger and the respiration rate should be recorded every minute by a radiographer, nurse, or anesthetist present in the magnet room.

Scout images of the tumor should now be acquired using a standard steady-state free precession sequence which is an MRI technique that uses steady states of magnetizations. The subject should then be brought out of the magnet, and the mask should be attached to a customized anesthetic circuit and air cylinders. The gas flow rate should be adjusted to be comfortable to the patient, who should then be returned back to the magnet. Now-a-second scout image should be acquired to check slice position. A delay should be introduced between acquisitions, calculated to keep the time resolution to one image per minute.[10]

Gas inhalation protocol

The breathing circuit design should be capable of providing a flow rate of at least 20 L/min. The gas flow should be continually modified by trained personnel throughout the examination to ensure it was sufficient. The mask should have valved inflow/outflow holes to prevent rebreathing, and to make breathing in and out equally comfortable.

A careful and complete explanation of the procedure should be made to each patient, so the hyperventilation does not panic them. Before the MRI examination, an evaluation for mask tolerance and gas tolerance should be undertaken, by practicing with the circuit and those unable to cope with either should not be allowed to proceed further.
The effects of breathing carbogen being at a maximum after 5-10 min, the initial imaging protocol used to acquire images during 5 min of air-breathing, 5 min of carbogen, and 5 min of air, with a 5 min transition period without imaging between them. This protocol further evolved into continuous imaging for 30 min: 10 min air, 10 min carbogen, and a further 10 min of air. However, the final protocol has been accepted to be 5 min air breathing, followed by 10 min carbogen, and 10 min air.\textsuperscript{10}

**Image analysis**

Regions of interest should be drawn from the $T_1$-weighted scout image, around the visible limits of the tumor. Color overlays are being used to help identify regions that increased significantly in signal intensity with the carbogen breathing.\textsuperscript{10}

The response of the hypoxic tumors as interpreted by BOLD MRI before and during the administration of radiosensitizers has been summarized in Flowchart 2.

**BOLD MRI: Merits and Demerits**

BOLD MRI has become a useful tool that can be used to interrogate the pathophysiology of tumors. Non-invasively, it can monitor real-time changes of tumor oxygenation during the administration of pharmacological treatments and can assess the maturation and the functional state of tumor blood vessels. It can be repeated whenever necessary and above all it does not require any exogenous contrast medium or radioisotope.

However, this technique has certain drawbacks. BOLD MRI is a non-quantitative method for assessing tumor pO$_2$, the rise in signal is not always the result of oxygenation and the signal changes are small, short-lived and may pose a challenge in understanding and interpretation.\textsuperscript{6,12} Hence, it alarms a need for higher level of training in observation and interpretation of the same.

**Conclusion**

Tumor hypoxia is a well-known biological entity that is often present in malignant solid tumors. Hypoxic tumors employ several different survival mechanisms, which may result in a loss of apoptotic potential, increased proliferative potential, and formation of new blood vessels that encourages the evolutionary selection for a more malignant phenotype. As such, hypoxia affects the curability of solid tumors, regardless of treatment modality.

Given the negative treatment and outcome problems associated with tumor hypoxia, a major goal for clinicians is to identify hypoxic tumors through a number of different diagnostic approaches.\textsuperscript{1} BOLD MRI is one such robust technique which is reliable, easy to use and can assess the tumor oxygenation without the use of any exogenous contrast agent. Thus, BOLD MRI is a valuable modality that provides functional and physiological information on tumor hypoxia which has prognostic and predictive value. Such an assessment with BOLD MRI will strengthen its valubility to the radiation oncologists and surgeons, heralding the possibility to implement this functional imaging technique for the diagnosis of hypoxic cancer in routine clinical practice.

**References**

7. Lee CT, Boss MK, Dewhirst MW. Imaging tumor hypoxia...