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Editorial

The current issue of Annals comprises of one review article, two interesting case reports and three original articles. The opening article by Dikshit *et al* is on a very common yet incompletely understood entity – vasovagal syncope (VVS). This review article deals in depth with the pathophysiology, evaluation and management of VVS. Despite the extensive research, the exact mechanism leading to syncope is still unclear. However, among the various theories postulated, the authors are of the opinion that blunting of cardiovascular sensitivity to orthostatic stress, inability of peripheral circulation to respond to autonomic vasoconstrictors and excessive local production of nitric oxide seem to be the most plausible explanations at present. In addition to these factors, dehydration, psychological factors may contribute to syncope by altering the normal regulatory physiological response. The authors have emphasised the importance of meticulous history taking to ascertain the cause of syncope. Quiet standing and head-up tilt table (HUT) test were the most commonly used investigations. Management options were increased salt and fluid intake, and drugs like corticosteroids, Nitric Oxide synthase inhibitors.

The next article by Balyan *et al* gives a new insight on the development of end-stage renal disease (ESRD) in patients with type 2 diabetes mellitus. Urinary albumin to creatinine ratio (ACR) is an established marker for early detection of microalbuminuria. Increased oxidative stress has been postulated as one of the contributors in the pathogenesis of diabetic nephropathy and its progression to ESRD. However, literature regarding their association is limited. In order to understand an inter-relationship between oxidative markers and microalbuminuria the authors have compared the level of oxidative stress in patients of diabetes mellitus with and without microalbuminuria by using various oxidative markers. A positive correlation was found between microalbuminuria and malondialdehyde (MDA) levels, and significantly reduced superoxide dismutase (SOD) and glutathione levels were observed in subjects of microalbuminuria.

Parveen *et al* from Jamia Hamdard University, New Delhi, have analysed the effect of individual antiepileptic drugs (AEDs) – carbamazepine, sodium valproate and levetiracetam on modulation of *Wnt* inhibitors in Indian women with epilepsy. The authors inferred that AEDs deteriorate bone health through enhanced sclerostin levels. The deleterious effect may or may not be related to receptor activator of nuclear factor kappaB ligand (RANKL) subject to type of AED used.

The fourth article by Bhethanabhotla *et al* from AIIMS, New Delhi is an attempt to address the unmet need in the management of pediatric Hodgkin disease. As the response to salvage chemotherapy before stem cell transplant is an independent predictor of survival, this retrospective study was done in an attempt to identify predictors of poor response to salvage chemotherapy in relapsed/refractory cases. The authors conclude that Stage 4 and bulky disease at relapse are high risk factors to predict incomplete response in these patients.

To conclude, we are presenting two intriguing case reports. One of them is regarding an atypical MRI finding. The classical hot cross bun sign is a well-known entity seen in neurodegenerative conditions

like multiple system atrophy- cerebellar type, secondary Parkinson's disease, spino-cerebellar ataxia type 1 and 2, etc. Reverse hot cross bun sign has been described earlier in patients with Wilson disease and pontine infarct. Jain *et al* report an unusual case of a 78-year-old male presenting with insidious onset progressive speech difficulty, who was clinically diagnosed as primary progressive aphasia (PPA). The MRI brain showed an atypical finding of reverse "hot cross bun" sign in pons, which has not been reported earlier in patients with PPA.

The next case report by Madhusudhan *et al* from AIIMS, New Delhi, is about a 26-year-old male who was diagnosed as acute cholecystitis with cholelithiasis. MRI showed a rare congenital anomaly of duplication of gall bladder which were united at the neck region. In the era of laparoscopic cholecystectomy, pre-operative detection of biliary anomalies is important to prevent unintended injuries and its associated morbidities.

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Review Article

Current Concepts of Pathophysiology of Vasovagal Syncope and its Evaluation and Management: A Review

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ABSTRACT

Vasovagal syncope (VVS) with a sudden, temporary loss of consciousness (LoC) is a common phenomenon in the young and the elderly. Though generally described as innocuous, it may lead to serious consequences in special category of people (pilots), or in the elderly in whom LoC may lead to a fall and serious injury. The topic has been copiously researched upon and discussed in medical literature over the last few decades, but the exact mechanisms which lead to the disability have yet to be fully agreed upon. Changes in cardiovascular baroreceptor sensitivity, aberrations in the complex interaction amongst the nucleus of the tractus solitarius and the nuclei around it, inability of the peripheral circulation to respond to autonomic vasoconstrictors, or excess production of vasodilators such as nitric oxide produced locally have been considered in its pathophysiology. Various extraneous situations like dehydration, exposure to heat stress, medications, psychological factors may adversely stress regulatory physiological responses and promote occasional episodes of VVS. More complex dysautonomia could be a reason for the recurrent VVS. Differences between brain structure of VVS sufferers and normal subjects have been proposed. Head-up tilt table (HUT) test is the most widely applied investigation for evaluating VVS episodes. Lower body negative pressure (LBNP) has also been used. Enhancement of the orthostatic stress may be done by simultaneous use of both, or with peripheral vasodilators. As to whether such an enhancement is necessary is debatable. Management with increased salt and fluid intake, corticosteroids, beta adrenergic receptor blockers, alpha adrenergic receptor stimulants, selective serotonin reuptake inhibitors, and nitric oxide synthase inhibitors have been tried with variable success.

Keywords: Vasovagal syncope, head-up tilt table, loss of consciousness, pilot, dysautonomia, orthostatic stress.

Introduction

Vasovagal syncope (VVS), a sudden loss of consciousness in apparently healthy individuals, though brief, and medically innocuous, is often interpreted as a catastrophic event by the sufferer. Recovery is full and rapid. It is more a symptom complex than a disease, but

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may leave behind a lurking doubt in the individual's mind about his/her sense of well being. There may be a loss of confidence in one's over all ability which becomes particularly relevant in certain categories of people such as aviators who may fear for their professional careers, further escalating the situational anxiety. Undeniably if a loss of consciousness occurs because of causes listed as "pathological" in Table 1, the matter is of grave concern. However, what is addressed in this review is the VVS which is a temporary aberration of body physiology, the mechanism of which is still as elusive as the Scarlet Pimpernel. Although the loss of consciousness (LoC) during VVS is "brief" and recovery is "spontaneous and complete"; in an occupation such as aviation, the condition may have life threatening consequences (1).

Definition and Types of Syncope

Syncope has been variously defined as "a temporary loss of consciousness" (2); "A sudden transient loss of consciousness with an inability to maintain postural tone that was not compatible with a seizure disorder, vertigo, coma, shock, and other states of altered consciousness" (3); "A sudden and brief loss of consciousness and postural tone from which recovery is spontaneous (4), or more recently as "A temporary loss of consciousness and postural tone" (5) or as "A sudden and transient loss of consciousness with spontaneous recovery" (6). It is obvious that basic concept of what defines syncope has remained unchanged over a period of about six decades, and is unlikely to do so in the future. Collectively stated: *"Syncope is a short duration, reversible loss of consciousness and postural tone following a rapid fall in blood pressure from which an individual recovers spontaneously, usually without any residual effects. In most instances it is associated with a change of posture from supine to erect"*.

Incidence

Vasovagal syncope, or the "common faint" occurs in apparently healthy young people (more in females, and during

Table 1: Causes of syncope (sudden loss of consciousness)

Pathological 1. Cardiovascular disease producing a sudden fall in cardiac output 2. CNS disorders such as transient ischemic attacks 3. Autonomic dysfunction: diabetes mellitus, postural tachycardia syndrome (POTS), shy-dragers syndrome
Physiological 1. Postural change from supine to upright 2. Prolonged standing 3. Heat stress 4. Dehydration/hemorrhage 5. Pain 6. Anxiety/fear 7. Micturition syncope 8. Medications 9. Insomnia/fatigue 10. Age related 11. Post space flight 12. Pregnancy *Most of the above (listed as Physiological) may produce a rapid reduction in the central blood volume leading to a sudden reflex decrease in heart rate in combination with peripheral.*

adolescence) with an incidence which has varied from about 6% (7) to as high as 25% (8). A very extensive report by da Silva (2014) (9) cites an incidence which varies from about 15% in children, to 39% in young adults and 23% in the elderly. Syncopes are classified as reflex (vasovagal), cardiac, neurological and others, with the vasovagal syncope as the most frequent. The percentage of VVS has not been clarified in the overall percentages quoted in this study. Lavania *et al* (10) using a questionnaire survey found a fairly high incidence of presyncope/ syncope (about 30%) in 134 healthy medical students of both sexes, mean age 19.3 years. However, their findings were confounded by the fact that none of the 41 students who had given positive history of a faint developed even presyncopal symptoms when subjected to passive upright standing. In our series of 166 head-up tilt table (HUT) exposures of 143 healthy military men (mean age 29.4 years) without a prior history of orthostatic intolerance, there was not a single episode of presyncope/ syncope. However, 22.8% of the 57 young, apparently healthy airmen with previous history of syncope/presyncope referred to our laboratory for evaluation, developed some symptoms of orthostatic intolerance, though not frank syncope during HUT (11). It is thus obvious that VVS is indeed an enigma as suggested by Dikshit (1) and its mode of occurrence remains far from being explained suitably. In apparently healthy elderly population (6th to 8th decades of life) a 6% to 11% incidence of unexplained syncope has been reported (6). Incidence in children may be as high as 10%-44% (12). Information on syncope in Indian children, and to that matter on cardiovascular response to orthostatic stress in normal Indian children is for all practical purposes unavailable.

Mammals other than man are generally not known to develop VVS as they do not need to operate in an upright posture as humans do. And even if they do so (giraffes reaching out for food by raising their necks to significant heights), the relatively low blood flow to their proportionately small brains size makes them more or less immune to below par perfusion of their brains (13). There is no known report of orthostatically induced VVS in the great apes, (our ancient cousins) probably again because of the relatively small size of their brain, and also the fact that they prefer to operate on all fours as a routine rather than in the erect posture which they may assume at will. Thus, it is almost impossible to use an animal model to study the intricacies of the physiology of postural change induced syncope. Emotional or fear-induced bradycardia and hemorrhage/severe fluid loss, though known to produce conditions close to the VVS seen in humans, but a loss of consciousness is not known to occur (14).

Gender and Race Differences in Occurrence of Syncope

Little information is available on these issues. Almost all our data on response to orthostatic stress and incidence of syncope has been obtained in male subjects (1, 11, 15-23). Patel *et al* (24) were unable to demonstrate gender difference in their male and female subjects during exposure to HUT. They did not report any incidence of syncope. On the other hand, Sachse (25) reported a higher incidence of orthostatic intolerance during 6 minutes of standing after supine rest in elderly women as compared to males in the same age group. Frank syncope was not seen. It is difficult to draw any major conclusions from her relatively limited study as she also reported that heart rate variability in both her groups (males and females) was similar. A large Korean study (26) comprising of 497 males and 547 females reported that females had a higher incidence of syncope/presyncope. A high incidence in young women has also been reported by others (6). da Silva (9) on the other hand while reviewing a very large data, opined that there was no gender difference. She had concentrated more on the overall incidence of various types of syncope conceding that the VVS was the most common variety though specific incidence for this entity was not recorded.

Little information is available on racial differences in the occurrence of syncope. Goldstein and Shapiro (27) reported that Blacks have a different response to orthostatic stress as compared with Asians and Whites, but there was no mention of VVS in their study. Some of our own observations are tabulated in Table 2. Some minor differences in the normal responses of the recorded parameters were seen though statistical comparisons were not made. Our orthostatic tolerance tests using either HUT (11, 20-23) or lower body negative pressure (LBNP) (19) were performed on normal healthy, physically fit military men. None of the subjects exposed to the stress for the conventional period of 20 minutes (HUT) or a maximum LBNP-50 mmHg for 5 minutes, showed any symptoms of discomfort or signs of impending VVS. However, as stated earlier 22.8% of the 57 military men who were evaluated in our laboratory for single episodes of presyncope /syncope did show abnormal response to HUT (11). A number of questions arise out of these findings:

1. Can these individuals be considered as a part of the so called "normal" population or condemned outright as those with abnormal cardiovascular reflex status?
2. Were they victims of a one off aberration in response to any one or more of the situational stresses listed under "Physiological" in Table 1?
3. What would be the chances of recurrence of VVS in such subjects?
4. How should such subjects be treated; with just observation or medication?

Many of these issues are important in those who work in sensitive environments, e.g. pilots. More often than not such findings are enigmatic. During evaluation of Indian Air Force pilots for cosmonaut training (23), one highly experienced test pilot did not have the expected increase in his heart rate and diastolic blood pressure during two 70^0 HUT tests to which he was subjected on two consecutive days. He was disqualified for the specialized training. Repetition of his HUT tests after three and six months did not reproduce the aberration seen earlier. He had never suffered a VVS during his 18 odd years as a fighter pilot. He was not grounded. As against our observations, Hickler *et al* (8) found an incidence between 20%-25% in their otherwise normal Caucasian subjects exposed to HUT. In the same context, Indian

Table 2: Response of healthy young males to LBNP - 40 mmHg and 70^o HUT. Values given are mean changes from pre-tilt

	LBNP -40 mmHg		**70^o Head-up tilt**	
	Caucasians	**Indians**	**Indians**	**Omani Arabs**
REF no	*28*	*21*	*11*	*32*
HR/min	+18	+9	+16	+19
SBP (mmHg)	-7	- 7	- 1	-6
DBP (mmHg)	+2	+3	+9	-6
PP (mmHg)	-5	-12	-10	-4

LBNP-Lower body negative pressure; HUT-Head-up tilt table; SBP- Systolic blood pressure; DBP-Diastolic blood pressure; PP-Pulse pressure.

military men did not exhibit any abnormal response to 5 minutes of -50 mmHg LBNP (21) while some of our Caucasian subjects did have mild prodromal signs of syncope when exposed to the same degree of stress (28). Based on these observations, could it be too far fetched to deduce that Indians tolerate orthostatic stress better than Caucasians, possibly because Indians are known to be heat adapted, and hence their physiology for handling body fluid shifts functions more efficiently? Only extensive studies can help to draw definite conclusions on the issue of ethnicity, orthostatic tolerance and syncope incidence. An interesting corollary to this may be that the response of different ethnic groups exposed to orthostatic stress be compared in places like the USA or Singapore where a very wide spectrum of different populations is found in abundance. Inclusion of female subjects will help to resolve the gender differences, if any.

Cardiovascular Response to Orthostatic Stress

When posture is changed from the supine to the erect, about 500 to 600 ml of blood is translocated from the central blood volume (CBV) in the chest to the periphery, mainly the lower limbs because of the normal gravitational effect on the blood column (29). This has been reiterated by many others (1, 15, 28), The orthostatic stress may be applied as postural change from supine to quiet standing (30, 31), HUT, (11, 15-18, 22, 23), or LBNP (6, 19, 21, 28). The well documented response to orthostatic stress consists of a tachycardia, increased diastolic blood pressure, minor changes in systolic blood pressure, increase in peripheral vascular resistance including splanchnic circulation, a decrease in stroke volume and the cardiac output (1, 11, 17, 20, 21, 28). More sophisticated evaluation of cardiovascular response of normal young Omani Arabs to 5 minutes of HUT was done by Jaju (32). While recording online heart rate variability and sympatho-vagal balance (LF/HF) during the HUT in her subjects, she clearly demonstrated that the overall baroreceptor sensitivity (during both up slope and down slope) diminished significantly while the LF/HF ratio increased significantly, the latter clearly indicating heightened sympathetic influence on the cardiovascular system. Increase in sympathetic activity was demonstrated as the highly significant increase in blood norepinephrine (NE). In the same context, incremental muscle sympathetic nerve activity which is a response to sympathetic stimulation has been recorded in human subjects with graded depletion of the CBV [Rea and Wallin (1989)] (33). In summary, a depletion of the CBV requires activation of the sympathetic system which must produce two seminal cardiovascular effects: an increase in heart rate and peripheral vasoconstriction in order to maintain adequate perfusion to the brain in order to avoid LoC. As a consequence, the typical observations include tachycardia, an increase in diastolic blood pressure, minor changes in systolic BP, and a fall in stroke volume, and despite the tachycardia, in the cardiac output because of the lowering of the stroke volume (11, 15, 17).

The Neural Arc for the Baroreceptor Reflex

The receptors which sense the changes in the circulating fluid volume and the arterial blood pressure are the low pressure cardio-pulmonary receptors (34-37), and the high pressure arterial baroreceptors (38-40), respectively. Also to be noted is the presence of endocardial sensory receptors particularly in the left ventricle, sub-served by vagal C fibres which have been associated with producing a bradycardia and hypotension under a set of circumstances which may be relevant to the topic in hand (38-40).

The natural physiological stimulus to the low pressure cardiovascular receptors (type B atrial receptors) is the stretch generated by the circulating fluid volume and hence they are also referred to as volume receptors (35-37). Their activation results mainly in an increase in heart rate (the Bainbridge effect), and a decrease in secretion of the anti diuretic hormone (ADH) by

the hypothalamus (35). As against this, a reduction in circulating fluid volume as also a decrease in the CBV which diminishes receptor stretch produces effects which are not diagonally opposite. Under these circumstances, there is an increase in forearm vascular resistance (FVR) without a change in heart rate or blood pressure (34, 37). The effects are accompanied by some increase in plasma catecholamines. These workers (34) subjected volunteers to LBNP of -5 mmHg and observed that the increase in FVR of their subjects had peaked even at this low grade of stress in which only a small depletion in CBV (not measured) would have occurred. Tripathi and Nadel (41) confirmed these findings with their LBNP experiment. In our own series of LBNP exposures, we observed that FVR in our healthy male Caucasian subjects had reached its zenith by the time LBNP of -30 mmHg had been achieved. Thereafter there was no further change in FVR even up to -50 mmHg LBNP stress (32). That sympathetic nerve activity indeed increases with a relatively small depletion of the CBV has been confirmed and has been attributed indisputably to the deactivation of low pressure cardiopulmonary receptors (33). The afferents from these receptors travel up the vagus nerves in both medullated (atrial type B and A), and C fibres to synapse with the second order neurons in the nucleus of the tractus solitarius (NTS). The neurotransmitter released when they are activated is glutamate (40, 42, 43).

