Background: Tuberculosis (TB) remains a significant global health concern and its diagnosis is challenging due to the limitations in the specificity and sensitivity of the current diagnostic tests. Exosomes are bioactive 30–100 nm vesicles produced by most cell types and are found in almost all human body fluids. Exosomal microRNAs (miRNAs) can transfer biological information between cells and tissues and may act as potential biomarkers in many diseases. In this pilot study, we assessed the miRNA profile of exosomes released from human monocyte-derived macrophages upon infection with Mycobacterium bovis Bacillus Calmette–Guerin (BCG).

Methods: Human monocytes were obtained from the peripheral blood of three healthy subjects and driven to a monocyte-derived macrophage (MDM) phenotype using standard protocols. MDMs were infected with BCG or left uninfected as control. 72 h post-infection, exosomes were collected from the cell culture medium, RNA was isolated and RNA-seq performed. The raw reads were filtered to eliminate adaptor and primer sequences and the sequences were run against the mature human miRNA sequences available in miRBase. MicroRNAs were identified using an E value < 0.01. miRNA network analysis was performed using the DIANA miRNA tool, miRDB and functional KEGG pathway analysis.

Results: Infection of MDMs with BCG leads to the release of several exosomal miRNAs. These included miR-1224, -1293, -425, -4467, -4732, -484, -5094, -6848-6849, -4488 and -96 all of which were predicted to target metabolism and energy production-related pathways.

Conclusions: This study provides evidence for the release of specific exosomal miRNAs from BCG-infected MDMs. These exosomal miRNAs reflect host-pathogen interaction and subversion of host metabolic processes following infection.

Keywords: Mycobacterium, Exosome, miRNA, Macrophage, Biomarker
would be of great practical value. BCG vaccines are live vaccines derived from a strain of *Mycobacterium bovis*, and has been shown that extracellular forms of BCG in the mucosal lymphatic tissues following oral vaccination [7].

Exosomal biomarkers are considered as possible novel diagnostic biomarkers especially in infectious diseases [8–10]. Exosomes are bioactive vesicles of 30–100 nm in diameter, which are secreted from most cell types and can be found in nearly all human bodily fluids [11, 12]. Exosomes enable cell-to-cell communication by shuttling various molecules including miRNAs between cells [12–14]. Different signatures of exosomal content have been reported in various diseases [12, 15–18] including cancers [19–23], acute myeloid leukemia (AML) [24, 25] and Alzheimer’s disease [26].

MicroRNAs are important regulatory molecules that play critical roles in pathological conditions [27, 28]. The role of miRNAs in modulation of innate and adaptive immunity and cellular responses to bacterial infection has been reported previously [29–32]. Various bacterial components, such as peptidoglycan (PG), lipoproteins and lipopolysaccharide (LPS) can affect the host’s miRNA expression levels [33, 34] and trigger inflammatory responses [35]. Functional miRNAs are capsulated in exosomes and delivered to recipient cells and subsequently cause specific modulation of their transcriptome [36]. Several studies have described the role of exosomes in TB [1, 8, 37–39]. Exosomes released from macrophages infected with M.t.b, as well as exosomes isolated from M.t.b-infected mice, promote both innate and acquired immune responses in vitro and in vivo [1, 8, 37–39]. The modulatory effects of exosomes released from M.t.b-infected macrophages has been reviewed [40] and indicate that they can stimulate production of inflammatory mediators and induction of apoptosis in recipient cells [28, 40].

Exosomal miRNAs have been proposed as potential biomarkers in numerous diseases such as cardiovascular disease, malignancies, and Alzheimer’s disease [15, 16, 41–43] although the role of exosomal miRNAs as potential biomarkers TB are not well described. In this pilot study, we profiled the exosomal miRNA of human macrophages after co-infection with *Mycobacterium bovis*, Bacillus Calmette–Guerin (BCG). We hypothesized that BCG-infected macrophages would secrete a specific set of exosomal miRNAs that may playing role in the pathogenesis of TB.

**Methods**

**Cell culture**

Peripheral blood was obtained from three healthy human donors who had no clinical manifestations of disease. Complete blood count (CBC), ESR, CRP and liver and kidney function tests were evaluated. To investigate prior exposure of TB, QuantiFERON-TB Gold (QFT®) and PPD tests were performed and all three healthy subjects were negative for latent TB. An institutional review board (IRB) from Dr. Masih Daneshvari Hospital, Tehran, Iran approved the study.

