Intimal Changes in the Cephalic Vein of Renal Failure Patients before Arterio-Venous Fistula (AVF) Construction

Mahmoud A. Wali, Refaat A. Eid, Madhu Dewan and Mohammad A. Al-Homrany

Departments of Surgery, Pathology and Medicine, Asir Central Hospital and College of Medicine, King Khalid University, Abha, Saudi Arabia

Abstract

Native cephalic vein remains the superior dialysis conduit, even thirty years after it was first described. However, up to 37% of hemodialysis patients develop progressive stenosis in the venous circuit of arterio-venous fistula (AVF), which may later cause thrombosis and occlusion. To study the pre-existing morphological changes in the wall of the cephalic vein before AVF construction, we collected 23 cephalic vein specimens from 3 normal, young trauma and twenty renal failure patients. The samples were collected at the time of vascular repair in the first group and AVF construction in the second group. Sections were prepared and stained for both light and transmission electron microscopy (TEM) examination. Compared with normal cephalic vein, all pre-access cephalic veins showed thickening of the wall and intimal hyperplasia. Other changes were loss of internal elastic lamina in 9 (45%) patients, loss of endothelial cell layer in 6 (30%), inflammatory cell infiltration of the wall in 5 (25%), mural calcification in 3 (15%) and telangiectasia in 2 (10%). Other ultrastructural changes observed were intimal hypertrophy, degeneration and loss of the endothelial cells, degeneration and fraying of smooth muscle cells (SMCs) and loss of wall components into the lumen. In conclusion, most of the apparently normal cephalic veins of renal failure patients showed morphological abnormalities at the time of AVF construction, which may well influence the outcome of shunts in terms of future stenosis and failure. It seems likely that the later development of AVF stenosis may be the result of pre-existing disease rather than the direct insult of arterialization.

Key words: cephalic vein, arterio-venous fistula (AVF), renal failure, intimal hyperplasia, stenosis, thrombosis, failure

Introduction

A well-functioning vascular access is the pre-requisite for chronic hemodialysis treatment (Janicki et al., 2001). A native arteriovenous fistula (AVF) is the first choice for hemodialysis access (Kingdon et al., 2001), and the cephalic vein remains the superior dialysis conduit, even 30
years after it was first described (Burdick and Maley, 1996). However, complications associated with the vascular access represent one of the most important causes of morbidity among hemodialysis patients (Neumann et al., 2001), and a major contribution to hemodialysis cost (De Marchi et al., 1996). While these complications are less frequent with AVFs than with synthetic grafts (Hirth et al., 1996), they account for 15% of hospital admissions among US hemodialysis patients at a cost of about $1 billion per year in 1996 (Feldman et al., 1996). In their review of dialysis practice in Hong Kong, Chin et al. found that 24.5% of the AVFs were never used due to primary failure and that 30.1% of the fistulas used had to be abandoned for various reasons (Chui et al., 2000). In addition, 22.8% of the patients experienced complications, the most common of which was thrombosis and stenosis (Chui et al., 2000). According to Bagolan et al. (1998), 35% of AVFs have either immediate or late complications, with thrombosis being the most frequent complication. Thrombosis usually results from stenotic lesion in the venous outflow system. In the report by De Marchi et al. (1996), 37% of their patients developed progressive stenosis in the venous circuit, which was complicated by thrombosis in some of them.

Recent studies on the pre-bypass long saphenous vein have suggested that the extent of pre-existing disease may contribute to the development of later stenoses and therefore affect the outcome of coronary and femoro-distal bypasses (Martin et al., 1991; Panetta et al., 1992; Davis et al., 1992; Davies et al., 1993). However, very little attention has been paid to the condition of the cephalic vein before the operation of AVF. In order to verify the condition of the vein wall before its use as an AVF, and to evaluate possible pre-existing morphological changes, mainly in the intima, we conducted this histological survey on samples of the cephalic vein used for AVF.