Endocardial receptors, particularly those located in the left ventricle have been known to decrease heart rate and vascular tone when stimulated chemically or by mechanical stretch (40). Their C fibre afferents also travel in the vagus nerve to synapse with the 2nd order neurons of the NTS. Excessive contraction of the left ventricle may stimulate these receptors mechanically to produce bradycardia and hypotension (44) seen in subjects with VVS.

By far the most powerful influence is exerted on heart rate and peripheral vascular tone by the arterial baroreceptors located in the carotid sinus and the aortic arch (40). For the purpose of this review which takes in to account the effects of de-activation of baroreceptors, only the effects of the carotid sinus high pressure baroreceptors have been entertained.

The carotid sinus arterial (high pressure) baroreceptors are stretch receptors which respond to pulsatile stretch generated by the systole. They function physiologically between a pressure range of about 50 mmHg to about 200 mmHg. The number of inhibitory impulses sent into the NTS along the IXth cranial nerve peak at the upper limit of pressure, and reach their nadir at 50 mmHg when deactivated (40). The neurotransmitter (glutamate) amount released by the nerve terminals on the NTS neurons is thus reduced if there is a decrease in the pulsatile pressure in the carotid sinus. This must logically happen on reduction in the CBV under various circumstances, most commonly on change of posture from supine to standing to reduce the inhibitory control on the heart and the vasculature with a simultaneous increase in sympathetic activation. The efferent pathway resorted to is either the stimulation or inhibition of the Xth nerve, and reciprocally inhibition or excitation of the sympathetic innervation of the heart and circulation (depending on whether there is activation or deactivation of the receptors).

Central Ramifications of the Reflex and the Neurotransmitters Involved

The baroreceptor afferents synapse with the second order neurons of the NTS. The excitatory neurotransmitter released by these nerve terminals is glutamate (40, 42, 45) which occupies ionotropic glutamate receptors on the NTS (42, 46). The glutamate is released at the afferent nerve terminals and finds its way to the ionotropic receptors on the NTS neurons to stimulate them (Fig. 1). It may be deduced that when the baroreceptors are deactivated (as during reduction in CBV) the volume of glutamate is reduced and vice-a-versa as the release of the glutamate is governed by the frequency of baroreceptor firing (42). As the

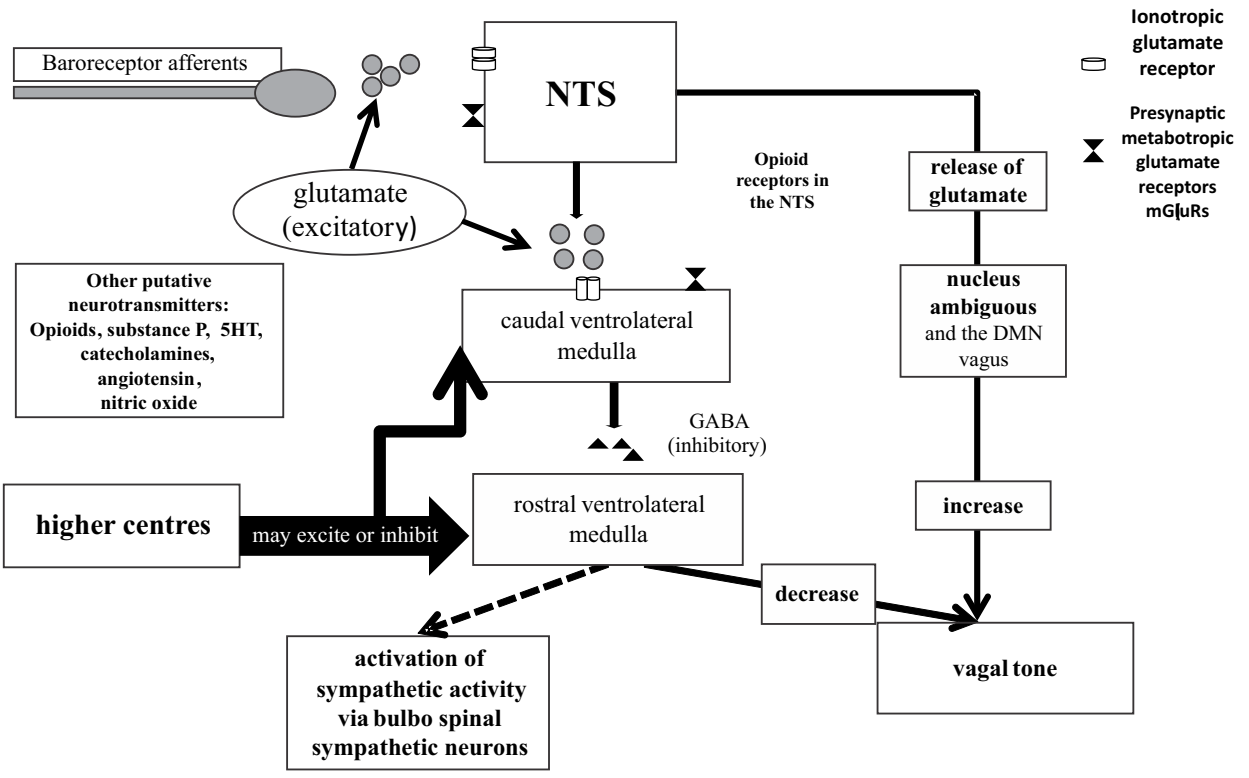

Fig. 1 : Central and neurotransmitter interactions in the NTS

Flow chart constructed on the basis of reference nos. 40, 42, 43, 45 to 48, inhibitory influence is shown by the dotted arrow.

stimulation of NTS neurons by baroreceptor afferents is responsible for the inhibitory effect on the heart and peripheral circulation, a deactivation of these receptors resulting in a decrease in release of the neurotransmitter will be responsible for releasing the inhibitory effect of the NTS on the heart and peripheral vascular tone. The occupation of ionotropic glutamate receptors on the presynaptic membrane of the NTS neurons is regulated by the generation of metabotropic glutamate receptors (mGluRs) thus making the process self limiting. Of the three types of mGluRs (Type I, II and III), type II are involved in limiting activity of glutamate at the presynaptic 2nd order neurons of the NTS.

There is evidence that the mGluRs are synthesized in the terminal portion of the afferent nerves synapsing with the NTS (46). The NTS in turn sends excitatory input (with glutamate as neurotransmitter) to the caudal ventrolateral medulla (CVLM) which in turn keeps the rostral ventrolateral medulla (RVLM) inhibited *via* gama amino butyric acid (GABA) as its transmitter. It has been postulated that this release of GABA in response to stimulation by glutamate from the NTS neurons is self regulated by mGluRs metabolizing the glutamate on the presynaptic membrane of the CVLM (45). The RVLM has a constant excitatory input to the bulbo-spinal sympathetic nerves innervating the heart and peripheral circulation, and inhibits the vagus, but this stimulatory effect is kept under inhibitory control by the CVLM. The NTS also sends excitatory input to the vagal motor nuclei, the Nucleus Ambiguous (NA) being mainly concerned with vagal innervation of the heart (40) and Fig.1. Therefore, the NTS and its associated inhibitory influence is constantly varying under the influence of glutamate even at rest. If and when the release of glutamate is restricted by a decrease in the afferent input by the various baroreceptors, the inhibitory

influence of the NTS reduces because the quantum of excitatory glutamate from the afferent nerve endings reduces. The NTS and the ventrolateral medulla are under influence of various higher centres which include the cortex, the hypothalamus, the limbic system and the raphe nuclei. A number of putative neurotransmitters are thought to be acting in this region. These include catecholamines, angiotensin, opioids, nitric oxide (NO) and substance P (47). Most of the conclusions drawn have been from small animal studies (42, 45-47). Opioid receptors are found in abundance in the NTS and it is the mu-type receptor of the available kappa, delta and mu-opioid receptors that has been linked to excitatory cardiovascular effect (48). That endogenously produced opioids may have a role in humans at the level of the NTS was suggested by Schobel *et al* (49) when they demonstrated in normal subjects that naloxone, an opioid receptor antagonist, enhanced a low pressure cardiovascular receptor induced reflex. But contrary to this Wallbridge *et al* (50) refuted the idea of endogenous opioids playing a role in cardiovascular control as they reported that their patients of recurrent syncope did not benefit from naloxone. Thus, endogenous peptides, opioid receptors and their role in central interactions at the level of the NTS are again confounding. The overall understanding though is that opioids influence the NTS in producing a cardio-inhibitory effect and this effect may be suitably neutralized by use of naloxone, an opioid blocker.

The NTS has serotonergic neurons which modulate cardiovascular response to a variety of stresses via a multiplicity of 5HT receptor types (51). Blockage of $5HT_{1A}$ and $5HT_3$ receptors reduces the brain stem 5HT uptake increasing the local levels of the neurotransmitter. This may induce a down regulation of post synaptic 5HT receptors (52) in the dorsal motor nucleus of the vagus where 5HT acts to produce bradycardia of baroreceptor stimulation. Knowledge of the role that 5HT plays as a neurotransmitter in the NTS/NA of the vagus is, therefore, confusing at best. The overall central interactions of the NTS and associated medullary neurons with the cortex, hypothalamus and the limbic system must be kept in mind while considering pathophysiology of "VVS or the common faint" which is addressed by this review.

A number of neurotransmitters in peripheral circulation need to be mentioned. Post ganglionic sympathetic NE is released at the sympathetic nerve endings and is mainly responsible for vasoconstriction of the veins and the arterioles (40). Application of even low degree of LBNP was accompanied by significantly raised blood concentration of catecholamines (34). A sudden withdrawal of sympathetic activity will thus be accompanied by a sudden depletion of catecholamines available for contraction of the vascular smooth muscle and produce a dilatation. NO is a local chemo-transmitter produced by endothelial cells of the arteries and arterioles and is a powerful vasodilator (40). It is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) because of the shear stress exerted on the endothelial cells by the blood flow. During sympathetic activation, the blood flow velocity may increase to generate shear stress which in turn increases the NO production in order to regulate the degree of vasoconstriction that would otherwise be brought about by sympathetic excitation (53). NO produces relaxation of vascular smooth muscle by producing cyclic GMP which in turn generates various protein kinases which expel intra cellular Ca^{++} from the smooth muscle cell, thus helping the process of dilation (54). Selective excessive synthesis of NO which may produce large scale vasodilatation causing hypotension as in VVS has not yet been reported, but excess synthesis of NO in patients of severe shock where in which large scale vasodilatation occurs is known (55).

A powerful peptide, endothelin, is expressed by endothelial cells of arterioles (40) and secreted as a local hormone taken up by endothelin receptors (ET_A) on the vascular smooth muscle. This normally opposes the

vasodilatation being produced by the NO also being secreted by the endothelium (56). Physiologically therefore, there is a continuous to-and-fro battle being waged locally between endothelin which vasoconstricts the vascular smooth muscle and NO which does the opposite. Endothelin by itself may self regulate its vasoconstrictor effect by occupying ET_B receptors which, though numerically much less in number than the ET_A receptors, are present on the vascular smooth muscle in some vascular beds. The ET_B receptors then help in the synthesis of NO by the smooth muscle cells.

Clinical Manifestations of Vasovagal Syncope and Factors Aggravating it

Most reports describing presentation of syncope have encountered symptoms and signs listed in Table 3 (8-11, 57). The episode often start as a prodrome with mild symptoms such as restlessness, visual blurring, sweating and may progress in severity to a LoC. Subjects have been known to slip directly into a LoC without the prodromal symptoms. The percentage reported for this is as high as 39% in patients who had developed the malady (58). Rarely convulsions may occur as a part of VVS (59, 60) though we have not observed any such in our subjects who developed syncope during HUT test or LBNP exposures. Recovery is spontaneous when the subject becomes supine after the LoC, and it is in his/her best interest that alacrity is not shown by care givers in putting the person upright. Some post syncope symptoms such as fatigue, confusion, disorientation have been reported in as much as 76% of patients (58). Such symptoms have been known to last for about 15-20 minutes in the recovery period (61). A family history is often forthcoming in syncope patients (58) and should be enquired into while evaluating an individual with syncope. There are two possibilities when the simple faint may prove to be dangerous. An awkward fall because of the LoC may cause a serious injury especially in the elderly. In fact a fairly high percentage of VVS patients have been reported to have had soft tissue injuries (58). In our experience, the few subjects who developed syncope or presyncopal symptoms did not have a fall or injuries, as they were in a laboratory situation and were promptly attended to. Patients who have had episodes of mild vertigo should also be tested with HUT as the accompanying dizziness could be a symptom of syncope (11). If syncope occurs in an individual in charge of a moving vehicle (e.g., an aeroplane in flight), a serious accident may result because of the ensuing loss of control. A VVS may be precipitated because of environmental factors such as fatigue, insomnia, dehydration, exposure to severe heat stress, painful conditions, medications and anxiety (Table 1). It is imperative, therefore, that a detailed history is obtained in a patient to ascertain if any such factors could have contributed to the episode. This is relevant in two ways. It helps to

Table 3: Common symptoms and signs during VVS

1.	Giddiness/light headedness
2.	Dizziness
3.	Restlessness/nervousness
4.	Headache/nausea/vomiting
5.	Abdominal discomfort
6.	Copious yawning
7.	Sweating
8.	Palpitations
9.	Blurring of vision
10.	Confusion
11.	Pallor
12.	Loss of consciousness without any of the above

distinguish between a one off episode and recurrent syncope, and in advising the sufferer as to the precautions that need be taken in order to avoid recurrence. In aircrew a more considered view may be taken while re-flighting if the s/he has been subjected to environmental /situational stress/s as listed.

The most prominent cardiovascular finding in a person who has developed LoC as a result of a VVS is a significant bradycardia. The precipitous fall in the blood pressure is often difficult to record as there is more often than not a time gap between the occurrence of the episode and the first BP measurement. By the time regular measurements are established the recovery is underway with regaining of consciousness. It is imperative that a patient of VVS is evaluated using on line monitoring technology (Fig.2). Without it, it is almost impossible to draw any definite conclusions about the condition in the given patient. The BP and heart rate take a variable time to come back to normal, the BP usually taking longer as compared with the heart rate.

Differential Diagnosis

In the differential diagnosis it is important to distinguish between VVS and the postural tachycardia syndrome (POTS) which again is more commonly seen in females. The symptoms of the latter condition are often quite similar to the prodrome of VVS except that LoC is a rare occurrence during POTS. Tachycardia, exercise intolerance, lightheadedness (dizziness), extreme fatigue, headache, blurring of vision are usually noted. In the upright posture, the heart rate increases by >30 /min within 5 to 20 minutes of the orthostatic stress while the blood pressure is maintained. The blood NE is markedly elevated to > 600 pg/ml. The symptoms tend to disappear quickly when the subject resumes supine position. Exposure to heat stress, dehydration, and anxiety inducing situations promote its onset (62, 63). The differences between the two conditions become particularly relevant in the Armed Forces personnel who are commonly exposed to various environmental stresses which are known to precipitate either. VVS is relatively innocuous and is not known to be associated with a permanent autonomic abnormality as such, while in POTS one of the possibilities is the permanent inadequacy of lower limb adrenergic innervation, be it because of antibodies to the $alpha_1$ receptors in the blood vessels or a genetic deficiency of NE transporters (63). On the other hand in VVS and /or orthostatic intolerance, response to orthostatic stress is only temporarily affected in people with an otherwise normal autonomic nervous system (64). It may be noted here that authors sometimes segregate VVS from orthostatic hypotension (65) while for the purpose of this review no such differentiation is being made while trying to understand this knotted issue.

Pathophysiology

The two cardinal features noted at the time of a VVS are: i) a decrease in peripheral vascular tone; and ii) bradycardia. This combination rapidly induces hypotension, reduces brain perfusion which, if it persists for a period of about 8 to 10 seconds, will result in LoC. This is in obvious contravention to the tachycardia and peripheral vasoconstriction that must normally occur as a reflex on change of posture from supine to erect in order to maintain brain perfusion and normal activity. It may be, therefore, helpful if the issue is analyzed under the two separate heads listed earlier.

As seen in Fig. 2, an initial decline of the peripheral resistance followed by a relatively rapid fall precedes the onset of bradycardia in a presyncopal/syncopal episode. A reflex peripheral vasoconstriction is produced by sympathetic activation because of deactivation of the arterial baroreceptors. This should normally help to maintain the volume availability/volume capacity ratio, and hence the venous return under circumstances which tend to cause peripheral vascular pooling (5). This is supported by experiments in which recording of

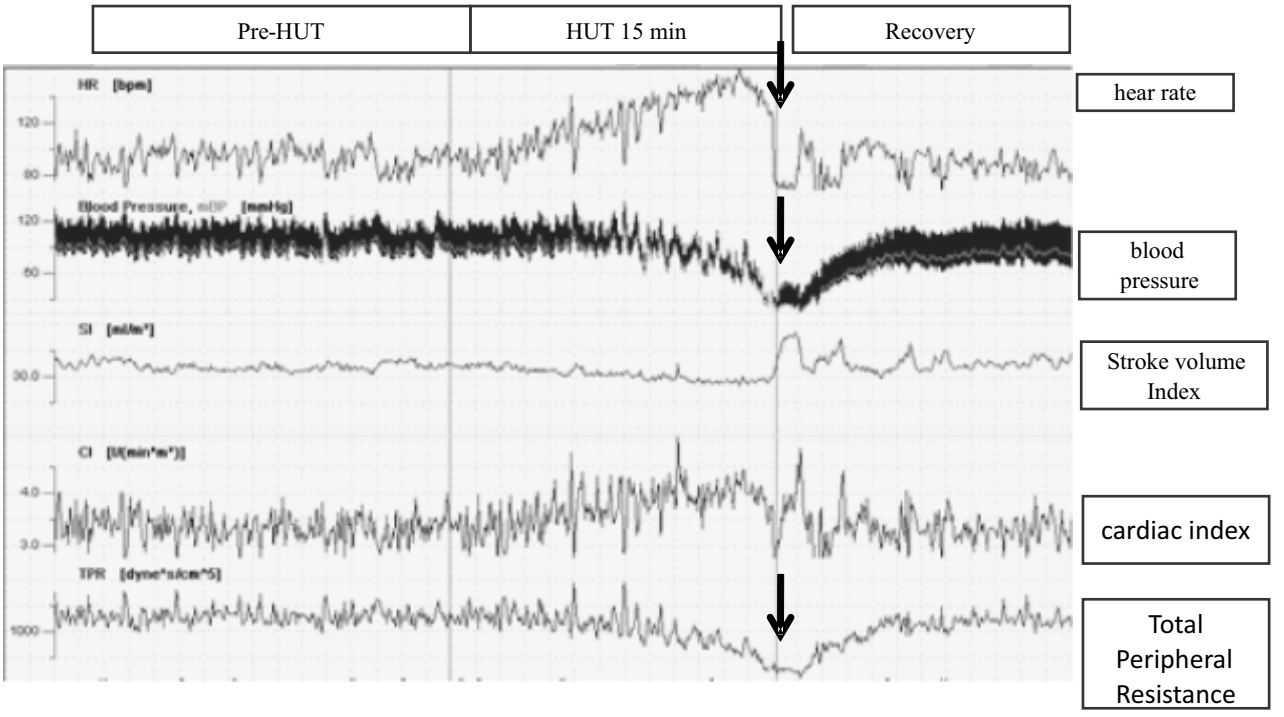

Fig. 2: On line record of a young male subject going in to VVS during HUT.
(Courtesy HOD Physiology and Clinical Physiology, College of Medicine, SQU, Muscat, Oman)
The arrows show the steep fall in the given parameters with the VVS. Note that the decline in the TPR and the BP started even as the tachycardia was accentuating indicating that the hypotension was initially because of the vasodilation. Despite this the stroke volume and the cardiac out put were maintained by the tachycardia.

muscle sympathetic nerve activity (MSNA) was shown to increase in response to peripheral vascular pooling (33, 34). Vadaddi *et al* (66) too reported an increase in MSNA with increasing depletion of the central blood volume. But what was intriguing was their observation that this happened not only in their normal subjects who did not develop syncope, but also in their patients of recurrent VVS. That MSNA activity was withdrawn at the time of presyncope/ syncope has been reported by (67). Therefore, widespread peripheral pooling including that in the splanchnic vessels which occurs at VVS is secondary to the withdrawal of sympathetic vasoconstrictor support to the peripheral vasculature. Mosqueda-Garcia *et al* (68) on the other hand reported that in patients of recurrent syncope, the decrease in baro-reflex sensitivity (BRS) and NE availability was accompanied by a near total disappearance of MSNA. It is difficult to explain the contrasts in the findings of Vadaddi *et al*, and Mosqueda-Garcia (66, 68). One possibility which may do so is the timing of recording of the MSNA during the episodes.