PBMCs were isolated by density gradient centrifugation using Ficoll-Paque (Invitrogen Corp., Carlsbad, CA). The monocyte-enriched layer was collected from the Ficoll:plasma interface and cells were cultured in Roswell Park Memorial Institute medium (RPMI 1640) (Gibco; Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 25 mM HEPES (Gibco), 100 units/ml penicillin (Sigma, Munich, Germany) and 100 μg/ml streptomycin (Sigma) in 5% CO₂ at 37 °C. After 4 h, non-adherent cells, which were mostly T lymphocytes, were removed. The adherent monocytes were washed with 1× PBS (Sigma) 2–3 times and were collected by centrifugation for 10 min at 400g. Macrophages were obtained by culturing the monocytes for 7–8 days in RPMI containing 100 ng/ml GM-CSF (Invitrogen) and 10% FBS with one medium change with GM-CSF on the 4th day as described previously [44].

**Infection assay**

On day 7 or 8, the GM-CSF medium was removed and replaced with fresh medium without GM-CSF for at least 4 h before infection. Uptake of bacteria was assessed by flow cytometry to determine the infection ratio required to obtain 85% infectivity as described previously [45, 46]. Ten flasks each containing 1×10⁷ cells were infected with opsonized BCG (obtained as a gift from Pasteur Institute of Iran, IPI) at an MOI (Multiplicity of infection) of 10 or were left uninfected as controls. Cells were incubated for 2 h at 37 °C in a 5% CO₂ before washing with 1× PBS containing amikacin (80 μg/ml) to eliminate possible free organisms. Subsequently, the cells were incubated in medium containing exosome-depleted FBS (10% final concentration) (System Bioscience, CA, USA) and 100 ng/ml GM-CSF for 72 h.

**Exosome isolation and characterization**

Exosomes were isolated from the culture supernatants of infected and uninfected cells 72 h post infection using total exosome isolation (TEI) reagent according to the manufacturer’s instructions (Invitrogen by the Thermo Fisher Scientific corporation, Waltham, MA, USA). Briefly, the cell culture media (CCM) were centrifuged at 300×g for 30 min and filtered twice through a 0.22 μm filter (Merck-Millipore, Billerica, MA, USA) to remove apoptotic bodies, dead cells and cell debris. The CCM was mixed with TEI solution at a 5:1 ratio. The samples
were incubated overnight at 4 °C and centrifuged for 1 h at 10,000×g. The pellet was re-suspended in 1 ml of PBS and stored at −20 °C. Purified exosomes were characterized by electron microscopy (Carl Zeiss NTS, Oberkochen, Germany) and nanoparticle tracking analysis (Malvern/Nanosight LM10. CA, USA).

**Exosomal RNA isolation and qualification**

Prior to RNA extraction, exosomes were again filtered through a 0.22 mm filter and treated with RNase A (5 μg/μl Fermentase, Thermo-Fisher, Boston, MA, USA) for 90 min at 37 °C to eliminate non-exosomal RNAs. Total RNA was isolated from exosomes using the total exosomal RNA and protein isolation kit (Thermo-Fisher) according to the manufacturer’s instructions. RNA concentration and purity was measured using a Nano-drop 2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The quality, yield, and size of extracted RNA was analyzed using capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies, Foster City, CA, USA).

**Small RNA library construction and miRNA sequencing**

Size selection and gel purification of the RNA samples was performed on a 15% Tris–Borate-EDTA (TBE) polyacrylamide/urea gel, RNAs were excised from the gel, purified as above and small RNA libraries constructed as described previously [47]. RNA libraries were run on a Roche 454 Genome Sequencer FLX according to the manufacturer’s instructions. The raw reads were filtered to eliminate adaptor sequences and the sequences, in FASTQ format, were run against the mature human miRNA sequence database available at miRBase using BLAST software. MicroRNAs were identified using an E value cutoff <0.01. MicroRNA pathway analysis was performed using the DIANA miRNA tool [48, 49], miRDB [50] and functional KEGG pathway database [51].

**Results**

**Exosome characterization and exosomal RNA preparation**

Exosomes were isolated from the culture supernatants from paired infected and uninfected monocyte-derived macrophages from healthy subjects. Exosomes were confirmed by scanning (SEM) and transmission electron microscopy (TEM) and characterized for size distribution by nanoparticle analyzer and demonstrated the expected size and morphology (Fig. 1).

Total exosomal RNA was extracted and the concentration measured. The amount of RNA isolated from 10 T75 culture flasks in each group varied from 1.3 to 1.8 μg from the infected and uninfected cell-derived exosomes with no significant differences between groups (p > 0.05). The extracted exosomal RNA was also qualified on an Agilent Bioanalyzer and the RNA population observed in the exosomes were predominantly from small RNAs (Fig. 2).