**Material and Methods**

During the period from June 2000 to October 2001, a total of 23 cephalic vein specimens were collected from 3 young trauma and 20 renal failure patients at Asir Central Hospital (ACH), in Abha, Kingdom of Saudi Arabia. The trauma patients who underwent repair of their upper limb arterial injuries and acted as normal controls were three males with the mean age of 24 ± 3.6 years (20–27 years). The renal failure patients were 8 males and 12 females with a mean age of 44.3 ± 16.2 years (16–70 years). These patients underwent construction of a primary direct arterio-venous fistula (AVF) for hemodialysis on the chosen limb. The fistulas were equally distributed between the right and left upper limbs, with 5 done at wrist and 15 at elbow. None of the patients had an existing or a previously documented history of deep vein thrombosis or superficial thrombophlebitis on the operated limb. Renal failure patients underwent pre-operative physical assessment and, if required, upper limb venography to assess the presence of a suitable cephalic vein at either the wrist or elbow. All the patients gave their informed consent for cephalic vein biopsy prior to surgery.

At the time of operation, two 2–3 mm circumferential segments were excised from a non-traumatized part of the cephalic vein in every patient. One specimen was collected in a small, labeled test tube containing 10% formalin for light microscopy examination and the other one was put in another vial containing 2.5% glutaraldehyde solution for electron microscopy (EM) examination. In the pathology laboratory, Paraffin sections were prepared and semithin sections
were stained with Hematoxylin & Eosin (H&E) and Masson’s trichrome stains. Slides were examined under the light microscope and representative sections were photographed using Olympus® PM 10 SP automatic micrographic system (Japan).

In the EM laboratory, specimens were cut into 2–3 mm cubes, immediately fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer (pH 7.2) and then kept in the refrigerator at 4°C for two hours. Samples were later post-fixed in 1% osmium tetroxide in sodium cacodylate buffer, dehydrated in an ascending series of ethyl alcohol and embedded in Spurr’s resin. Semithin sections, prepared using diamond knives and stained with toluidine blue, were examined under the light microscope. Ultrathin sections, prepared with diamond knives and stained with uranyl acetate and lead citrate, were examined at 80 kv under the transmission electron microscope “TEM” (Jeol® 1200 EX, Japan).

**Results**

Hematoxylin & Eosin (H&E) sections of the normal cephalic vein showed regular wall with regular intimal surface. The normal ratio of intima to media was in the range of 1 : 5, with an intact endothelial cell layer. The normal intimal and medial smooth muscle cells (SMCs) were well developed and regularly disposed (Fig. 1A). In renal failure patients, sections of the cephalic vein showed irregularity of the wall and the lumen with marked focal or diffuse hypertrophy. This was mainly due to intimal thickening and atrophy of both the intimal and medial SMCs with reversal of the normal intima: media ratio to be in the range of 5 : 1 (Fig. 1B). All sections showed marked intimal thickening or hyperplasia. The intimal thickening appeared to be due to marked infiltration by collagen fibers and hyaline material with disruption of the normal palisade arrangement of the intimal SMC layer. This was apparent in sections stained with Masson’s trichrome which showed marked collagen infiltration of the hypertrophied intima with accumulation of layers of intimal SMCs close to, or along the de-epithelialized luminal surface (Fig. 1C). Sections stained with Verhoff von Gieson’s stain showed loss of the internal elastic lamina with the presence of fragmented and disrupted elastic fibers scattered across the wall (Fig. 1D). Loss of the internal elastic lamina was evident in 9 (45%) patients. Other changes were partial or complete loss of the endothelial cell layer in 6 (30%), mucoid or myxoid degeneration in 6 (30%), inflammatory reaction with the presence of erythrocytes and histiocytes in the wall in 5 (25%), intimal calcification in 3 (15%) and telangiectasia in 2 (10%).