In a normal situation, the neurotransmitter NE in this case is released at the nerve terminals on receiving excitatory nerve signals. Some of it occupies the adrenergic receptors to produce vasoconstriction while some spills over into the plasma (and can be measured). Most of the unused NE is picked up by the NE transporters (NET) and repacked in to the NE vesicles at the nerve endings for subsequent use. Also available in the cytoplasm of the vesicle is a Vesicular Monoamine Transferase which sends the NE collected in the cytoplasm to the vesicles which are being recharged by a protein Dynamin (66). These authors (66) went further to demonstrate that in patients who were diagnosed with recurrent VVS, the availability of NE at the sympathetic nerve terminals was significantly reduced despite exaggerated sympathetic nerve activity as recorded by microneurography. They

attributed this to reduced presence of tyrosine hydroxylase at the nerve terminals. This enzyme catalyses the conversion of tyrosine to DOPA which progresses further to form NE via dopamine. As against this, in normal subjects, there appeared to be enough NE spill over and adequate availability of NET molecules. In the same context, it has been suggested that one possibility in the pathophysiology of POTS is a deficiency of NET in the lower limb vasculature (65). Carrying the argument further, it may be hypothesized that reduced availability of the GTPase (Dynamin) at the nerve terminals will result in reduced number of vesicles for NE and hence could be responsible for limited availability of NE when required despite sufficient volleys of sympathetic nerve impulses, creating another situation of dysautonomia. To date, we are unaware of objective evidence for this hypothesis.

Reflecting on the evidence cited so far, it may be concluded that in normal individuals, and perhaps in those who have an occasional /incidental syncope, the response to central blood volume depletion is a strong sympathetic response indicated by an increase in the MSNA with spill over of NE into circulation. At the time of syncope, this sympathetic activity is suddenly withdrawn, leading to wide spread vasodilation which drastically reduces venous return, causing hypotension, and diminished brain perfusion. As against this, the peripheral vasodilatation preceding syncope in patients with recurrent syncope seem to behave differently in that despite high degree of MSNA, the NE availability at the adrenergic receptor level is compromised, and this produces the vasodilatation which contributes to the compromised brain perfusion. The above findings may be taken as sufficient evidence that pathophysiology of recurrent VVS (and POTS) is very clearly a dysautnomia, not an innocuous condition (namely an incidental VVS) in which there is a temporary loss of cardiovascular control followed by complete recovery. A conclusion that the former disability(s) exist (s) in an individual may be arrived at by obtaining a detailed clinical history. Laboratory investigations will clinch the diagnosis. Those diagnosed with these melodies, therefore, must be declared permanently unfit for professions such as flying.

The evidence thus far suggests that at the time of occurrence of the faint, there is a sudden reduction in peripheral vascular tone brought about by a withdrawal of sympathetic vasoconstriction. What then is responsible for this vasoconstriction withdrawal? Bechir *et al* (69) reported that BRS (ms/mmHg) calculated using Fast Fourier Transformation was significantly lower in subjects who had suffered VVS as compared with normal subjects, at rest as well as during central blood volume depletion by LBNP. They argued that it was responsible for relatively poor MSNA activity during central blood volume depletion in these subjects of VVS, though this may not be the only cause and effect relationship. Arguing against this, Furlan *et al* (70) commented that *"Baroreflex function can be depressed by suprabulbar central influences and also by vagal, somatic, or sympathetic afferents"*. (Perhaps a more appropriate expression could be Baro-reflex dysfunction rather than depression). The complex interactions around the NTS have been described earlier. For the withdrawal of the vasoconstrictor tone which should be enhanced during CBV depletion in order to maintain the volume availability/volume capacity ratio close to normal, activity of the CVLM needs to be enhanced (Fig. 1). This in fact is in contravention to the normal physiological role of this nucleus which is supposed to be activated by increased baroreceptor afferent input, not during reduced input which is the case under these circumstances. In that case, an influence other than the CVLM must directly inhibit the RVLM (thus rapidly withdrawing the sympathetic excitatory response) or excite the CVLM which in turn would increase its inhibition of the RVLM. Perhaps higher central influences (Fig.1) play this decisive role. Recently, advanced methods in neuroimaging have been used to study subjects who had

common faints as against those who did not have such a history. Beacher *et al* (71) found that the medullary and mid brain volumes were reduced in patients who had had syncope while Kim *et al* (72) using voxel-based morphometry and regional volumetry, reported smaller volumes of insular cortex in VVS patients. An extensive review has further suggested that the frontal lobe has connections with the NTS and the hypothalamus, and hence any dysfunction in the former may affect sympathetic outflow (73). In that review, the authors have opined that cortical thickness of those who have common syncope was different to those who suffered from POTS. All these reports suggest a complex higher centre dysfunction in patients with VVS and allied maladies. Does than this demands that based on radiographic findings, ALL those who have suffered a syncope must be dubbed, anatomically and physiologically abnormal? If so even those who have reported VVS brought about in the presence of situational disturbances (Table 1) must be labeled as permanent suspects. At this point of time, however, such a conclusion seems too far fetched to be implemented, especially in specific professions such as aviation. It may also be impractical to evaluate each and very person with VVS in such a detailed manner to determine his/her fallibility as physiologically normal.

NO is a well established endothelial relaxation factor (EDRF) responsible for arteriolar dilatation (40). Normally, there is a reciprocal control between vasoconstriction (adrenergic) and vasodilation (NO induced) in the peripheral vasculature which helps to maintain adequate tissue perfusion at all times. During CBV depletion, in order to maintain blood pressure, it is the vasoconstrictor element which must dominate in order to maintain diastolic blood pressure (arteriolar constriction), and support venous return by inducing venoconstriction. But if NO induced vasodilation overcomes the adrenergic vasoconstrictor effect, the resulting vasodilation will contribute to a possible VVS. That this happens in young adults with recurrent VVS was elegantly demonstrated by Stewart *et al* (74). They concluded that in patients with recurrent VVS, there is large scale vasodilation, particularly in the splanchnic bed which is mediated by NO which is secreted by neuronal NO via nitrergic nerves, a nomenclature suggested by Toda *et al* (75) to describe neuronal NO synthesized in postganglionic parasympathetic nerves to distinguish them from cholinergic nerves. In their subjects Stewart *et al* were able to reverse the vasodilation by administering NO synthase inhibitors, and in fact have suggested that this may be a valid therapeutic approach in treating patients of recurrent VVS.

It has been mentioned earlier that endothelin produced locally by endothelial cells help to generate NO by influencing ET_B receptors while at the same time producing vasoconstriction by occupying ET_A receptors (56). It may be hypothesized that in some individuals prone to recurrent VVS, the ET_B receptors overwhelm the ET_A receptors numerically. This may then make the endothelin released to induce more NO secretion, and hence vasodilatation, in lieu of its expected physiological action of vasoconstriction.

These arguments may explain the pathophysiology of recurrent VVS to an extent. But what about the occasional/incidental syncope in otherwise normal individuals? Could there be other mechanisms which induce such events? During sympathetic stimulation, there is an increase in velocity of blood flow. This induces shear stress on the endothelial cells, prompting them to generate NO (40). It could be hypothesized that in otherwise normal individuals who develop a VVS under a set of environmental / situational stresses (Table 1), the shear effect produced by the stress may be of such a high order that the NO generated may be quantitatively enough to overcome adrenergic vasoconstriction and cause a peripheral pooling which is a preamble to progressive decrease in the venous return. Not only are the peripheral effects of NO relevant in cardiovascular control,

but as argued by Zanzinger (76), NO has an important role in manipulation of peripheral vascular tone by interfering with sympathetic activation of peripheral circulation at the level of the NTS and its ramifications to provoke pathophysiological vasodilation which progresses to VVS. The above discussion thus brings out emphatically the role of uncontrolled vasodilation, whether because of withdrawal of sympathetic vasoconstriction, or because of actions of NO at the centre or at the periphery, as a major contributor to the pathophysiology of VVS.

The bradycardia of VVS is the other major contender in the causation of VVS, and in all probabilities is reflex in origin. The most consistent explanation for the reflex bradycardia is that it is probably a response to excessive stimulation of the ventricular mechanoreceptors chemically or mechanically (the Bezold-Jarisch reflex). This occurs because the "near empty ventricles" beat vigorously to stimulate the mechanoreceptors in their walls. The afferents are carried by vagal C fibres to the NTS from where they orchestrate the bradycardia (38, 44, 57) to set up the cardio depressor part of the reflex. (The latter's assertion that mechanoreceptors which affect the heart rate are to be found in the urinary bladder, the intestines, and the lungs may help to explain the syncope which may be brought about by micturition, defecation and a bout of coughing).

Certain factors such as prolonged standing (even in normal individuals), exposure to heat stress, use of vasodilator medications etc tend to promote the venous pooling. The hydrostatic pressure exerted by the peripheral pooling would accentuate the loss of fluid into the interstitial space, further compromising the venous return. Greater this loss, more is the sympathetic nerve activity as measured by microneurographic recording techniques (66, 67). It is conceivable, therefore, that exaggerated sympathetic activity occurs. This in turn will produce powerful contractions of the ventricles which as such are relatively "empty" because of the diminishing venous return, and thus precipitate the bradycardia adding to the already established lowered vascular tone. VVS could be the outcome. This hypothesis supported by Yamanouchi *et al* (77) who found that in their subjects with a history of VVS, the left ventricular (LV) volume as measured using 2D echocardiography reduced quite significantly during HUT while that of their normal subjects did not. They carried these findings forward by suggesting that it may indeed be the very low LV volume during CBV depletion that may precipitate the reflex bradycardia seen during VVS. Surprisingly, however Novak *et al* (78) have refuted this theory by demonstrating that the ventricular chamber size measured using echocardiography did not change during a presyncope or a frank syncope. In another study, eight healthy young men were depleted of their CBV using LBNP until they reached presyncopal level. 2D echocardiography at this stage showed only a 28% decrease in LV volume, not a near empty ventricle. On the basis of these findings the authors concluded that Bezold Jarisch reflex is not brought into play when VVS occurs (79). Perhaps if the degree of stress applied by them was severe enough to precipitate a frank syncope, they may have found "near empty" ventricles. There is therefore an ambiguity in the occurrence of a significant bradycardia as a pathophysiologic event during VVS. That the Bezold Jarisch reflex is responsible for the syncopal bradycardia is indeed apocryphal has been suggested by Fitzpatrick *et al* (80). Some of their heart transplant patients developed near syncopal episodes with bradycardia on exposure to a decrease in CBV. They have argued that this suggested partial vagal innervation of the transplanted donor heart, but there was no evidence to suggest that these hearts could have developed afferents from the LV mechanoreceptors which are necessary to bring on the Bezold Jarisch reflex. Nevertheless, in the classical VVS scenario (Fig. 2), there is no doubt that bradycardia forms an important event in the progression of VVS. This conclusion is strengthened by the findings of a study by

Santini *et al* (81) who were able to prevent the bradycardia in their subjects who developed syncope during HUT exposure with intra venous administration of atropine to block post ganglionic muscarinic cholinergic receptors. In conclusion, bradycardia is an integral part of VVS. As to whether it is induced because of the Bezold Jarisch reflex or because of the complex interactions in the region of the NTS is a matter of debate.

Baro Reflex Sensitivity (BRS) and Syncope

"BRS is defined as the change in inter beat interval (IBI) in milliseconds per unit change in BP" (82). In principle, it indicates as to how the sinus code autonomic innervation reacts to oscillations in the blood pressure on a beat to beat basis. This has also been referred to as cardio-vagal BRS (83). Jaju (32) using noninvasive online recording of blood pressure and ecg (RR intervals) computed the BRS in normal young Omani Arabs using the Sequence method. In this, the computer identifies a minimum of four consecutive beats with either a widening of their R-R intervals (Up events) or with a reduction in their R-R intervals (down events), and calculates the change in the time (msecs) per 1 mmHg change in the systolic BP. She reported that the BRS reduces from about 24 msec/mmHg to about 10 msec/mmHg at the end of 5 minutes of 70^0 HUT indicating a sympathetic dominance on the SA node. Others have also reported similar figures in normal subjects (83). The BRS has been found to be low even in resting conditions in those with poor orthostatic tolerance and the index does not change much on exposure to orthostatic stress (69, 84). In fact it has been suggested that the defect may lie at the sensory receptor level where the changes in the pulsatile pressure are not sensed adequately (69). Surprisingly though the same authors (69) do not report a decrease in the BRS of their normal subjects during orthostatic stress. A low resting BRS does not augur well for SA node nervous control, and has been associated with serious heart disease and hypertension (82, 85, 86). On the other hand, not much difference was noted in the BRS of normal subjects and patients of VVS at rest or during HUT (Table 4). How does one then use BRS in the assessment of VVS subjects, and how relevant is it? Is it best to leave BRS as a prognostic measure in serious heart disease rather than link its presence to the so called innocuous VVS? Theoretically sufferers from an occasional VVS at the time of evaluation should have a normal robust BRS which decreases during HUT, and recovers rapidly to the prepostural change status later as in normal people who have never had a syncope (Fig. 3). That those with recurrent syncope and poor orthostatic tolerance have a low BRS value which does not change much during HUT has already been established (69, 83). Where does that leave the so called "Normal" individual who may have had an incidental VVS probably precipitated by a series of environmental factors in terms of his/her cardiovascular reflex status? Perhaps if such people are subjected to prolonged orthostatic stress (which should produce a syncope in most) and their parameters recorded (including the cardio-vagal BRS), their response and recovery after the VVS episode would closely resemble the so called normal (Fig. 3), while those with conditions such as recurrent VVS/POTS would show a different pattern. A study by Cheng *et al* (85) seems to support this view. The BRS of their normal subjects and patients of VVS were about 24 msec/mmHg and 22 msec/mmHg. This parameter reduced in both groups when exposed to HUT. While the post tilt BRS of normal subjects recovered to near pre-tilt value, that of the VVS subjects continued to remain low. Such an investigation is likely to help in reflighting of highly trained aircrew who may have had a VVS and have been grounded on the mere suspicion that such an event may recur while in flight and lead to a catastrophic event. Alternatively use of BRS may be confined for use in patients with heart disease such as myocardial infarction, hypertension where a low BRS has been associated with high morbidity/mortality (82, 86, 87).

Table 4: BRS of normal subjects and those who had VVS episodes (Data courtesy HoD Physiology and Clinical Physiology, Sultan Qaboos University)

Normal			+ve tilt		
Gender/age	**BRS rest**	**BRS Tilt change**	**Gender /age**	**BRS rest**	**BRS Tilt change**
F 40	17.2	11.2 **-6.0**	M31	6.8	7.0 **+0.2**
F69	20.5	11.5 **-9.0**	F45	12.9	10.36 **-2.54**
M57	6.2	4.54 **-1.7**	M33	--	5.3
F55	4.4	8.48 **+4.08**	M27	20.2	18.14 **-1.8**
F39	12.6	12.42 **-0.18**	M33	12.3	9.2 **-3.1**
F36	6.1	4.9 **-1.2**	F43	7	9.3 **+2.3**
			F54	9.2	7.32 **-1.88**

(Data courtesy HoD Physiology and Clin. Physiol. CoM and SQUH, SQU).