**Infection with BCG significantly modulates the miRNA content in infected cell-derived exosomes**

Using an E value <0.01 as the cut-off, 44 and 47 miRNAs were identified in the infected and uninfected macrophage-derived exosomes, respectively (Table 1). 38 of these miRNAs (~78%) were present in both infected and uninfected exosomes but 11 miRNAs (miR-1224, -1293, -425, -4467, -4732, -484, -5094, -6848 and -6849, -96 and -4488) were predominantly expressed in exosomes derived from M.tb infected cells. The fold-differences in expression of the 11 miRNAs differentially expressed between infected and non-infected macrophages are shown in Table 2.

**Differentially expressed miRNAs were associated with pathways related to pathogenesis of mycobacterial infection and intracellular survival**

To examine the target pathways affected by the differentially expressed miRNAs in the infected macrophage-like group, miRNA network analysis was performed. Pathway analysis showed differential activation of pathways related to mycobacterium invasion, intracellualr survival, energy production machinery and immunity reactions (Table 3). Most of the target genes regulated by these miRNAs were involved in the cell infection process and energy production pathways (Table 4). A subgroup of these differentially expressed miRNAs (miR-484, -5094, -425, -1293, -6848, -6849) had the most profound effect on the pathways activated by BCG infection (Table 5).

**Discussion**

In the current study, we assessed the exosomal miRNAs released from human macrophages following infection with BCG. We detected a group of 11 exosomal miRNAs (miRs-1224, -1293, -425, -4467, -4732, -484, -5094, -6848, -6849, -96 and -4488) that were differentially expressed in infected cells. These miRNAs are involved in several key pathways including central carbon metabolism, fatty acids and sugar metabolism, amino acid metabolism, bacterial invasion related pathways and cell signaling pathways. This suggests that host pathways implicated in immune surveillance are modulated to enable bacterial survival within infected macrophages.

Recent studies have highlighted the role of exosomes as a vehicle for the transfer of proteins, lipids as well as biologically active miRNAs to distant cells. Exosomes may also act as novel biomarkers in several diseases such as acute myeloid leukemia (AML), ovarian cancer, asthma and sarcoidosis [20, 24, 36]. The miRNA profile
Fig. 1 Exosome characterization by scanning (SEM) and transmission electron microscopy (TEM) and nanoanalyzer. Exosomes were isolated by total exosome isolation reagents and passed twice through a 0.22 mm filter before being analyzed for morphology by SEM (a) and TEM (b). The size distribution of the isolated exosomes was further analyzed by nanoanalyzer (c) and showed the size distribution of the exosomes as 70 ± 3.4 nm. The results are representative of three independent experiments.

Fig. 2 Exosomal RNA qualification. Total RNA was extracted from the exosomes and the quality of the exosomal RNA was assessed by running the samples on an Agilent Bioanalyzer. The RNA population observed in the exosomes were predominantly from small RNAs. The result is representative of three independent experiments.
and some aspects of exosomal content has recently been examined in TB patients [18, 37, 52, 53]. In these studies, the levels of serum free miRNAs [54], macrophage cell miRNAs [55] and exosomal protein content [39] were evaluated. The expression of 14 miRNAs in M.tb-infected macrophages were significantly altered and depended upon the infective strain (Beijing/W or non-Beijing/W strains) [55] and did not overlap with those reported here from M.tb-infected macrophage-derived exosomes. There were no overlapping miRNAs found in the serum of infected patients [54] and the target pathways were distinct from those seen here. This highlights the potential for detecting strain-specific infection using exosomal miRNAs.

Exosomal transport of miRNAs enables their stability and delivery throughout the body [56]. Exposure of cells to various bacterial components affects the host miRNA profile [34] and this is also evident in the miRNA profile of released exosomes reported in this study. This will potentially cause significant functional modulation of

Table 1  Exosomal miRNA content in infected and uninfected macrophage-derived exosomes

| miRNA name                  | Common miRNAs according to expression level |
|-----------------------------|---------------------------------------------|
| Infected exosomal miRNAs    | mir-486, mir-21, mir-146a, mir-92a, mir-146b, let-7i, mir-423, mir-378a, let-7f, let-7g, let-7a, mir-155, mir-320a, mir-191, let-7b, mir-24-, mir-26a, mir-423, mir-140, mir-30d, mir-148a, mir-101, mir-221, mir-103a, let-7e, mir-28, mir-1307, mir-151a, mir-148b, mir-26b, mir-27a, mir-532, let-7d, mir-361, mir-99b, mir-342, mir-941, mir-911 |
| Uninfected exosomal miRNA   | mir-21, mir-146b, mir-146a, let-7f, let-7i, let-7a, mir-378a, let-7g, let-7b, mir-26a, mir-191, mir-423, mir-30d, mir-155, mir-320a, mir-511, mir-423, mir-1307, mir-101, mir-24-, mir-92a, mir-99b, mir-532, let-7d, mir-486, let-7e, mir-103a, mir-221, mir-26b, mir-140, mir-148b, mir-28, mir-151a, mir-941, mir-342, mir-27a, mir-361 |