Under the EM, sections of the normal cephalic vein showed regular luminal and endothelial surface with normal ratio of intima/media and intact internal elastic lamina (Fig. 2A). Veins of renal failure patients showed marked irregularity with elongation and invagination of the intimal surface. The endothelial cells looked atrophied and irregular together with loss of the underlying internal elastic lamina and disruption of the intimal SMCs layer. Areas of intimal thickening showed abnormal accumulation of fragmented SMCs among a background of increased amount of fragmented collagen and dispersed elastic fibers (Fig. 2B). Affected areas also showed elongated, degenerated, partially attached, or separated, endothelial cells (Fig. 2C). With further changes, endothelial cells appeared vacuolated with thin, finger-like cytoplasmic processes (Fig. 2D).
Fig. 1. Cross sections of normal and renal failure cephalic veins (Light Microscopy). A. Section of a normal vein showing the normal proportion of intima (I) and media (M) and the intact endothelial cell layer (A: Adventitia, L: Lumen) (H&E stain: × 515). B. Cephalic vein of renal failure patient showing thickening of the wall due to marked intimal hyperplasia (I). Note the atrophied media (M) and the reversed intima/media ratio (L: Lumen) (H&E: × 515). C. Cephalic vein of renal failure patient showing the collagenous (C) fibrous tissue infiltration of the intimal hyperplasia. Note the accumulation of layers of intimal smooth muscle cells (SMCs) (arrows) towards the luminal surface (L: Lumen) (Masson’s trichrome: × 1030). D. Section of a pre-access cephalic vein showing irregularity of the wall, atrophy of the media (M), intimal hyperplasia (I) and loss of the internal elastic lamina (A: Adventitia, L: Lumen) (Verhoff von Gieson’s stain: × 515).
Fig. 2. Intimal layer of normal and pre-access cephalic veins (Electron Microscopy). A. A normal endothelial cell (E) with elongated nucleus (N) rests on the basal lamina (arrows) of the endothelial layer. Note the regular internal elastic lamina (star) between the tunica intima (I) and the tunica media (M) (L: Lumen) [× 7333]. B. Pre-access cephalic vein showing increased endothelial surface area due to elongation and invagination of the wall. Endothelial cells (E) are pyknotic and irregular. The sub endothelial layer lost the internal elastic lamina with increased amount of amorphous collagen fibers (C) and irregular smooth muscle cells (S) (L: Lumen) [× 3429]. C. Elongated, irregular endothelial cells (E) with spiky projections (arrows). Note the thickened, irregular intima with abnormal smooth muscle vacuoles (V) (L: Lumen) [× 3600]. D. Spiky, irregular endothelial cell (E) showing separation of the surface projections (arrows), pyknotic nucleus (N) and vacuolated cytoplasm (V) (L: Lumen) [× 9000].
At higher magnifications, affected endothelial cells looked swollen with pyknotic nuclei and degenerate cytoplasm. They accumulated large intra-cellular vacuoles which compressed the cytoplasm. With the increase in the intracellular pressure, the cells ballooned, partially separated from the intimal surface and erected on a narrow stalk (Fig. 3A). This progressed further until the cells peeled off the intimal surface and fell into the lumen, exposing a bare, de-epithelialized intimal surface (Fig. 3B). With the loss of the protective endothelial cell layer, chunks of the different wall components were lost to the lumen. Extra-cellular components like elastic fibers were seen accumulating on or exposed to the bare intimal surface, associated with fragmentation of the sub-intimal SMCs and collagen and elastic fibers (Fig. 3C). At other areas, degenerate SMCs were also seen sticking on the de-epithelialized intimal surface and directly exposed to lumen (Fig. 3D).

With the loss of the endothelial cell barrier, intra-luminal blood components, like platelets, started to adhere to the bare intima (Fig. 4A). Also, red blood cells (RBCs) infiltrated the intimal surface and lay free among degenerated, disintegrated collagen/elastin lattice of the vein wall (Fig. 4B).

**Discussion**

If at all possible, constructing a native fistula, either in the forearm or upper arm, will serve the patient better than a prosthetic graft (Burdick and Maley, 1996). The National Kidney Foundation (US) has identified the use of arteriovenous grafts (AVG) and the interventions required to maintain their potency as two major causes of increased expenditure in the management of hemodialysis access in end-stage renal disease patients. They have issued an appeal for the increased use of native arteriovenous fistulae (AVF) (Ascher et al., 2001).