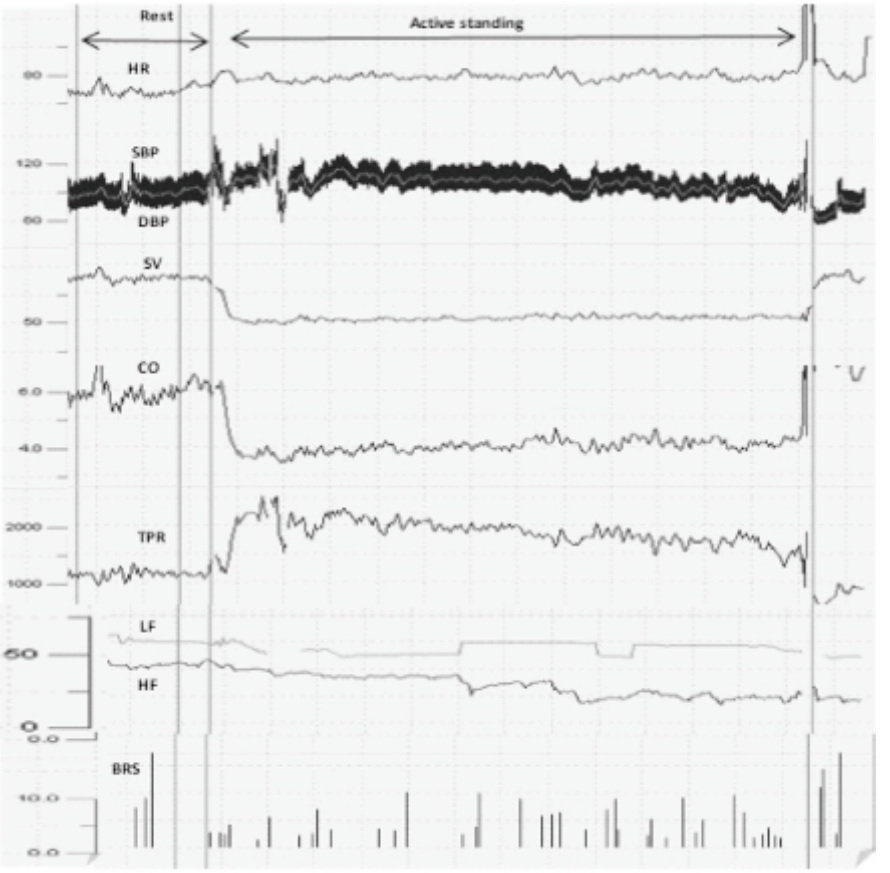

Fig. 3: Response of a normal healthy individual to change of posture from supine to standing. Note the robust BRS at rest (last tracing) which reduces during standing, and recovers to near original level when back to supine.
(Record courtesy HoD Physiol and Clin Physiol, CoM,SQU, Muscat, Oman).

Thus far two major aspects which contribute to the pathophysiology of VVS have been dealt with: namely the peripheral vasodilation and the bradycardia. However these cannot be looked at in isolation.

Hormonal secretions change during orthostatic stress and alter further if a VVS occurs. A number of hormones are released when CBV reduces (as during applied orthostatic stress). Jaju (32) showed that plasma NE in the blood of her normal subjects increased significantly after 5 minutes of HUT exposure, and this correlated well with autonomic parameters of sympathetic excitation namely LF nu (low frequency normalized units) and LF/HF ratio. In patients who suffer recurrent syncope, plasma epinephrine levels are much higher at near syncope than normal subjects and contribute to the greater degree of vasodilation by counteracting the sympathetic vasoconstriction, and to the more vigorous ventricular contractions as shown by greater LV shortening fraction (which may stimulate cardiac mechanoreceptors) (88). Epinephrine may also cause the over bearing dilation in muscle vascular beds by stimulating beta adrenergic receptors. Others have found that plasma catecholamines, ACTH, Arginine vasopressin (AVP), plasma renin activity and aldosterone increase in normal subjects in whom near VVS is precipitated by using a combination of HUT and LBNP (Jacob *et al* 1998) (89). Plasma cortisol and prolactin levels increased in those who developed VVS during HUT, the change being attributed to activation of serotonergic neurons in the region of the NTS, show casing the latter as the main objective of study (90). The role of cortisol as a stress hormone during VVS is understandable, but in the current context the conjoint increase in prolactin has been used only to high light the serotonergic neuron activity in the brain, and not for any cardiovascular effects of prolactin. Jardine *et al* (91) report simultaneous increases in blood AVP and atrial natriuretic peptide (ANP) in their patients of recurring syncope during HUT. They opined that the vasodilation at the time of VVS could have been contributed to the increase in ANP. This is difficult to reconcile with as the natural stimulus for ANP secretion is an increase in atrial filling, not a reduction in heart volumes which occurs with lowering of the CBV. During VVS, the atrial volume would be further depleted, thereby removing the natural stimulus for ANP release. The arguments thus far have shown that the reduced vascular tone and cardiac depression which occur at syncope are interwoven with endocrine changes which are probably triggered by the central ramifications in the region of the NTS, the CVLM and the RVLM, whether it be inhibition of sympathetic vasoconstriction, or stimulation of the vagus, or a combination of both.

Post Space Flight Orthostatic Intolerance

A significant percentage of astronauts /cosmonauts returning to earth after space flight (micro gravity environment) are known to develop symptoms and signs of orthostatic intolerance amounting to presyncope. Full spontaneous recovery occurs in a few days. Frank VVS is uncommon. Disturbance in baro-reflex activity in terms of gain in the baro-vagal reflex has been suggested as the root cause (92, 93). A detailed investigation was carried in 13 astronauts before, during and post space flight (94). In flight, the astronauts themselves performed carotid baroreceptor stimulation and deactivation experiments using neck suction and pressure. The data reflected an increase in sympathetic activity in flight, and not a gain in the baro-vagal reflex as reported by others (92, 93). However, the vagal part probably starts to regain its edge on return to earth and may take as long as a week to re-establish itself. Perhaps on landing the upswing of the vagal influence produces the presyncopal effects such as dizziness and other symptoms and signs. An important contributing factor may be the poor anti-gravity muscle tone because of the zero gravity environment. This may fail to support the peripheral circulation on returning to earth. Other factors suggested are a loss in circulating

plasma volume, and vestibular stimulation which result in vomiting and add to fluid loss (94). It is surprising that no mention has been made by any of the authors on the role, if any that ANP may have in the physiological effects of space flight. There is a significant increase in the CBV during space flight and also an increase in the heart volume content (93) as the gravitational effect on the blood column is absent in space. This should act as a powerful stimulus for the release of ANP from the atria, and probably could account for the diuresis observed in the early phase of zero G flight. The high blood levels of ANP may persist on return to earth to cause vasodilation as also the cardio-vagal reflex gain post flight to produce orthostatic intolerance. The only damning factor may be the short half life of ANP (about 2-5 minutes). It may be interesting to study the role of ANP after simulated microgravity conditions produced on earth by water floatation or prolonged bed rest.

The elderly are known to have a high incidence of syncope, especially in the 70 years and more age group (6, 9, 64). It is important to differentiate between the classical VVS and a variety of clinical reasons which may present as a syncope in the elderly, particularly cardiovascular and neurological disorders, and age-induced autonomic dysfunction (Table 1). BRS has been found to decrease with advancing age particularly in the "Down" events, and thus may be a factor which determines the higher incidence of VVS in the elderly (95). VVS is the most common cause of syncope in the elderly and may occur under a variety of circumstances such as change of posture, defecation, coughing, sneezing and micturition. Most patients present with bradycardia as the primary manifestation. What makes it dangerous is the serious injury it may cause to the patient if s/he sustains a fall. Further, the aged are more prone to develop dehydration. They may be consuming medications (diuretics) which may contribute to the dehydration, and lower vascular tone (96). As such this group is handicapped physiologically by a lower circulating fluid volume, making them more susceptible. Another issue to be noted in the care of the elderly is the possibility of their developing VVS after a meal which may induce secretion of vasodilator substances leading to sudden hypotension. Perhaps age induced aberrations in the autonomic nervous system may produce this dysautonomia (14). It is pertinent to note that more often than not, history of the classical prodrome or of past history of having had fainting spells is not forthcoming because of prevalent cognitive deficiencies (57, 97). This makes diagnosis of the condition difficult. Further, the VVS has to be carefully distinguished from other more serious causes of LoC in the elderly namely cardiovascular and neurological events (98). All these issues make VVS a serious proposition in the aged.

Evaluation of VVS

A scrupulous history of the syncopal event is usually enough to distinguish the VVS under discussion here from syncope attributed to various cardiovascular, neurological and other clinical disorders (57, 65, 98). Eliciting information on exposure to various physiological/environmental stresses (Table 1) is essential to accurately attribute the cause of the VVS as it will help to determine as to whether the episode could be classified as "Innocuous". The information is helpful in the management of such cases by offering advice on preventive avoidance to the stress. It may or may not be essential to further investigate the episode if the history is able to reveal the possible cause. Though in the elderly it may be prudent to use provocative tests to bring on the episode if possible (57). However, we believe that even if there is a single episode of VVS in a specified population such as aircrew, detailed testing is warranted.

Logically the tests that reduce the CBV would be most effective in challenging the cardiovascular baroreceptor reflex mechanism. These include 1. Quiet standing; 2. Head-up tilt table (HUT) test; 3. Lower body negative pressure (LBNP); 4. Combined HUT and LBNP;

and 5. HUT + vasodilator drug or LBNP and vasodilator.

Quiet standing is a simple test which does not require special laboratory set up, and can be easily carried out using basic equipment (30), as also using sophisticated monitoring equipment (Fig. 3). Standing dislocates about 400-500 cc of blood from the CBV to the periphery to deactivate the cardiovascular baroreceptors and set up a reparative reflex response. In the POTS, the HR increases by >30 beats /min without any appreciable change in the blood pressure, but with typical symptoms of dizziness, palpitations, sweating after about the 5th minute. LoC is very rare. If a syncope was to occur, there would be symptoms of dizziness, discomfort, a fall in BP followed by a sudden bradycardia and LoC. Repeated measurements need to be made to keep track of the developing situation.

By far the most tried and tested method of evaluating a patient of syncope is the HUT. The degree of tilt can vary from 20^0 to 70^0. The latter is most frequently used as hydrostatically it produces the effects of a near vertical tilt (tangent of 70^0 is close to 1, i.e. of 90^0) while avoiding the severe discomfort and perception of falling over felt by the subject when tilted by 80^0 or more. We have used 70^0 HUT extensively (15-18, 22, 23, 32) to study cardiovascular reflex status as have others (8, 12, 59). The usual duration of HUT stress is 15 to 20 minutes though duration of 5 minutes as well more prolonged (>30 min) have also been used. The longer duration tilts may be used to provoke a syncopal like situation or frank syncope because longer the duration of the stress, greater is the loss of circulating fluid volume into the periphery. The rate of application of the HUT may be maintained constant by using electrically operated HUT table. Ideally it should take about 15 seconds. Faster rate of application may bring about vestibular stimulation which may influence cardiovascular response.

A normal response to HUT consists of an increase in mean arterial pressure, in the diastolic BP and in the in heart rate, and with a fall in the pulse pressure (11). The stroke volume index and the cardiac output index reduce significantly by 33% and 18%, respectively (17). (The stroke volume was measured using LE ejection time method). If HUT induced reflex syncope occurs, the BP starts decreasing after a variable time on assuming HUT. The heart rate (HR) may increase just before syncope and plunges at the time of syncope along with the BP (Fig. 2).

It is difficult to arrive on a consensus regarding sensitivity, specificity and diagnostic yield of the HUT as it depends upon the technique used (99). HUT has acceptable sensitivity and specificity when calculated in patients with true VVS (100, 101). Some authors have recommended a combination of HUT with vasodilator drugs (sublingual nitrates or intra venous infusion of isoproterenol) to provoke VVS during HUT especially in those who may have had an incidental episode. A recent systematic review gives the 66% of overall positivity rate for syncope with nitroglycerine challenge and 61% for isoproterenol challenge (102). Others opine that drug provocation during tilt helps to reduce the total duration of the tests (103). Importantly there are those who recommend drug free (non provocative) tilt over pharmacological challenge as it provides better diagnostic yield (100, 101). We too would like to support this view.

When should a HUT be done? We opine that it will be definitely helpful in confirming diagnosis in patients with recurrent syncope and in those apparently normal individuals in selective occupations (pilots) who have had an incidental episode of syncope. Our belief is strengthened by a similar opinion by others (102). It is also useful in evaluating elderly patients with a possible occurrence of VVS because often such patients are unable to give accurate history of the episode (102).

Apart from HUT, we have used lower body negative/suction pressure (LBNP/LBSP)

(15, 19-21, 104) to test cardiovascular responses to graded displacement of the CBV in to the periphery. Blood volume displaced by LBNP of -40 mmHg and 70^0 HUT is similar at about 500-600 ml. The suction may be applied in a graded manner so that the volume of blood displaced increases in a graded manner. This has helped in deciphering the role played by low pressure cardiopulmonary receptors in FVR. At suction pressure below 20 mmHg there is an increase in the FVR without a change in HR, while by -30 mmHg, tachycardia induced by deactivation of arterial baroreceptors sets in without a further change in the FVR (21, 34). There are slight differences in the reflex response to the two types of stresses (Table 2). The greater increases in the tachycardia and the diastolic pressure during HUT (11) are attributed to the natural gravitational effect under which the reflex mechanisms normally operate in day to day life (105) making it a better natural stimulus, while during LBNP (21) the subject is in a horizontal position thus eliminating the change in hydrostatic pressure normally brought about by gravity while in the upright posture. This makes HUT a better tool for investigation of the reflex mechanism under discussion.

A combination of HUT and LBNP (-20 and -40 mmHg for 10 minutes each) has been applied simultaneously to elicit cardiovascular responses to CBV displacement in to the periphery (106). 84% of the subjects who underwent this procedure developed presyncopal effects. The authors argue that this technique is better as the duration of exposure to the stress is markedly reduced. Against this, we would argue that the type of VVS in apparently normal but high risk profession individuals that we are looking at, should fall in to the 16% of those who do not develop any symptoms. This argument seems too farfetched to classify only those in the latter category to be "safe". We would agree more with those who recommend drug free tilt over pharmacological challenge as it provides better diagnostic yield (100, 101).

Principles of Management of VVS

A detailed approach to management of various types of syncope may be found in the ESC Guidelines for the diagnosis and management of syncope 2018 (100). It may not be necessary to treat vigorously all those with history of VVS (64, 65). A useful approach for those who may suffer from an occasional VVS brought about by environmental situations such as dehydration, prolonged standing, and heat stress exposure would be to ensure that the discrepancy between the availability of circulating fluid volume and the vascular volume capacity ratio is brought back towards as far as possible. This may be easily done by increasing fluid and salt intake as the basic measure. They should be advised on avoidance of such situations in order to prevent recurrence. This should suffice for subjects who are aircrew with a single or occasional history of VVS. The approach should be useful for patients diagnosed with POTS, a distant cousin of the prodrome of VVS. In addition, reassurance and upper torso exercise training has been found to be helpful. As a precautionary measure, such patients are advised to stand up slowly using support. More stringent measures may not be necessary, and if required may be decided upon by the clinician.

In more persistent recurrent VVS, various medications have been tried with variable success. The principle applied was to modulate available knowledge of various neurotransmitters associated with functioning of the NTS. Schobel *et al* (49) suggested that blocking opiod receptors in the NTS region could be helpful in augmenting the cardiopulmonary deactivation reflex. However, their suggestion was refuted (50). That serotonin may have a role to play in the etiology of VVS was proposed by Jordan (51) and accordingly paroxitine, a selective serotonin uptake inhibitor was tried in the treatment of recurrent syncope with some success (52). However, another analysis by Raj and Koffin did not particularly find paroxitine particularly useful (65). The use of beta blockers was attempted in order to reduce

the powerful ventricular contractions which may be responsible for stimulating ventricular mechanoreceptors (69) that form the afferent limb of the Bezold Jarisch reflex. But the results of the use of this class of drugs was not very encouraging (107). Midodrine an alpha adrenergic receptor agonist, has been found to be useful (107). This would help to limit the vasodilation as a major contributor to the pathophysiology of VVS.

The role of NO in causing vasodilation during VVS has been elaborated earlier (74-76). In fact, Stewart *et al* (74) demonstrated that use of NO synthase inhibitors was helpful in attenuating the vasodilation during of VVS and is deemed promising as a tool for use in patients of recurrent VVS.

There have been attempts to improve responses of patients with recurrent VVS by orthostatic training (84). Patients of VVS were asked to lean against a wall with their feet placed 15 cm away from the wall. Duration of each session was 30 minutes. The authors reported an improvement in 74% of those who completed the training. The success was apparently limited by a relatively low compliance by the volunteers.

Verheyden *et al* (108) gave daily HUT exposure training to 17 VVS patients of recurrent VVS with the hypothesis that repeated HUT stress will improve vasoconstrictor response. They found that after about 4 exposures the their subjects were free of VVS and prodrome, and they remained symptom free for long as 6 weeks if just quiet standing was continued at home. This could indeed be a useful approach in tackling the problem.

Yoga training affects sympatho-vagal balance positively (109). If VVS is an indication of temporary autonomic dysfunction, practice of yoga should help patients of VVS. That it actually does so was reported by Gunda *et al* (2015) (110) in female subjects with syncope. Heart rate variability drifts towards vagal dominance signaling alteration in the autonomic control of the sinus node. Theoretically this may increase resting cardio-vagal baro-reflex sensitivity there by helping to alleviate symptoms of presyncope and syncope in these subjects. Our own experience showed that the three cosmonauts (one Indian and two Russian) who flew the joint Indo Soviet space mission in 1983 did not react adversely to orthostatic stress on return to earth (111). They had been trained to do yoga exercises during their preflight training and during the space flight. As to whether it was partly the yoga training that helped them cope with rigors of space flight cannot be ascertained as this was only one such experiment. It may be interesting to give a serious thought to this aspect when ISRO sends up an Indian manned space flight in the near future.

Conclusions

Despite the extensive research and material available on VVS, the entity remains baffling. As to what is a "Safe VVS" especially in certain types of populations such as aircrew still remains partially unanswered. It can be concluded that VVS is a result of a temporary failure of reflex control of the cardiovascular system. The search for the means of overcoming this shortfall on a permanent basis in subjects who suffer VVS is still ongoing.

Conflict of Interests

Nil.

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Oxidative Stress is Independent Factor for End-stage Renal Disease in Type 2 Diabetes Mellitus Patients

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ABSTRACT

An imbalance between oxidant and antioxidants is thought to precede the development of renal lesions. The aim of present study is to determine the relationship between oxidative markers and severity of microalbuminuria in patients with type 2 diabetes mellitus (T2DM). A total of 100 T2DM patients (50 males and 50 females) participated in this study. They were screened for microalbuminuria along with oxidative status in patients. Microalbuminuria was detected by measuring the albumin to creatinine ratio (ACR) in urine samples. Patients were divided into two groups; normoalbuminuria (n=36) and microalbuminuria (n=64) as per the ACR levels. No difference in the groups was observed in terms of age, sex, glycated hemoglobin (HbA1c) and blood pressure. The level of oxidative stress was significantly higher in microalbuminuria group of T2DM patients. A significant correlation was observed between ACR and lipid peroxidation (MDA) levels. We conclude that oxidative stress is one of the important mediators of end-stage renal disease (ESRD).