Differentially expressed exosomal miRNAs (p < 0.05) obtained from three independent experiments. 44 and 47 miRNAs were identified in the M.tb-infected and -uninfected macrophage-derived exosomes with a copy number >20

| miRNA name                  | Log Fc | Log CPM | p value | FDR    |
|-----------------------------|--------|---------|---------|--------|
| miR-96                      | 5.89073| 2.930204| 0.000245| 0.000186|
| miR-1224                    | 5.217967| 3.015217| 0.000519| 0.000361|
| miR-1293                    | 3.171918| 14.19242 | 0.0001093| 0.000066|
| miR-4467                    | 3.174797| 12.62878 | 0.0001093| 0.000066|
| miR-6848                    | 4.143784| 3.760948 | 0.0002233| 0.000138|
| miR-6849                    | 3.118348| 7.30397  | 0.000268 | 0.000164|
| miR-4488                    | 3.184877| 5.073045 | 0.000404 | 0.000248|
| miR-425                     | 3.481839| 4.249725 | 0.000572 | 0.000360|
| miR-4732                    | 2.578799| 8.273334 | 0.000616 | 0.000360|
| miR-484                     | 2.389806| 7.714758 | 0.000566 | 0.000360|
| miR-5094                    | 5.125813| 1.680093 | 0.002864 | 0.001423|

FC fold change, CPM read per million, FDR false discovery rate

Table 2 Differentially expressed microRNAs in the exosomes released specifically from M.tb-infected cells

Table 3 Functional pathways identified in mRNA targets for miRNAs

| Pathways in bacterial digestion |
|-------------------------------|
| Proteosome                     | 0.0139 | 3 |
| Ubiquitin-mediated proteolysis | 0.0057 | 7 |

Pathways in infection process

| Pathways | p value | Number of miRNAs in each pathway |
|----------|---------|----------------------------------|
| Bacterial invasion of epithelial cells | 0.0310 | 7 |
| Endocytosis | 0.0337 | 8 |
| ECM-receptor interaction | 0.00153 | 4 |
| Adherents junction | 0.00730 | 4 |
| Inositol phosphate metabolism | 0.00201 | 4 |

Pathways in amino acids metabolism

| Pathways | p value | Number of miRNAs in each pathway |
|----------|---------|----------------------------------|
| Phenylalanine, tyrosine and tryptophan biosynthesis | 0.000713 | 3 |
| Valine, leucine and isoleucine degradation | 0.0310 | 6 |
| Lysine degradation | 0.0421 | 6 |

Pathways for energy production and sugar metabolism

| Pathways | p value | Number of miRNAs in each pathway |
|----------|---------|----------------------------------|
| Central carbon metabolism | 0.00435 | 5 |
| 2-Oxocarboxylic acid metabolism | 0.0165 | 3 |

Cell signaling pathways

| Pathways | p value | Number of miRNAs in each pathway |
|----------|---------|----------------------------------|
| Phosphatidylinositol signaling system | 0.0489 | 5 |
| TGF-beta signaling pathway | 0.0489 | 4 |
both near and remote recipient cells [33]. Since the nutrients required for efficient M.tb growth and proliferation inside the host cell are restricted [57], bacteria sequester the host’s cell metabolism by activation of virulence-associated factors [57]. These may trigger regulatory miRNA networks that control the carbon and nitrogen metabolism in cells [58, 59] and also determine their release into exosomes.

Fatty acid biosynthesis and associated downstream metabolites may be affected by exosomal miRNAs induced by BCG treatment. Thus, one of the dysregulated exosomal miRNAs found in this study, miR-1224, is involved in the regulation of lipid metabolism. miR-1224 regulates the expression of lipid-related genes that are directly regulated by transcription factors such as SP1 [60]. In support of this, studies have shown an abundance

| Pathways in fatty acid metabolism | Gene | p value |
|----------------------------------|------|---------|
| Fatty acid biosynthesis          | 2    | 0.0000000000684 |
| Fatty acid metabolism            | 12   | 0.00000154 |
| Fatty acid elongation             | 7    | 0.00738 |
| Steroid biosynthesis             | 6    | 0.00631 |
| 2-Oxocarboxylic acid metabolism  | 6    | 0.0165 |