It has been shown that 37% of hemodialysis patients develop a progressive stenosis in the venous circuit of the AVF, which later leads to thrombosis and occlusion (De Marchi et al., 1996). In a large study, the immediate failure rate ranged from 7.7% to 20.7%, depending upon the type of AVF constructed, mainly due to thrombosis. Thrombosis was also responsible for 87.2% of the late failures (Kinnairt et al., 1977). It has been shown that hemodialysis patients have elevated plasma levels of platelet-derived growth factor, monocyte chemoattractant protein-1 and interleukin-6 (De Marchi et al., 1997). These proteins are thought to be involved in the neointima formation and regulation of the proliferation of vascular SMCs, which is the key mechanism in the pathogenesis of fistula stenosis (De Marchi et al., 1996). In their study, Liani et al. (1996) found a significant increase of platelet surface glycoproteins in hemodialysis patients with frequent AVF obstruction. The expression of these platelet receptors might be related to the prothrombotic tendency of those patients who suffer more occlusions and thrombosis of their AVFs (Laini et al., 1996). Weiss et al. (2001) found significant intimal hyperplasia in all failed native AVFs recovered at time of surgical revision or resection. These intimal hyperplastic lesions showed histochemical localization of oxidative stress markers and growth factors, known to contribute to intimal hyperplasia.

The pre-operative diagnosis of diseased vein is possible in some cases by the use of duplex ultrasonography or venography (Panetta et al., 1992). However, intra-operative measures, like
Fig. 3. Advanced changes in the endothelial cells and intimal layer. A. A degenerating endothelial cell (E) which is swollen, erected on a narrow stalk with a large single vacuole (V) and compressed rim of cytoplasm (arrows) (L: lumen) [× 9286]. B. Separation of a degenerated endothelial cell (E) with pyknotic nucleus (N) and fraying of the intimal surface (arrows). Note the thickened, degenerated sub-endothelial intima which contained fragmented, collagen fibers (C) [× 7273]. C. Accumulation of dense elastic fibers (arrows) on the intimal surface. Note the irregular, fragmented smooth muscle cells (S), elastic (e) and collagen (C) fibers of the thickened intima (L: Lumen) [× 4167]. D. Completely damaged intimal surface (arrow) with a spiral or ball of smooth muscle cell (S) ready to separate completely into the lumen (L) [× 5143].
visual inspection, gentle palpation and routine irrigation with heparin-saline solution are also required. If pre-existing cephalic vein disease is suspected or identified, one should anticipate an increased risk of shunt failure (Panetta et al., 1992). Several reports have evaluated the importance of altered pathophysiologic condition and structure of the saphenous vein in the genesis of graft stenosis (Cheanvechal et al., 1975; Marin et al., 1991; Panetta et al., 1992; Davies et al., 1992). The presence of pre-existing disease, including those changes resulting from previous thrombophlebitis, has been shown to reduce the patency of the grafts containing these alterations (Panetta et al., 1992). Davies et al. (1992) showed that pre-operative assessment of vein wall compliance can be used to predict vein grafts at risk of failure. Correlative histological analysis of veins with decreased compliance showed that such veins had increased fibrosis at time of insertion and a subsequent increased rate of failure. Individual variables such as increased intimal thickness, increased wall thickness, decreased vein diameter and the presence of occult calcification in the vein wall, were associated with an increased incidence of vein graft lesions and failure (Marin et al., 1993).