Keywords: Type 2 diabetes mellitus, microalbuminuria, diabetic nephropathy, albumin to creatinine ratio, oxidative stress.

Introduction

The incidence of diabetes mellitus is increasing globally. Besides, it is also one of the leading causes of end-stage renal disease (ESRD). According to the International Diabetes Federation (IDF), approximately 425 million adults are presently living with diabetes and by 2045, this will reach to 629 million (1). Type 2 diabetes mellitus (T2DM) is associated with older age, obesity, family history of diabetes, impaired glucose metabolism, and physical inactivity. The disease usually begins with either insufficient insulin secretion or its impaired action. Uncontrolled diabetes leads to a number of short and long-term complications such as ketoacidosis, diabetic retinopathy, nephropathy, neuropathy, and many other complications.

Renal damage (nephropathy) is a serious complication of diabetes mellitus and is the leading cause of ESRD. In diabetes, kidney disease is characterized by progressive albuminuria, decline in glomerular filtration rate (GFR), hypertension and high risk of cardiovascular mortality and morbidity (2). Microalbuminuria is an early marker of progressive nephropathy that can be measured in spot urine as albumin to creatinine ratio (ACR). In general, ACR has slightly better diagnostic accuracy than urine albumin concentration alone for detection of albuminuria in many populations (3). Moreover, ACR appears to be the best marker for early identification of diabetic nephropathy. Screening for albuminuria is recommended in patients at increased risk for chronic kidney disease (CKD), those with

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hypertension, diabetes mellitus, cardiovascular disease and a family history of CKD.

Diabetic nephropathy is characterized by excessive accumulation of extracellular matrix in kidney. The basement membranes are thickened and the glomerular mesangial matrix and the tubulointerstitial space are expanded. Increased oxidative stress in diabetes contributes to the pathogenesis of diabetic nephropathy and its progression to ESRD. However, relatively little information is available to define the association between oxidative stress and microalbuminuria in human subjects. It is, therefore, pertinent to understand the inter-relationship between oxidative markers and microalbuminuria in T2DM patients. It would be clinically useful to identify the associations of oxidative stress with severity of microalbuminuria, so that it can be used as a determining factor for assessing nephropathy in patients with diabetes.

Materials and Methods

A total of 100 T2DM patients (50 males and 50 females) were recruited from the medical OPD of HAHC Hospital from July 2017 to May 2018. T2DM was diagnosed as per the criteria laid down by the American Diabetes Association (ADA) (4). Based on their clinical history, subjects with the following manifestations were excluded from the study: patients with high blood pressure, other causes of albuminuria such as fever, urinary tract infection, congestive heart disease, and hematuria. The anthropometric data were collected for each participant at the time of recruitment and informed consent was obtained. The study was ethically approved by the Institutional Ethics Committee (IEC) of Jamia Hamdard, New Delhi.

Study Assessment

After an overnight fasting, blood and urine samples were collected for biochemical analysis. The biochemical parameters such as fasting glucose level, lipid profile, liver, and kidney function tests were determined using Siemens Xpand plus biochemistry autoanalyzer. The glycated hemoglobin (HbA1c) level was evaluated in Bio-Rad D-10$^{(TM)}$high-performance liquid chromatography-based system. The oxidative markers such as reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and malondialdehyde were analyzed by spectrophotometer using standard protocols(5).

Albumin-creatinine Ratio (ACR) Measurement

Urinary albumin and creatinine levels were analyzed in random spot urine samples by Siemens Xpand plus biochemistry autoanalyzer and ACR was calculated. ACR is considered as an early marker of progressive nephropathy. As per ADA and National Kidney Foundation guidelines, microalbuminuria is defined by urinary ACR level at the range in between 30 to 300 μg/mg in both men and women(3).

Statistical Analysis

For each variable, values were expressed as mean ± SEM. The statistical analysis was carried out in GraphPad Prism, ver. 5.0 software. To detect possible relationships between ACR and other variables, Pearson's correlation coefficient (r) was calculated. At 95% confidence interval, $p<0.05$ was considered as statistically significant.

Results

In present study, out of 100 T2DM patients, 64% of patients were diagnosed to have microalbuminuria and rest 36% had normal albumin levels (normoalbuminuria) (Table 1). Moreover, 36.1% and 42.1% of smokers were present in the normoalbuminuria and microalbuminuria groups, respectively. No differences in the groups were observed in terms of age, sex, HbA1c level and blood pressure.

The biochemical parameters such as total cholesterol, triglyceride, high-density

Table 1: Baseline characteristics of patients with type 2 diabetes mellitus

	Normoalbuminuria (n=36)	Microalbuminuria (n=64)
Sex (M/F)	14/22	33/31
Smoker/non-smoker (n)	13/23	27/37
Alcoholic/non-alcoholic (n)	7/29	13/51
Age (years)	48.5±13.3	46.1±14.3
SBP/DBP (mmHg)	128.4±8.1/83.3±5.1	131.4±7.4/ 83.2±5.5
HbA1c (%)	10.4±1.5	10.8±1.93
ACR (µg/mg)	14.06±5.2	120.8±80

SBP-systolic blood pressure; DBP- Diastolic blood pressure; ACR- Albumin-creatinine ratio.
All values are expressed as mean±SD; $p<0.05$ statistical significant.

Table 2: Biochemical parameters in T2DM patients in normoalbuminuria and microalbuminuria groups

	Normoalbuminuria (n=36)	Microalbuminuria (n=64)
Total cholesterol (mg/dL)	218.9±30.2	219.3±30.7^{ns}
Triglyceride (mg/dL)	194.6±25.1	186.5±26.7 ^{ns}
HDL-C (mg/dL)	35.3±6.4	33.8±4.4 ^{ns}
LDL-C (mg/dL)	149.0±19.3	143.2±21.4^{ns}
VLDL-C (mg/dL)	38.9±5.8	37.3±8.6 ^{ns}
SOD (U/mL)	6.58±3.6	5.20±3.8*
GSH (µmol)	62.3±10.1	54.6±9.2 ^{ns}
MDA (µmol)	174.3±30.1	216.0±32.2**
Catalase (KU)	178.1±28.2	175.1±25.2^{ns}

Data were expressed as mean±SEM. HDL-C- High density lipoprotein-cholesterol; LDL- Low density lipoprotein, VLDL- Very low density lipoprotein, SOD- Superoxide dismutase, GSH- Glutathione; MDA- Malondialdehyde. $p>0.05$ [not significant (ns)], * $p<0.05$, ** $p<0.001$.

lipoprotein cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were analyzed in T2DM patients of normoalbuminuria and microalbuminuria groups. No statistically significant difference was observed in these parameters between the two groups (Table 2). The assessment of oxidative stress revealed a significantly lower levels of antioxidant enzymes in patients with microalbuminuria than the normoalbuminuria. Lipid peroxidation values were significantly higher in diabetic patients with microalbuminuria as compared to normoalbuminuria. Reduced GSH level in plasma did not show any statistically significant difference amongst the groups, however the values in microalbuminuria group were slightly lower as compared to normoalbuminuria group.

Further, to decipher the relation of different associated variables with microalbuminuria, a correlation study was carried out by using

Table 3: A correlation study in between albumin-creatinine ratio (ACR) and other associated factors

	Correlation coefficient (r)	p value
Age (years)	0.12	0.34
Sex	0.11	0.36
SOD(U/mL)	-0.10	0.43
GSH(µmol)	-0.11	0.36
Catalase(KU)	0.001	0.99

SBP-systolic blood pressure; DBP- Diastolic blood pressure;
ACR- Albumin-creatinine ratio. All values are expressed as mean±SD; $p<0.05$ statistical significant.

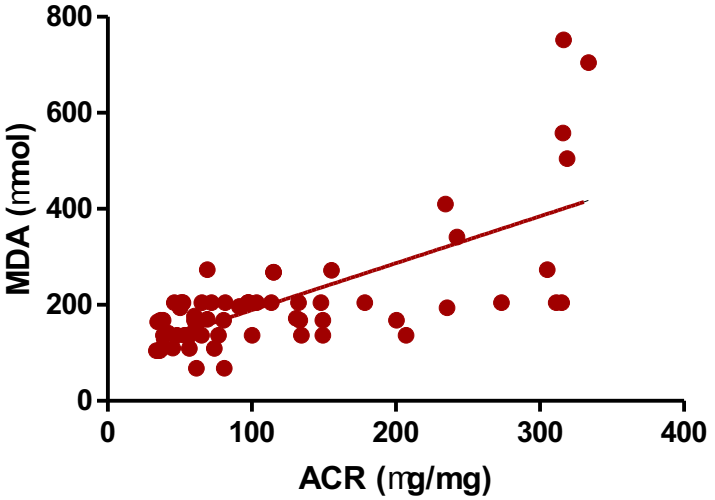

Fig. 1: A correlation study (Pearson) in between albumin-creatinine ratio and plasma MDA levels in T2DM patients. (r +0.69, p <0.001).
ACR- Albumin-creatinine ratio; MDA- Malondialdehyde.
+ value showed positive correlation, p value <0.05 is significant correlation.

GraphPad Prism, ver. 5.0 software. In this study, only malondialdehdyde levels showed a positive correlation with ACR (r +0.69, p <0.001) (Table 3 and Fig. 1).

Discussion

The outcome of this study suggests that oxidative stress is one of the mediators related to vascular complications like diabetic nephropathy in T2DM patients. We excluded the hypertensive patients in this study as it is a well-established confounding factor for ESRD in T2DM patient (6). However, 64% of T2DM patients were diagnosed as having microalbuminuria in this study. Microalbuminuria is a risk factor for development of ESRD in diabetic patients. ACR is an established marker for revealing microalbuminuria in patients and it was statistically increased in microalbuminuria group. The HbA1c level in microalbuminuria and normoalbuminuria group was not statistically different that indicates that glycoxidation itself does not lead to the change in the level of oxidative stress in diabetic patients. Further, total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C values also were not significantly different in microalbuminuria group of patients as compared to normoalbuminuria group (p>0.05). The evaluation of oxidative stress level in both the groups of T2DM patients revealed that malondialdehyde (MDA) levels were significantly higher in microalbuminuria group as compared to normoalbuminuria group (p <0.001). MDA is routinely measured as an indicator of oxidative stress. Hyperglycemia in T2DM patients resulted in the generation of ROS, increasing oxidative stress in affected tissues, which are damaged by the consequent activation of nuclear factor kappa B, along with advanced glycation end product (AGE) formation and activation of the protein kinase C and hexosamine pathways that lead to the increased level of MDA in patients (6-8). The reduced GSH levels were decreased in subjects with microalbuminuria as compared to normoalbuminuria patients. A significant decreased in levels of SOD was observed in subjects of microalbuminuria group as compared to normoalbuminuria (p <0.05). SOD is required for scavenging the superoxide radicals (9), therefore reduced activity of SOD leads to increased levels of superoxide radicals which will result in decreased levels of reduced GSH(10). Oxidative stress continued in diabetes by hyperglycemia and the glycoxidation products such as HbA1c and AGE and the absence of an appropriate compensatory response from the endogenous antioxidant network has been implicated in systemic endothelial dysfunction (6,11). Microalbuminuria is considered a marker of endothelial dysfunction (12). The correlation study between ACR and MDA levels showed a positive correlation which proves that oxidative stress is one of the risk factors for diabetic nephropathy. The oxidative stress in diabetic patients and altered antioxidant status, cellular milieu of renal system is disrupted causing renal lesions and damages that lead to the development of diabetic nephropathy due to disruption of endothelial glycocalyx through the reactive oxygen species (ROS), cytokines and vascular endothelial growth factors(13,14).

Conclusion

We conclude that diabetic patients have considerable higher risk of developing renal impairment and should be regularly monitored to expedite early detection of diabetes-induced nephropathy. Strategy for targeting the oxidative stress in T2DM patients may be an appropriate therapy for diabetic nephropathy. The outcome of this study would help in defining new targets for the prevention and treatment of diabetic nephropathy.

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Conflict of Interest

We confirmed that there are no known conflicts of interest associated with this publication.

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Carbamazepine, Sodium Valproate and Levetiracetam Modulate *Wnt* Inhibitors in Indian Women with Epilepsy

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ABSTRACT

Background: Antiepileptic drug (AED) therapy has been claimed to deteriorate bone health. Majority of the research was inclined towards vitamin-D deficiency as the patho-mechanism. However, after the role of *Wnt* in bone metabolism was discovered, it has paved way for investigating the role of *Wnt* inhibitors in mediating effects on bone accrual. Recently, we have reported the modulation of two *Wnt* inhibitors, sclerostin and dickkopf-1 (DKK-1), following AED therapy in Indian women with epilepsy, however, the subgroup analysis for individual drug is elucidated in this report.

Methods: Individual analysis for our earlier cross-sectional study on three AEDs, carbamazepine (CBZ), sodium valproate (SVP) and levetiracetam (LTM), on sclerostin and dickkopf-1, and their correlation with receptor activator of nuclear factor kappaB ligand (RANKL) and serum 25-hydroxy vitamin D (25OHD) was assessed in Indian women with epilepsy.

Results: We observed enhanced sclerostin and 25OHD levels with all three AEDs while serum RANKL was higher with SVP and LTM only. Further, serum DKK-1 levels were lowered with CBZ and LTM. Sclerostin showed a positive correlation with RANKL in CBZ group, while DKK-1 presented no such relationship.

Conclusion: As sclerostin is more specific than DKK-1, we may conclude that these drugs may compromise bone health through disturbance in *Wnt* signaling mechanisms.

*Keywords: s*clerostin, DKK-1, RANKL, 25OHD, sodium valproate, carbamazepine, levetiracetam.

Introduction

Wnt signaling plays an important role in development and maintenance of many organs and tissues, including bone. *Wnt*/β-catenin or canonical pathway appears to be particularly important for bone biology (1). *Wnt* signaling is tightly regulated by members of several families of secreted antagonists including sclerostin and dickkopf-1 (DKK-1) (2). Previous reports have identified vitamin-D inactivation as a major cause of antiepileptic drugs (AEDs) induced metabolic bone diseases (3). However, it is required to explore newer mechanisms that may aid in understanding the pathophysiology of AED induced bony consequences better. The

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objective of our study was primarily to perform sub-group analysis on our previously published report on the modulation of *Wnt* inhibitors and receptor activator of nuclear factor kappaB ligand (RANKL) following AED monotherapy. It is well-known that DKK-1 and sclerostin, secreted by osteocytes, block the effect of *Wnt* on osteoblasts thereby reducing bone formation. Sclerostin has been reported as a strong and independent risk factor for osteoporosis related fractures among postmenopausal women (4). On the other hand, RANK/RANKL pathway plays a critical role in bone remodeling and higher RANKL levels corresponds to increased bone resorption. Several studies provide evidence of association of these markers with bony consequences (5-7). This study aims to look for evidence of any association between *Wnt* inhibitors and three AEDs.

Method

The study includes sub-group analysis of data of a cross-sectional analytic study conducted at Neuroscience Centre, AIIMS, New Delhi and has been published recently (8). Parveen *et al* included 32 adult women with epilepsy treated with sodium valproate (SVP), carbamazepine (CBZ) or levetiracetam (LTM) as monotherapy for at least a year. All women gave their consent in accordance with declaration of Helsinki. Serum was employed for the analysis of *Wnt* inhibitors (Sclerostin and DKK-1) and RANKL using human specific ELISA kit from Sincere Biotech (Beijing, People's Republic of China) and 25OHD procured from Siemens (Mumbai, India).

Data contained both continuous and categorical variables. Therefore, mean±SD or median with interquartile range (IQR) were used for continuous data whereas frequency with proportions were used for categorical data for their presentation. Normality of the continuous variables was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Analysis of variance (ANOVA) test was used between treatment groups and post-hoc analysis was done by using Tukey's honestly significant difference test. ANOVA test and post-hoc Dunnett t-test was used for comparison between individual drug and control. For this comparison, RANKL was converted to log form as data was not normally distributed. The Spearman rank correlation was used to assess the correlation between the variables. The two-sided p-value less than 0.05 was considered as statistical significant. The statistical software IBM SPSS Version 22.0 (Chicago, USA) was used for entire analysis.

Results

Demographics and Clinical Characteristics

Thirty-two women with epilepsy were included in the study. Based on the drug treatment, patients were stratified into individual drug treatment groups comprising CBZ (n=08), SVP (n=10) and LTM (n=14). There was no statistical difference in age and duration of treatment among groups receiving various AEDs (Table 1). Patients receiving LTM achieved highest seizure control (78.6%) at mean total daily dose of 1250 ± 353.55 mg/kg as compared to CBZ (62.5%) and SVP (50%).

Biochemical and Correlation Analysis

Sclerostin levels were higher in patients undergoing treatment with all three AEDs while serum RANKL levels were significantly higher in SVP group and LTM but not with CBZ. 25OHD levels were lowered in patients undergoing treatment with all three AEDs studied in individual AED analysis. Serum DKK-1 levels were significantly lowered with CBZ and LTM but not with SVP (Table 2). The mean 25OHD level was significantly more among the serum levels of patients treated with CBZ (mean difference, 4.11) and LTM (mean difference, 4.11) in comparison to SVP. The mean sclerostin was significantly more in LTM in comparison to CBZ (mean difference, -3.60) while mean DKK-1 was significantly less in CBZ compared to SVP (mean difference,

Table 1: Demographical and clinical characteristics of women with epilepsy included in the clinical study

	Women with epilepsy on AEDs		
Characteristics	**CBZ n=08**	**SVP n=10**	**LTM n=14**
Age (mean ± SD, years) (range)	26 ± 6.39 (20-36)	27.7 ± 6.13 (20-39)	24.64 ± 5.03 (20-38)
Type of seizure *Generalized tonic clonic* *Partial* *Febrile* *Juvenile myoclonic* *Absence* *Partial seizure with secondary generalization*	 5 3 0 0 0 0	 5 3 0 0 1 1	 9 3 1 1 0 0
Family history	0	0	1
Seizure -free period (mean ± SD, months) (range)	29.12 ± 29.33 (3-72)	23.5 ± 39.41 (1-133)	23.5 ± 15.65 (1-41)
Total daily dose of AEDs (mg/day)	400	500	1250 ± 353.55
Duration of t reatment (mean ± SD, months) (range)	71.5 ± 51.07 (24-190)	48.6 ± 40.57 (13-132)	40.07 ± 22.24 (12-72)
Degree of control on AEDs n (%) *Controlled (seizure free for › 1 yr or more)* *Uncontrolled*	 5 (62.5%) 3 (37.5%)	 5 (50%) 5 (50%)	 11 (78.6%) 3 (21.4%)

Note: The results are expressed numbers, % or Mean ± SD.
AEDs- Anti-epileptic drugs; CBZ- Carbamazepine; SVP- Sodium valproate; LTM- Levetiracetam.

-147.2). Sclerostin had a significant positive correlation with RANKL in CBZ group (Fig. 1), while DKK-1 showed no such relationship with any of the AEDs studied.

Discussion

There is increasing evidence that AED therapy is associated with alteration in bone metabolism and mineralization suggesting bone loss. However, there is no generalized mechanism responsible for such consequences. In our previous study, alteration in *Wnt* inhibitors was reported following AED therapy in Wistar rats (9) and in Indian women with epilepsy (8). The present study analyzed the effect of individual drugs *viz* SVP, CBZ and LTM on modulation of *Wnt* inhibitors in persons with epilepsy. Similar to previous study by Gifre *et al* (10) evaluating the effect of glucocorticoids on modulation of *Wnt* inhibitors, the present study also reported differential effects with the type of

Table 2: Bone turnover markers (RANKL and 25(OHD) and *Wnt* antagonists (Sclerostin and DKK-1) in persons with epilepsy on AED monotherapy in comparison to age-matched healthy controls

Bone turnover markers	CBZ	SVP	LTM
RANKL ($pgmL^{-1}$)	371.37 (347.11)	688.81 (469.78) ###	482.94 (475.16) ###
25OHD ($ngmL^{-1}$)	26.40 (4.45)###	22.29 (2.89)### a	26.40 (4.00)###
Sclerostin ($ngmL^{-1}$)	15.11 (2.09)###	16.79 (2.62)###	18.71 (4.90)###b
DKK-1 ($pgmL^{-1}$)	749.88 (57.46)### c	897.14 (126.30)	800.65 (690.96)###

RANKL- receptor activator of nuclear factor kappaB ligand; DKK-1- Dickkopf-1; 25OHD- 25-hydroxy vitamin D; CBZ- Carbamazepine; SVP- Sodium valproate; LTM- Levetiracetam.

Note: Data are expressed as mean (SD); RANKL was converted to log RANKL for comparison between CBZ, SVP and LTM vs Control. Values of control group for RANKL, 25OHD, sclerostin and DKK-1 has been taken from Parveen *et al* 2018 for comparison.

###p ‹ 0.001; CBZ, SVP, LTM vs control (ANOVA followed by post- hoc Dunnett t-test)
[a]SVP vs LTM (p=0.037, ANOVA followed by post-hoc Tukey HSD Multiple Comparisons Tests)
[b]LTM vs CBZ (p=0.007, ANOVA followed by post-hoc Tukey HSD Multiple Comparisons Tests)
[c]CBZ vs SVP (p=0.019, ANOVA followed by post-hoc Tukey HSD Multiple Comparisons Tests).

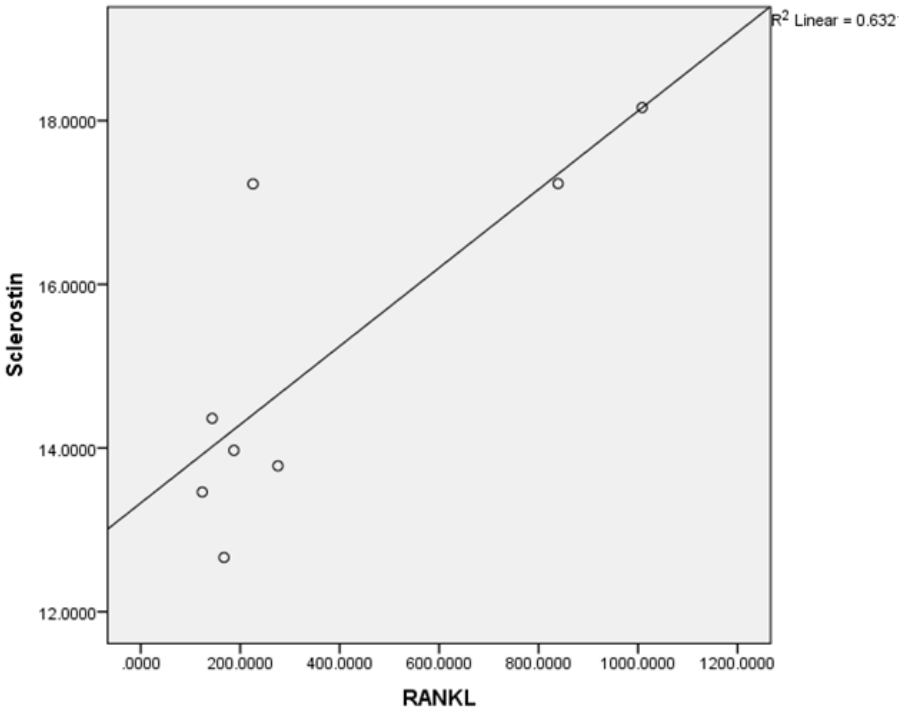

Fig. 1: Positive correlation between Sclerostin and RANKL in treatment with CBZ.
Correlation is significant at the 0.01 level (2-tailed).

Wnt inhibitor evaluated. While there were higher sclerostin levels with all three AEDs, DKK-1 levels were lowered with CBZ and LTM. Highest sclerostin levels were reported with LTM while lowest DKK-1 levels with CBZ. However, in our study, the observed lower DKK-1 levels following AED therapy in persons with epilepsy was not significant with SVP in individual AED analysis. DKK-1 plays a critical role during skeletal development but it is not highly expressed in adult bone unless activated by an insult (11). Sclerostin expression is limited primarily to the skeleton whereas expression of DKK-1 is more widespread (12).

The higher serum RANKL observed in our study following SVP is in agreement with pre-clinical study demonstrating increased RANKL levels in rats treated with valproate (13). RANKL activates and/or induces various transcription factors including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), c-Fos and nuclear factor of activated T-cells c1 (NFATc1), which act as positive modulators of osteoclast differentiation (14). RANKL increases NFATc1 protein levels by post-translational modification. RANKL stimulates NFATc1 acetylation via histone acetyltransferases (HATs). RANKL-mediated NFATc1 acetylation is increased by the histone deacetylase (HDAC) inhibitors (15). Various AEDs such as valproic acid, CBZ, oxcarbazepine, lamotrigine and levetiracetam are known to exert histone deacetylase inhibitory (HDACi) properties (16). Hence, increased RANKL levels by SVP and LTM could also be related to its HDAC inhibiting property (17). Though, CBZ didn't exert significant rise in serum RANKL levels but showed an increasing trend. The role of *Wnt* inhibitors and RANKL in bony consequences following AED therapy is proven in our previous report where sclerostin was positively correlated with RANKL, while no such effects were observed with DKK-1 (8). Now the individual analysis of drugs showed that the positive correlation observed between sclerostin and RANKL is due to CBZ. 25OHD was lowered with all three AEDs compared to control, however, the serum levels were not in the deficient range. Interestingly, our study showed that decrease in 25OHD was highest in SVP group as compared to CBZ and LTM treatment group. However, our study had limitation as the baseline data for 25OHD was not available and we compared the results with healthy controls.

To conclude, AEDs may compromise bone health through alterations in *Wnt* pathway *via* enhanced sclerostin levels which may or may not be related to RANKL depending upon the type of AED investigated.

List of Abbreviations

AEDs-	Antiepileptic drugs
CBZ-	Carbamazepine
SVP-	Sodium valproate
LTM-	Levetiracetam
RANKL-	Receptor activator of nuclear factor kappaB ligand
DKK-1-	Dickkopf 1
NF-κB-	Nuclear factor kappa-light-chain-enhancer of activated B cells
HDAC-	Histone deacetylase
NFATc1-	Nuclear factor of activated T-cells c1
CPCSEA-	Committee for the Purpose of Control and Supervision of Experiments on Animals
ANOVA-	Analysis of variance

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Author Contributions Statement

BP is responsible for enrolling patients, biochemical and statistical analysis and writing draft of the manuscript; MT is responsible for screening patients and editing the manuscript; DV is responsible for conception of study, scientific support and final editing of manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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Predictors of Poor Response to Salvage Chemotherapy in Relapsed/ Refractory Pediatric Hodgkin Lymphoma- A Retrospective Analysis from Tertiary Cancer Centre in India

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ABSTRACT

Background: Previous studies identified prognostic factors for survival in relapsed pediatric Hodgkin lymphoma (HL) who received salvage chemotherapy followed by autologous stem cell transplant (ASCT). However, data regarding predictors of poor response to salvage chemotherapy is limited.

Methods: We conducted retrospective study in all relapsed HL treated from January 2003 to December 2013. Logistic regression analysis was done to identify predictors of response to salvage chemotherapy. Cox regression analysis was done to identify prognostic factors for Freedom from treatment failure (FFTF) and overall survival (OS).

Results: Forty six patients had relapsed HL. Among 45 patients who received salvage chemotherapy only 34 (73.4%) underwent ASCT. Stage 4 disease (p=0.02) and bulky disease at relapse (p=0.03) were predictors of poor response to salvage chemotherapy. FFTF and OS at 5 yr for entire cohort were 50.1% and 63.3%, respectively, while the same for patients who underwent ASCT were 66.3% and 80.7%, respectively. Among ASCT patients, those who had primary refractory /early relapse [HR-4.7, (95% CI-1, 22); p=0.05] had significant impact on 5 yr FFTF whereas disease status at transplant (CR vs. No CR) had significant impact on 5 yr OS [HR-4.6, (95% CI-1.03, 20.5); p=0.04].

Conclusions: Identification of predictors of poor response to salvage chemotherapy is an unmet need in the management of pediatric HL since complete response (CR) before transplant is independent predictor of survival. Stage 4 and bulky disease at relapse are high risk factors to predict incomplete response. Future trials should explore newer agents for effective salvage for these patients to attain complete response before ASCT.

Keywords: relapsed hodgkin lymphoma, risk factor, poor response.

Introduction

Pediatric Hodgkin lymphoma (HL) is one of the childhood malignancies with excellent cure rates with multiagent chemotherapy and radiotherapy. However, small subset of 5-10% of patients relapse after initial treatment (1). Treatment of these patients includes salvage

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chemotherapy followed by high dose chemotherapy and autologous stem cell transplant (ASCT) based on stage at relapse, time of relapse (early vs. late relapse) after initial diagnosis and response to salvage chemotherapy (1). There is wide heterogeneity in the salvage chemotherapy regimen used in relapsed/ refractory pediatric HL by various pediatric oncology groups (2-6).

Most of the studies in relapse/refractory HL have reported outcomes of high dose chemotherapy and autologous stem cell transplant which are retrospective in nature (1, 3, 5-13). Response to salvage chemotherapy is considered an important prognostic factor for long term outcome (14). Other factors which were prognostic for survival were early relapse (< 12 months from completion of treatment), elevated lactate dehydrogenase, presence of B symptoms, extranodal involvement and stage at relapse (1, 5, 10, 15).

There is limited data regarding patients who received salvage chemotherapy but did not undergo ASCT due to progressive disease and hence overestimating the outcome of relapsed refractory HL. These are the patients who are likely to receive newer drugs like brentuximab, vedotin, nivolumab or participate in clinical drug trials. Identification of these patients prior to salvage chemotherapy may enable them to get initiated on newer regimens or drugs to elicit a response and further proceed to transplant, rather than initiating the same after failure of salvage regimens. Data regarding the predictors of poor response to salvage chemotherapy is limited and unmet need in relapsed HL.

We conducted a retrospective study to identify prognostic factors predicting response to salvage chemotherapy as well as survival in relapsed/refractory pediatric HL.

Methods

This retrospective study was performed in a tertiary cancer centre and was approved by Institutional Ethics Committee. We included patients with tissue diagnosis of HL, age ≤18 years at diagnosis who relapsed or had progressive disease after first line of chemotherapy between January, 2003 and December, 2013.

Data Collection

Medical records of all patients diagnosed with HL were retrieved. Data regarding the presenting symptoms, baseline laboratory parameters [complete blood count, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), and serum albumin], histological subtype, and date of start and completion of chemotherapy were recorded. Staging was done using modified Ann Arbor classification (16).

Definition of Primary Refractory and Relapsed Hodgkin Lymphoma

Patients who progressed during first line therapy or who progressed after initial response within 3 months of completion of therapy were defined as having primary refractory disease. Progressive disease was defined as appearance of any new lesion or increase in size of pre-existing disease site on CECT or PET-CT imaging. Patients who experienced relapse from 3 months to 12 months after completion of therapy were defined as early relapse and those who relapsed after 12 months were defined as late relapse.

Evaluation and Treatment of Relapse

All patients who relapsed had complete re-staging with contrast enhanced computed tomography of neck, chest, abdomen and pelvis

along with bone marrow aspirate and biopsy. Modified Ann Arbor classification was used for re-staging (16). Patients with primary refractory disease and advanced stage (IIB-IV) at relapse irrespective of timing of relapse (early vs. late) underwent salvage chemotherapy and based on response underwent ASCT. Patients with late relapse and early stage (stage IA, IB and IIA) at time of relapse received only salvage chemotherapy. These patients were excluded from analysis as they were not intended for consolidation with ASCT.

Response assessment with CECT or PET-CT was done after 2-3 cycles of salvage chemotherapy. Response was defined on CECT or PET-CT as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). CR was defined as resolution of previous involved disease site on CECT or absence of any metabolic activity or uptake less than uptake in mediastinal blood pool on PET-CT, partial response was defined as >50% decrease in size of measurable disease on CECT or decrease in size and uptake on PET-CT imaging, progressive disease was defined as appearance of any new lesion or increase in size of pre-existing disease site on CECT or PET-CT imaging (17).

Statistical Analysis

Freedom from treatment failure (FFTF) for the entire was defined as time from date of relapse to disease progression, relapse or death from any cause. For FFTF post transplant (patients who underwent transplant were included) was defined as time from date of transplant to disease progression, relapse or death from any cause. Overall survival (OS) was defined as time from date of relapse to date of last follow-up or death from any cause. Data was censored on 31st December, 2016. Logistic regression analysis was used to identify factors predicting response to salvage chemotherapy. Cox proportional hazards model was used for univariate analysis to identify predictive factors for FFTF and OS. Factors with significance with p value <0.05 were considered for multivariate Cox regression analysis to identify predictive factors for FFTF and OS. Stata (v 11.2, Statacorp, Chicago, IL, USA) was used for data analyses.

Results

A total of 50 patients had relapse of HL. Four patients had late relapse and stage 2A at the time of relapse; they received salvage chemotherapy, attained CR and they were not candidates for ASCT as they were at early stage at the time of late relapse. These patients were excluded from the analysis. Of the remaining 46 patients, 13 (28.3%) had primary refractory disease, 14 (30.4%) had early relapse and 19 (41.3%) had late relapse. Baseline characteristics of patients at diagnosis are given in Table 1. Disease and treatment characteristics at the time of relapse are given in Table 2.

A total of 45 patients received salvage chemotherapy. Twenty seven patients (58.6%) received platinum based salvage chemotherapy while 14 received ABVD (30.4%) as shown in Table 2. One patient did not receive any salvage chemotherapy and died of progressive disease. Median follow up of cohort was 41 months (range-4-141 months).

Response to Salvage Chemotherapy

Overall response rate to salvage chemotherapy was 59.9 % with CR in 20 (44.4%) patients and PR in 7 (15.5%) patients. Twenty-one patients received second salvage chemotherapy and of them 4 attained CR (19%) and 5 attained PR (23.8%). Thirty four (76.3%) patients received ASCT (CR-21, PR-11, and PD- 2).

Table 1 : Baseline characteristics of pediatric Hodgkin lymphoma at diagnosis

Baseline characteristic (N=46)	Median, N (%)
Median Age (yr) (range)	12 (4-18)
Sex · Male · Female	 36 (80.7) 10(19.3)
B symptoms	37 (71.1)
Stage · I · II · III · IV	 2 (4) 15 (30) 24 (48) 9 (18)
Bulky disease	15 (30)
Extranodal disease (excluding Liver, Bone marrow)	10(19.3)
Bone marrow involvement	2 (4)
Liver involvement	4 (3.85)
Spleen involvement	23 (44.2)
Subtype · Nodular sclerosis · Mixed cellularity · Lymphocyte rich · Lymphocyte depleted · NLPHL · Unspecified	 10(19.3) 28 (56.4) - 1(2) - 11(22.3)
Involved field radiotherapy	12 (23)
ABVD like regimen	40 (76.9%)

Table 2 : Characteristics at the time of relapse

Characteristic (N=46)	Median, N (%)
Timing of relapse · Primary refractory disease · Early relapse · Late relapse	 13 (28.3) 14 (30.4) 19 (41.6)
Stage · I · II · III · IV	 1 (4.4) 12 (26.7) 17 (37.8) 14 (31.1)
Extranodal disease	11(21.1)
Bone marrow involvement	4 (7.7)
Liver involvement	7 (13.4)
Spleen involvement	21 (46.7)
Salvage chemotherapy · Platinum based chemotherapy · ABVD · Other	 27(60) 14 (31.2) 4 (8.8)

Table 3: Logistic regression analysis for predictors of poor outcome (N=45)

Parameter	CR*, n (%)	No CR, n (%)	OR(95% CI)	P value
Gender			3.9	0.1
· Male(n=36)	19(52.8)	17(47.2)	(0.8-24)	
· Female(n=9)	2 (22.2)	7 (77.8)		
B symptoms			1.5	0.5
· Present(n=32)	14(43.7)	18(56.2)	(0.4-5.7)	
· Absent(n=13)	7(53.8)	6(46.2)		
Bulky disease at baseline			0.6	0.5
· Present(n=12)	7(58.3)	5 (41.7)	(0.2-2.2)	
· Absent(n=33)	14(42.4)	19(57.6)		
Extranodal disease at baseline			3.9	0.1
· Present(n=9)	2(22.2)	7 (77.8)	(0.7-20.1)	
· Absent(n=36)	19(52.8)	17(47.2)		
Spleen involvement at baseline			0.3	0.06
· Present(n=21)	13(61.9)	8 (38.1)	(0.1-1.04)	
· Absent(n=24)	8(33.3)	19(66.7)		
Stage 4 disease at baseline			2.6	0.2
· Present(n=7)	2(28.6)	5(71.4)	(0.5-17.7)	
· Absent(n=37)	19(51.3)	18(48.7)		
Timing of relapse			2.2	0.2
· Primary refractory/Early relapse(n=26)	10(38.4)	16(61.6)	(0.7-7.7)	
· Late relapse (n=19)	11(57.9)	8 (42.1)		
Extranodal disease at relapse			2.4	0.2
· Present(n=10)	3(30)	7 (70)	(0.5-10.4)	
· Absent(n=35)	18(51.4)	17(48.6)		
Spleen involvement at relapse			1.1	0.9
· Present(n=18)	8(44.4)	10 (55.6)	(0.3-3.5)	
· Absent(n=27)	13(48.2)	14(51.8)		
Stage 4 disease at relapse			5.1	0.02
· Present(n=15)	4(26.7)	11(73.3)	(1.3-23.9)	
· Absent(n=30)	17(56.7)	13(43.3)		
Bulky disease at relapse			7.1	0.03
· Present(n=10)	2(20)	8(80)	(1.2-42)	
· Absent(n=35)	19(54.3)	16(45.7)		

CR- complete response

Factors predicting complete response to chemotherapy

On logistic regression analysis, stage 4 disease at relapse (p=0.02) and bulky disease at relapse (p=0.03) were predictors for not attaining CR to salvage chemotherapy (Table 3).