There have been many studies on the morphological changes that occur in veins that have been used for arterial grafting. Only in the last 25 years, has the pre-bypass vein structure been investigated (Brody et al., 1972; Cheanvechal et al., 1975; Batayias et al., 1977; Thiene et al., 1980; Waller and Roberts, 1985). Qaetano et al. (1980) found that morphological changes were

Fig. 4. Effect of loss of the endothelial cell barrier. A. Aggregation of blood platelets (P) lying on the denuded intimal surface and underneath a separated endothelial cell (E) [x 6667]. B. Migration of red blood cells (R) into the vein wall through luminal clefts. Note the amorphous texture of the intima (stars) with fragmented smooth muscle cells (arrows) [x 4000].
Intimal changes in the cephalic vein

present almost constantly in the walls of the fresh “normal” saphenous veins before their use as aortocoronary conduits. In other studies it was reported in 12%–87% of long saphenous veins prior to their use as bypass conduits (Brody et al., 1972; Cheanvechal et al., 1975; Batayias et al., 1977; Qaetano et al., 1980; Thiene et al., 1980; Waller et al., 1985; Davies et al., 1992; Panetta et al., 1992; Ascher et al., 2001). It was also reported in three out of four cephalic vein samples prior to their use for lower limb bypasses (Davies et al., 1993). The main morphological findings consisted of increased wall thickness due to intimal fibrosis and medial sclerosis (Qaetano et al., 1980; Panetta et al., 1992; Marin et al., 1993), which did not correlate with age or sex of the patients (Waller and Roberts, 1985). According to Panetta et al. (1992) and Marín et al. (1993), the most striking histopathological finding was the presence of increased number of intimal smooth muscle cells (SMCs), as we well demonstrated in the present study. Seventy percent of the grafts that contained these cells began to fail and their presence increased the risk of failure almost 30 times (Panetta et al., 1992). Marín et al. (1993) also suggested a connection between the increase in these intimal SMCs of pre-bypass saphenous veins and those that compose mature hyperplastic vein graft stenoses. Unsuspected pre-existing saphenous vein disease may, therefore, set the stage for intimal hyperplastic changes that occur after arterialization, and might contribute to the development of progressive proliferation leading to graft occlusion (Qaetano et al., 1980; Panetta et al., 1992).

Although little has been written about the pre-existing cephalic vein disease, it is apparent that the presence of similar pre-existing morphological changes may affect the future behavior of shunts (AVFs), particularly with regard to the risk of stenosis, thrombosis and occlusion (Davies et al., 1993). Current theories have been put forward to explain these changes, if a defective endothelium is envisaged. The first is that repeated episodes of thrombophlebitis cause endothelial damage and transmural injury. The second suggests that these veins have an inherently defective endothelium. Both mechanisms put together involve platelet activation. Release of platelet-derived factors may then cause either migration or activation of pre-existing smooth muscle cells (SMCs) or their precursors (Walker et al., 1984; Schwartz et al., 1985; Gorstein, 1989). An alternative hypothesis is that the endothelium remains intact and that SMCs themselves and lymphocytes secrete cell mediators for the SMCs following a stimulus (Lindner et al., 1991; Hansson et al., 1989). Because loss of the endothelial cell layer is one of the early morphological changes in the vein wall, as suggested in this study, we are in favor of the first hypothesis. In their animal model of using the cephalic vein as an arterial graft, Boerboom et al. (1990) found that platelet inhibition did not decrease the extent of intimal hyperplasia. The presence of adherent platelets and the amount of fibrin correlated inversely with the amount of endothelium present during the first 14 days. Therefore, with the loss of the endothelial cell layer, platelets and fibrin will adhere to the bare intima, as we demonstrated in this study. Although none of the cephalic veins in our group of renal failure patients showed any evidence of clinical varicosities, some of the changes we described here are similar to those which we reported earlier in varicose veins (Wali and Eid, 2002).
Conclusion

In conclusion, most of the apparently “normal” cephalic veins have been shown to have morphological abnormalities at the time of arteriovenous fistula (AVF) construction, especially in the form of intimal hyperplasia and partial or complete loss of the endothelial cell layer. This may well influence the outcome of shunts in terms of future stenosis and failure due to thickening of the wall, adherence of platelets to the bare intima and activation of intimal smooth muscle cells. It seems likely that stenosis development may be the result of pre-existing disease rather than as a direct result of arterialization insult.

References


Intimal changes in the cephalic vein


(Received May 26, 2003; Accepted July 19, 2003)