Freedom from treatment failure and overall survival

FFTF at 5 yr for the entire cohort was 49.8% and OS at 5 yr was 66.3%. FFTF post transplant and OS at 5 yr for patients who underwent ASCT were 65.3% and 80.7%, respectively.

Table 4: Univariate analysis for FFTF and overall survival at 5 yr for relapsed/refractory Hodgkin lymphoma

	FFTF at 5 yr			OS at 5 yr		
	Estimate	**HR (95% CI)**	**P value**	**Estimate**	**HR (95% CI)**	**P value**
Gender		2.3	0.04		2.3	0.09
· Male (N=36)	54.9±0.08	(1.0-5.5)		65.6±0.08	(0.85-6.3)	
· Female (N=9)	30.1±0.14			66.7±0.19		
B symptoms		1.1	0.8		0.9	0.8
· Present(n=32)	61.5±0.13	(0.4-2.6)		69.2±0.12	(0.3-2.4)	
· Absent(n=13)	43.6±0.08			63.1±0.08		
Extranodal disease at baseline		2.5	0.04		0.6	0.4
· Present(n=9)	16.6±0.13	(1-5.6)		83.3±0.15	(0.1-2.4)	
· Absent(n=36)	57.3±0.08			61.1±0.08		
Spleen involvement at baseline		0.9	0.8		1.1	0.7
· Present(n=21)	48.4±0.13	(0.4-2.6)		60.7±0.15	(0.4-2.8)	
· Absent(n=24)	49.2±0.12			68.0±0.12		
Bulky disease at baseline		1.2	0.6		1.8	0.2
· Present(n=12)	53.8±0.13	(0.5-2.9)		58.7±0.14	(0.7-5.0)	
· Absent(n=33)	47.6±0.08			67.2±0.08		
Timing of relapse		2.1	0.07		1.2	0.6
· Primary refractory/Early relapse(n=26)	36.2±0.09	(0.9-5.6)		62.2±0.10	(0.5-3.3)	
· Late relapse (n=19)	68.4±0.10			68.4±0.10		
Bulky disease at relapse		0.9	0.8		1.2	0.8
· Present(n=10)	50.0±0.15	(0.3-2.4)		53.3±0.17	(0.4-3.6)	
· Absent(n=35)	48.8±0.08			68.2±0.08		
Spleen involvement at relapse		1.0	0.8		1.4	0.4
· Present(n=18)	43.7±0.09	(0.4-2.4)		53.8±0.17	(0.5-3.5)	
· Absent(n=27)	52.5±0.11			68.2±0.08		
Stage at relapse		3.3	0.004		2.8	0.04
· Stage 2 and 3 (n=30)	63.1±0.09	(1.5-7.6)		72.1±0.08	(1.04-7.6)	
· Stage 4 (n=15)	23.4±0.11			49.3±0.13		
Response to chemotherapy*						
· Complete response (n=21)	78.5±0.09	-	-	90.5±0.06	-	-
· Partial response (n=11)	36.3±0.14	4.9(1.4-17.1)	0.01	58.5±0.16	3.8(0.8-17.5)	0.08
· Progressive disease (n=13)	7.1±0.09	17.8(5.6-56.4)	<0.0001	8.7±0.08	11.8(4.9-65.5)	<0.0001

*One patient who did not receive salvage chemotherapy was excluded from analysis

Prognostic factors for survival for entire cohort

On univariate analysis, female sex, extranodal disease at baseline, stage 4 disease at relapse and chemorefractory disease had inferior FFTF (Table 4). On multivariate analysis, stage 4 disease at relapse (p=0.047) and response to salvage chemotherapy (p<0.0001) had significant impact on FFTF (Table 5, Fig. 1). For OS only response to salvage chemotherapy (p<0.0001) had significant impact on OS (Table 4).

Prognostic factors for survival for patients who underwent transplant

Among patients who underwent transplant, patients receiving more than 1

Table 5: Multivariate analysis for FFTF at 5 yr

	FFTF at 5 yr		
	HR	**95% CI**	**P value**
Sex			0.25
· Male (N=36)	1	-	
· Female (N=9)	1.74	0.6,4.6	
Stage at relapse			0.047
· Stage 2and 3 (n=30)	1	-	
· Stage 4 (n=15)	2.4	1.1,6.9	
Response to chemotherapy			
· Complete response (n=21)	1	-	-
· Partial response (n=11)	4.3	1.2,15	0.02
· Progressive disease (n=13)	16.6	5.1,53.8	<0.0001
Extranodal disease at baseline			0.9
· Absent(n=36)	1	-	
· Present(n=9)	2.4	0.9,6.8	

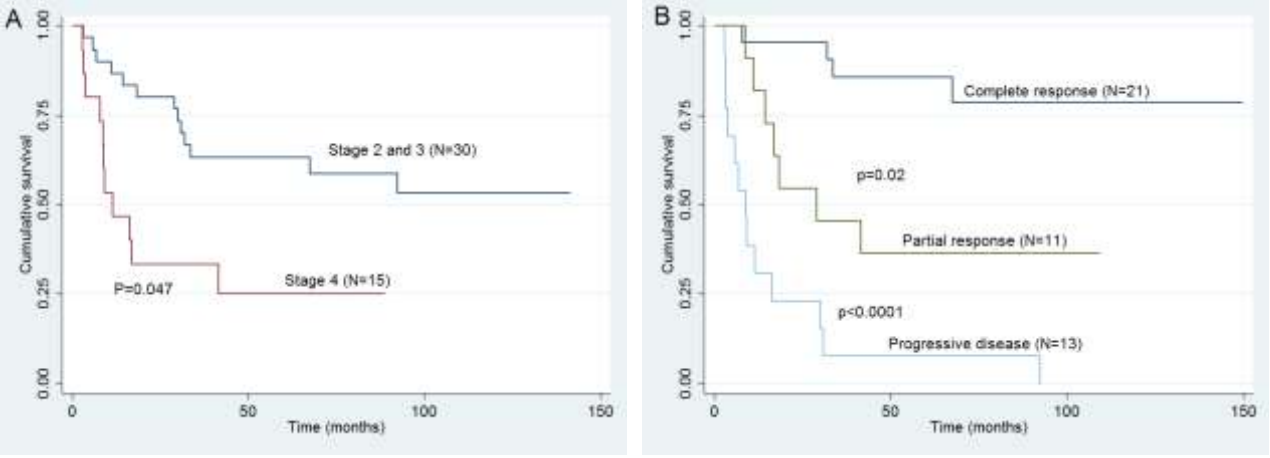

Fig. 1: Kaplan Meier survival graphs for 5 yr FFTF based on (A) Stage 2 and 3 vs. stage 4 at relapse (B) Response to salvage chemotherapy (complete response vs. partial response vs. progressive disease).

salvage chemotherapy to attain any response (CR and PR) [HR-3.8, (95% CI-1.2, 12.4); p=0.02], disease status at transplant (CR vs. No CR) [HR-3.4, (95% CI- 1.1, 11.1); p=0.04] and those who had early relapse/primary refractory disease [HR-5.4 (95% CI-1.2-24.8); p=0.03] had significant impact on 5 yr FFTF post transplant in univariate analysis, whereas only disease status at transplant (CR vs. No CR) alone had significant impact on 5 yr OS [HR-4.6, (95% CI-1.03, 20.5); p=0.04] (Supplementary Table 1). On multivariate analysis only patients with primary refractory disease/early relapse had inferior 5 yr FFTF post transplant as compared to late relapse [HR-4.7 (95% CI-1, 22); p=0.05] (Supplementary Table 2).

Discussion

With the availability of salvage chemotherapy followed by high dose chemotherapy with stem cell rescue, significant

Supplementary Table 1: Univariate analysis for FFTF and OS at 5 yr for transplant patients

	FFTF at 5 yr			OS at 5 yr		
	Estimate	**HR (95% CI)**	**P value**	**Estimate**	**HR (95% CI)**	**P value**
Gender		1.5	0.68		1.4	0.42
· Male (N=28)	66.8±0.09	(0.4-5.8)		75.9±0.08	(0.3-7.1)	
· Female (N=6)	62.5±0.21			66.6±0.27		
B symptoms		1.2	0.8		1.2	0.8
· Present(n=25)	60.6±0.10	(0.3-4.2)		77.5±0.08	(0.2-5.7)	
· Absent(n=9)	77.8±0.13			88.8±0.10		
Extranodal disease at baseline		2.5	0.2		0.6	0.6
· Present(n=6)	66.6±0.13	(1-5.6)		50±0.35	(0.1-2.4)	
· Absent(n=28)	70.9±0.08			76.9±0.08		
Spleen involvement at baseline		1.8	0.3		1.8	0.4
· Present(n=18)	58.3±0.12	(0.5-6.1)		74.5±0.13	(0.4-7.4)	
· Absent(n=16)	72.2±0.12			86.5±0.08		
Bulky disease at baseline		1	0.9		1.9	0.3
· Present(n=9)	61.7±0.10	(0.3-3.7)		77.8±0.13	(0.5-8.3)	
· Absent(n=25)	51.8±0.08			81.9±0.08		
Timing of relapse		5.5	0.03		1.9	0.2
· Primary refractory/Early relapse(n=20)	44.3±0.12	(1.2-24.8)		71.2±0.10	(0.5-8.3)	
· Late relapse (n=14)	79.6±0.13			92.4±0.10		
Bulky disease at relapse		0.7	0.6		1.2	0.8
· Present(n=7)	71.4±0.17	(0.2-3.3)		68.5±0.18	(0.2-6.1)	
· Absent(n=27)	63.3±0.09			84.4±0.07		
Spleen involvement at relapse		2.3	0.2		2.0	0.3
· Present(n=14)	41.6±0.2	(0.7-8.1)		69.2±0.12	(0.5-7.6)	
· Absent(n=20)	65.3±0.1			88.6±0.07		
Stage at relapse		2.3	0.17		1.6	0.5
· Stage 2 and 3 (n=8)	41.6±0.20	(0.7-8.1)		87.5±0.11	(0.3-8.4)	
· Stage 4 (n=26)	65.8±0.10			79.1±0.08		
Disease status before transplant		3.5	0.03		4.6	0.04
· Complete response (n=22)	80.8±0.08	(1.1-11.4)		90.1±0.06	(1.03-20.5)	
· No complete response (n=12)	38.1±0.09			63.5±0.14		
No of lines of salvage therapy		4.0	0.02		1.9	0.3
· 1 (n=24)	71.6±0.10	(1.2-12.9)		82.4±0.08	(0.5-7.9)	
· >1 (n=10)	22.5±0.18			77.1±0.14		

proportion of relapsed HL patients can be cured. However, not all the patients reach up to the phase of consolidation with ASCT. Previous studies reported the outcomes of only patients who underwent ASCT (1, 3, 7-11). Our study included all the patients with relapsed/refractory HL for outcome analysis.

OS and FFTF of the entire cohort is less than that reported in previous studies (1,5-10,13,14). However, in our study, patients who underwent transplant had similar survival as compared to previously reported studies (1, 3, 5, 10, 18). Inclusion of all patients with relapsed/refractory HL gives more realistic outcome.

Supplementary Table 2: Multivariate analysis for FFTF at 5 yr for transplant patients

	FFTF at 5 yr		
	HR	**95% CI**	**P value**
Timing of relapse			0.06
· Late relapse (n=14)	1	-	
· Primary refractory/Early relapse(n=20)	4.4	0.9,22.0	
Disease status before transplant			0.07
· Complete response (n=22)	1	-	
· No complete response (n=12)	3.0	1.3,9.7	
No. of lines of salvage therapy			0.4
· 1 (n=24)	1	-	
· >1 (n=10)	1.8	0.5,6.9	

Complete response prior to ASCT is an important prognostic factor for survival as demonstrated in previous studies (1, 10, 14). Identification of predictors of poor response to salvage chemotherapy is an unmet need in the management of relapsed pediatric HL. We identified stage 4 and bulky disease at relapse as independent predictors of achieving complete response to salvage chemotherapy. Our results show that a significant proportion of patients (26.1%) do not reach upto the stage of consolidation with ASCT. These are the patients who did not respond despite receiving multiple lines of salvage chemotherapy. Notably, the subsequent complete response rate in those who fail the first salvage regimen was only 20% in our cohort. Thus, relapse patients with these high risk features are the ones who are candidates for exploring newer agents such as Brentuximab vedotin and Bendamustine so as to attain complete response before consolidation with ASCT.

Stage 4 disease at relapse and chemorefractory disease were independent predictors of inferior FFTF and OS in our cohort. Timing of relapse (early vs. late) was not a significant prognostic factor in our study as seen in previous studies. Notably in our study 50% patients of stage 4 disease at relapse did not reach up to consolidation with ASCT. Since stage 4 disease also has poor response to salvage chemotherapy, this subgroup of patients requires newer agents as salvage to attain complete response.

Disease status at time of transplant was independent predictor of OS. This further suggests that to attain complete response with first salvage chemotherapy is an important milestone to have better long term survival.

Ours is the first study to evaluate risk factors to predict complete response to salvage chemotherapy in pediatric HL, and has analyzed outcome of relapsed HL in a real life situation by including all patients with relapse/refractory disease, and not just those who reach upto the stage of transplant. To conclude, complete response to salvage chemotherapy is an important predictor of long term survival in relapsed pediatric HL, and with salvage chemotherapy majority of patients reach upto the stage of transplant. Stage 4 and bulky disease at relapse are high risk factors to predict incomplete response. Effective salvage chemotherapy regimen to attain complete response before ASCT is an unmet need in these patients. Future trials exploring newer agents such as Brentuximab and Bendamustine should be explored specifically as salvage options in these high risk patients prior to subjecting them to other conventional chemotherapeutic options.

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Case Report

Reverse 'Hot Cross Bun' Sign in Primary Progressive Aphasia - An Atypical MRI Feature

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ABSTRACT

Primary progressive aphasia (PPA) is thought to be a disorder of focal cortical degeneration which occurs as a result of lobar atrophy of dominant frontal and temporal lobe. We report a case of a 78 year old male patient presenting with progressive language affection predominantly and clinically diagnosed as PPA but magnetic resonance imaging (MRI) brain showed an unusual finding of reverse 'hot cross bun' sign in pons in T2 weighted (T2W) / diffusion weighted image (DWI)/ T2 fluid attenuated inversion recovery (FLAIR) axial views. This is the first case report of reverse 'hot cross bun' sign in a case of PPA to the best of our knowledge.

Keywords: Primary progressive aphasia, reverse 'hot cross bun' sign, 'hot cross bun' sign.

Introduction

Primary progressive aphasia (PPA) is thought to be a disorder of focal cortical degeneration which occurs as a result of lobar atrophy of dominant frontal and temporal lobe. PPA is divided into three variants on the basis of clinical, brain imaging and histopathological evidence. Left perisylvian atrophy is the hallmark radiologic feature. Our patient presented with progressive language affection predominantly and clinically diagnosed as PPA but MRI brain showed an unusual finding of reverse 'hot cross bun' sign. To the best of our knowledge this sign has never been reported earlier in PPA.

Case Presentation

A 78 year old right handed, retired school teacher, hypertensive male presented with one and half year history of insidious onset, progressive speech difficulty in form of effortful, non-fluent speech with intermittent pauses. He had grammatical errors in spontaneous speech as well as in writing. Comprehension for complex sentences was impaired but preserved for a single word. Naming and repetition were mildly affected. Fund of information and word knowledge were normal. Conversation discourse was moderately impaired. However, grammatical and phonological errors were more common in writing than in spontaneous speech. He had problems with syntax comprehension. Naming and repetition were mildly disturbed with preserved word comprehension and object knowledge. His past history was unremarkable except for hypertension which was well controlled with medications. He was independent for his daily routine activities.

The general and systemic examination

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was unremarkable. On higher mental function testing, his Mini–Mental State Examination (MMSE) was 24/30 and Frontal Assessment Battery (FAB) was 8/18 suggestive of frontal lobe dysfunction. Language function showed intact comprehension along with preserved reading ability with decreased speech output, grammatical errors in writing and perseveration. There was severe word finding difficulty, impaired fluency with wrong usage of grammar intermittently. A diagnosis of primary progressive aphasia was made based on clinical history, neuropsychological and detailed language function testing. His routine investigations including hematological and metabolic profile were normal. Serum TSH and vitamin-B12 were normal.

CT Head and MRI brain with diffusion weighted image (DWI) with contrast was done. There was no evidence of acute/sub-acute or chronic infarct.

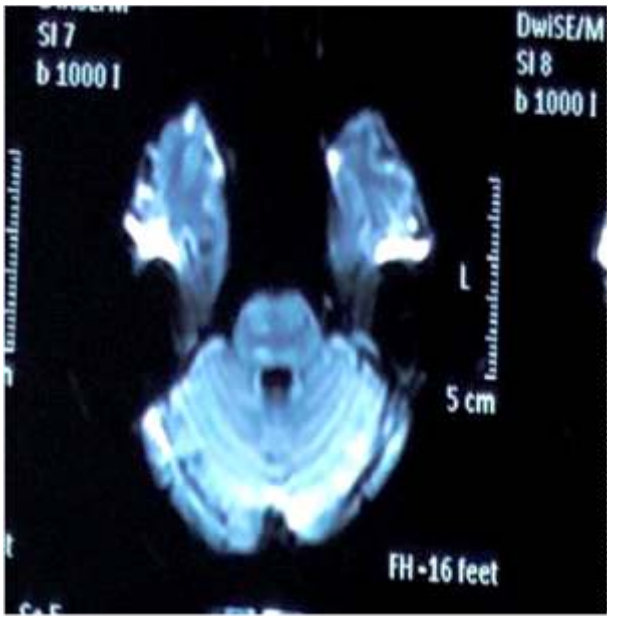

Fig. 2: MRI of the brain, DWI axial section, showing presence of reverse 'hot cross bun' sign at mid pons.

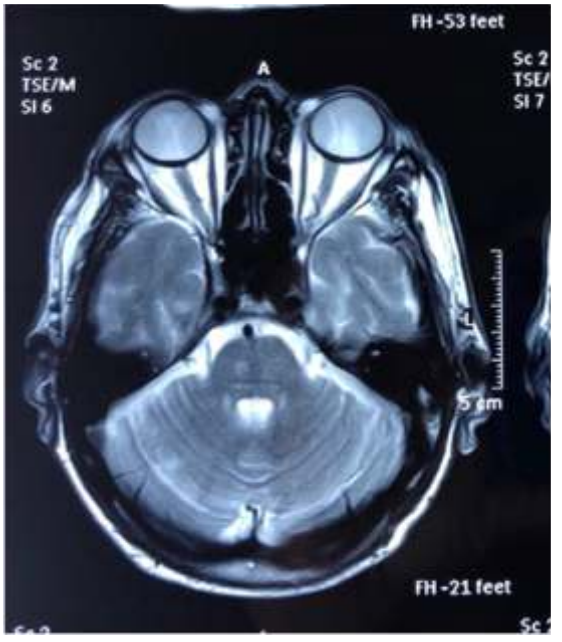

Fig. 1: MRI of the brain, T2-weighted axial section, showing presence of reverse 'hot cross bun' sign at mid pons.

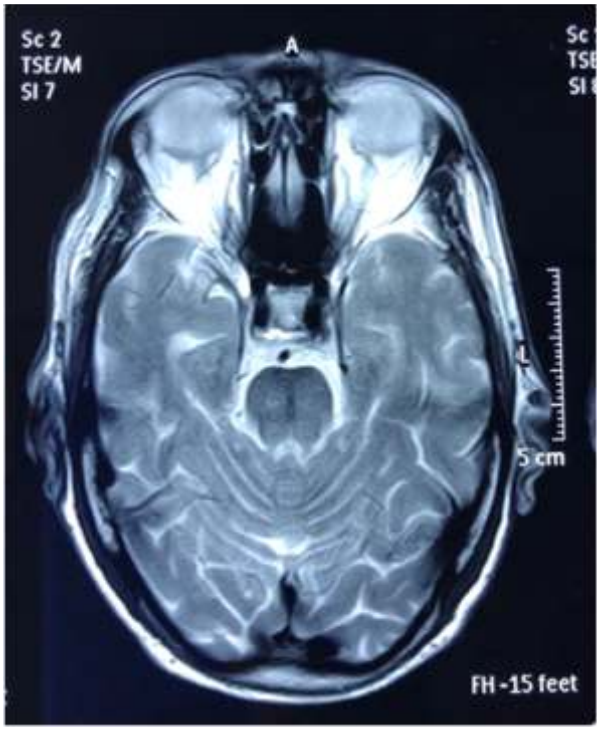

Fig. 3: MRI of the brain, FLAIR axial section, showing presence of reverse 'hot cross bun' sign at mid pons.

MRI brain showed diffuse cerebral atrophy. There was an interesting finding of reverse 'hot cross bun' sign in pons in T2 weighted (T2W) (Fig. 1) / DWI (Fig. 2) / T2 fluid attenuated inversion recovery (FLAIR) (Fig. 3) axial views. Speech therapy was instituted and he was managed with antihypertensive drugs.

Discussion

Primary progressive non-fluent aphasia is a progressive language disorder associated with atrophy of the frontal and temporal regions of dominant hemisphere (1). Primary affliction is in the language domain which differentiates it from the aphasic form of Alzheimer disease. It is important to differentiate these two entities as the former carries a better prognosis. PPA has been diagnosed and divided into different subtype according to Mesulam PPA diagnostic criteria (2). The main components of language involved are spontaneous speech output, anomia, fluency, and grammar. Other domains are relatively unaffected and the patient remains independent for his activities of daily living. There can be a subsequent progression into dementia over a decade. Early stages of the disease may not reveal any abnormal neuroimaging features but left perisylvian atrophy has been described as a hallmark feature.

Our case presented with progressive predominant language affection and we found a unique reverse 'hot cross bun' sign in MRI brain. 'Hot cross bun' sign is a cruciform-shaped T2W hyperintensity through the middle of pons due to degeneration of the cerebellopontine and tranverse pontine fibers. Reverse 'hot cross bun' sign is hypointensity in the intersecting lines and hyperintensity in the quadrangles forming a shape of a hot cross bun, which can be best appreciated on T2 and FLAIR image. 'Hot cross bun' sign is seen in multiple system atrophy–cerebellar type, spino-cerebellar ataxia (SCA 1/ 2), secondary Parkinson disease, cerebrotendinous xanthoma (CTX), progressive multifocal leucoencephalopathy (PML) in HIV seropositive patient and also in neurosarcoidosis (3).

Reverse 'hot cross bun' sign has been described earlier in a patient with pontine infarct (4) and Wilson disease (5) but not in PPA so far.

Conclusion

Our case presented with progressive predominant language affection and we found a unique reverse 'hot cross bun' sign in pons in T2W/DWI/ T2 FLAIR axial views of MRI brain. This is the first case report of reverse 'hot cross bun' sign in a case of PPA to the best of our knowledge. The treatment of PPA remains conservative with speech therapy as the mainstay. The prognosis is said to be better in PPA when compared to other neurodegenerative disorders like Alzheimer disease.

Acknowledgment

We have not received substantial contributions from non-contributors and no contributor has been omitted.

Conflict of Interest

We have no conflict of interest to declare.

Source of Funding

None.

Ethical Clearance

Written informed consent was obtained from the patient and relatives of patient for publication of this case, reports and any accompanying images. We are ensuring that, this study manuscript has not been submitted and published elsewhere.

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Duplication of the Gallbladder: A Rare Congenital Anomaly

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ABSTRACT

Biliary tract shows a large number of anatomic variations and duplication of the gallbladder (GB) is a rare congenital anomaly. Although it is very often detected incidentally, it may present with complications due to gall stone disease. Careful identification of this anomaly on imaging is important to prevent any serious surgical complications. We here report a case of duplicated GB in a 26-year-old male, who presented with right hypochondriac pain and detected to have two GB on magnetic resonance imaging (MRI).

Keywords: Gallbladder duplication, cholelithiasis, biliary anomalies.

Introduction

Duplication of the gallbladder (GB) is a rare congenital anomaly of the biliary tract with an incidence of approximately one in 4000 births (1). It may be detected incidentally on imaging done for other reasons or patients may present with symptoms due to any pathology affecting the GB (2). As many surgeries of GB are done laparoscopically, pre-operative identification of biliary anomalies, including duplicated GB is important to prevent inadvertent biliary and arterial injuries and associated morbidity and mortality (3). We report a case of duplicated GB with gall stones in both of them, diagnosed on magnetic resonance imaging (MRI).

Case Report

A 26-year-old male presented with history of pain in right hypochondrium associated with fever and nausea for four months. There was no history of jaundice, loss of weight or loss of appetite. Clinical examination was unremarkable. Liver function tests were unremarkable, except for mild elevation of serum alkaline phosphatase (253 IU/L; reference: 35 – 114 IU/L) with normal white blood cell counts and serological studies for viral hepatitis and dengue were negative. Ultrasonography (USG) showed GB full of calculi with posterior shadowing. There was no dilatation of intrahepatic biliary ducts and bile duct was normal. Since the serum alkaline phosphatase was elevated, MRI with magnetic resonance cholangiopancreatography (MRCP) was done to look for bile duct calculi.

MRI showed double gallbladders, which were united at the neck region (Fig. 1). One showed multiple calculi (signal voids on T1 and T2 weighted images) and the other showed a single calculus with sludge (seen as hyperintense bile on T1-weighted images). The wall was normal. There was no evidence of cholecystitis. Cystic duct could not be visualized. The biliary ducts were normal. A final diagnosis of duplicated GB with cholelithiasis in both sacs

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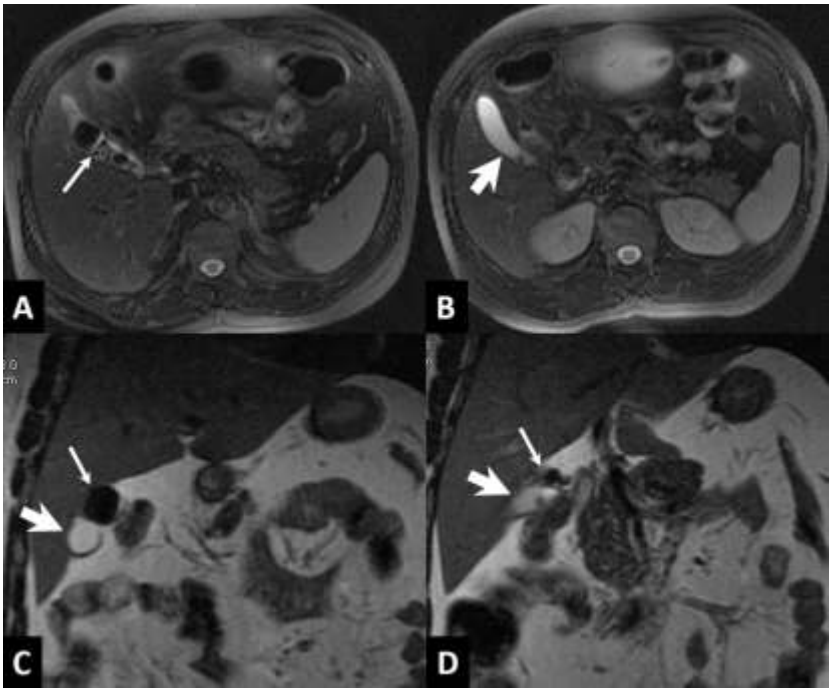

Fig. 1: A & B: Axial T2-weighted MR images showing two gallbladders, one with multiple calculi (thin arrow in A) and the other with solitary calculus (thick arrow in B). C & D: Coronal T2-weighted MR images showing both gallbladders (thick and thin arrows) with two separate necks and cystic ducts (arrows in D).

was made and the patient was referred for surgery.

Discussion

Duplication of the GB is an unusual congenital anomaly which occurs due to splitting of cystic primordium of the GB or due to the growth of an accessory bud from the biliary primordium during embryogenesis (1, 4). This results in the formation of two epithelium lined sacs which are either partially joined or completely separate from each other.

There are many classifications of duplication of GB. Boyden (1) classified them into two groups based on the presence of two separate cystic ducts. In the first – *vesical fellea divisa* – the GB is bilobed with common neck and no separate cystic duct. A longitudinal septum or invaginating cleft separates the lumen into two chambers (Fig. 2). In these cases both gall bladders share a common embryological origin (primordium). In the second type – *vesical fellea duplex* – there are two separate GB and cystic ducts. It has two subtypes – one in which two cystic ducts join together before joining the common bile duct (Y-type) and the other in which two cystic ducts separately join the common bile duct (H-type) (Fig. 3). In the second type a double embryological origin (dual primordium) is considered.

Clinical presentation is non-specific and the incidence of cholelithiasis appears similar to that of general population (5). They may be incidentally detected on imaging. USG shows two cystic structures in the GB fossa (6). However, classification is difficult as cystic duct is usually not identified. Further, the second sac may be missed if there is extensive shadowing due the calculi in one sac. MRI with MRCP will better demonstrate the duplicated GB and its type (7).

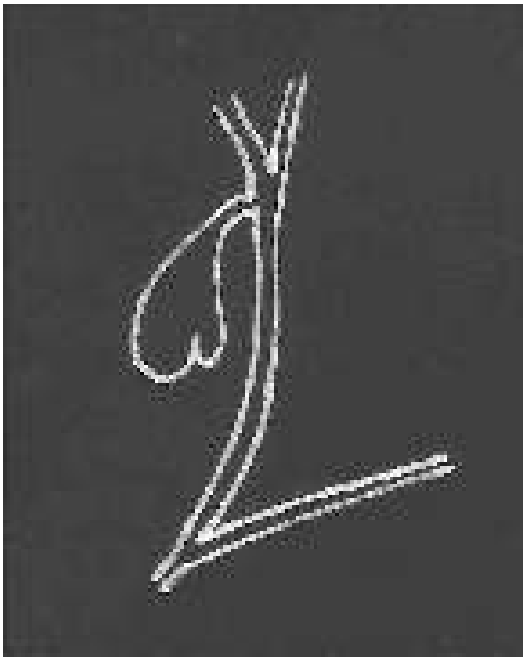

Fig. 2: Diagramatic representation of vesical fellea divisa. The gall bladder (GB) is bilobed with common neck and single cystic duct.

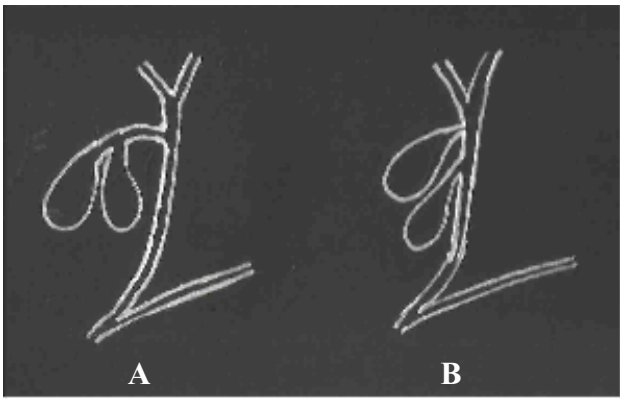

Fig. 3: Diagramatic representation of vesical fellea duplex. There are two separate GB and cystic ducts. In Y type cystic ducts join together before joining common bile duct (CBD) (A) and in H-type the two cystic ducts join CBD separately (B).

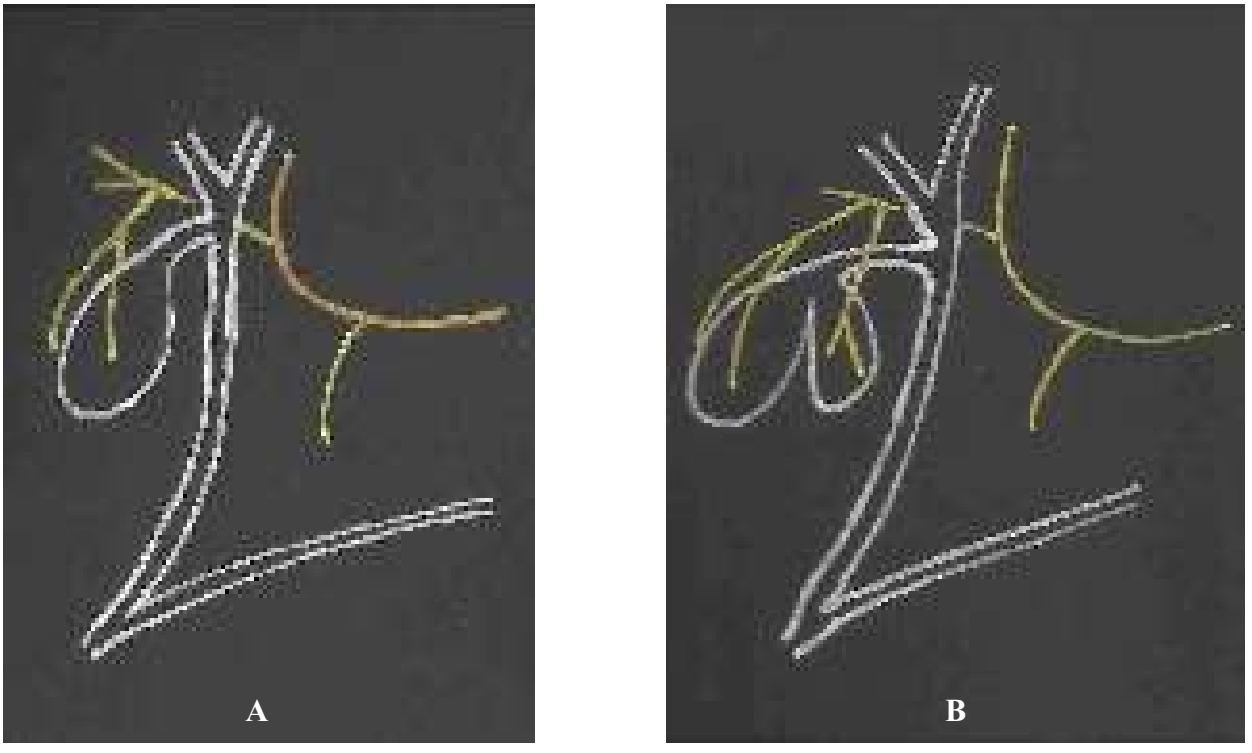

Fig. 4: Diagramatic representation of normal GB with single cystic artery (A). In duplication of GB there may be anatomic variations of hepatic arteries like multiple cystic arteries (B).

Duplicated GB does not need any treatment unless complicated by calculi or mass. In symptomatic cases, surgery is the treatment of choice. It may be done by open or laparoscopic approaches (8). Pre-operative diagnosis is important to prevent any inadvertent complications because of associated anatomical variations of cystic duct and hepatic arteries (9, 10, 11) (Fig. 4).

In conclusion, duplicated GB is a rare congenital anomaly and mostly asymptomatic. Imaging with USG or MRI is very useful in its detection and classification. Pre-operative diagnosis is helpful in avoiding complications during surgery.

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