| Pathways in amino acids metabolism | Gene | p value |
|-----------------------------------|------|---------|
| Phenylalanine, tyrosine and tryptophan biosynthesis | 4 | 0.000713 |
| Valine, leucine and isoleucine degradation | 12 | 0.0310 |
| Lysine degradation | 16 | 0.0421 |

| Pathways of energy production and sugar metabolism | Gene | p value |
|---------------------------------------------------|------|---------|
| Central carbon metabolism in cancer | 19 | 0.00435 |
| 2-Oxocarboxylic acid metabolism | 6 | 0.0165 |
| Glycosaminoglycan biosynthesis—heparan sulfate/heparin | 9 | 0.0113 |
| Proteoglycans | 49 | 0.0337 |

| Pathways in infection | Gene | p value |
|-----------------------|------|---------|
| Bacterial invasion of epithelial cells | 23 | 0.0310 |
| Endocytosis            | 51   | 0.0337 |
| ECM-receptor interaction | 16 | 0.00153 |
| Adhesers junction      | 21   | 0.00730 |
| Inositol phosphate metabolism | 15 | 0.0201 |

| Cell signaling pathways | Gene | p value |
|-------------------------|------|---------|
| Phosphatidylinositol signaling system | 21 | 0.0489 |
| TGF-beta signaling pathway | 18 | 0.0489 |

| Pathways in bacterial digestion | Gene | p value |
|---------------------------------|------|---------|
| Proteasome                      | 3    | 0.0138 |
| Ubiquitin mediated proteolysis  | 7    | 0.00566 |

Table 4 The number of genes targeted by the miRNAs in the altered pathways

| Pathways in fatty acid metabolism | Gene | p value |
|----------------------------------|------|---------|
| Fatty acid biosynthesis          | 2    | 0.0000000000684 |
| Fatty acid metabolism            | 12   | 0.00000154 |
| Fatty acid elongation             | 7    | 0.00738 |
| Steroid biosynthesis             | 6    | 0.00631 |
| 2-Oxocarboxylic acid metabolism  | 6    | 0.0165 |

| Pathways in amino acids metabolism | Gene | p value |
|-----------------------------------|------|---------|
| Phenylalanine, tyrosine and tryptophan biosynthesis | 4 | 0.000713 |
| Valine, leucine and isoleucine degradation | 12 | 0.0310 |
| Lysine degradation | 16 | 0.0421 |

| Pathways of energy production and sugar metabolism | Gene | p value |
|---------------------------------------------------|------|---------|
| Central carbon metabolism in cancer | 19 | 0.00435 |
| 2-Oxocarboxylic acid metabolism | 6 | 0.0165 |
| Glycosaminoglycan biosynthesis—heparan sulfate/heparin | 9 | 0.0113 |
| Proteoglycans | 49 | 0.0337 |

| Pathways in infection | Gene | p value |
|-----------------------|------|---------|
| Bacterial invasion of epithelial cells | 23 | 0.0310 |
| Endocytosis            | 51   | 0.0337 |
| ECM-receptor interaction | 16 | 0.00153 |
| Adhesers junction      | 21   | 0.00730 |
| Inositol phosphate metabolism | 15 | 0.0201 |

| Cell signaling pathways | Gene | p value |
|-------------------------|------|---------|
| Phosphatidylinositol signaling system | 21 | 0.0489 |
| TGF-beta signaling pathway | 18 | 0.0489 |

| Pathways in bacterial digestion | Gene | p value |
|---------------------------------|------|---------|
| Proteasome                      | 3    | 0.0138 |
| Ubiquitin mediated proteolysis  | 7    | 0.00566 |

Table 5 The number of target genes regulated by the differentially expressed miRNAs in the dysregulated pathways

| Pathways | Differentially expressed miRNA | Number target genes |
|----------|-------------------------------|---------------------|
| Bacterial invasion pathway | hsa-miR-484 | 15 |
| Fatty acid biosynthesis pathways | hsa-miR-1293 | 1 |
| Fatty acid metabolism | hsa-miR-425-5p | 4 |

| Pathways | Differentially expressed miRNA | Number target genes |
|----------|-------------------------------|---------------------|
| Fatty acid metabolism | hsa-miR-425-5p | 4 |
| Fatty acid metabolism | hsa-miR-1293 | 1 |
| Fatty acid metabolism | hsa-miR-6849-1 | 1 |

| Pathways | Differentially expressed miRNA | Number target genes |
|----------|-------------------------------|---------------------|
| Trp/phe synthesis | hsa-miR-484 | 1 |
| Fatty acid elongation | hsa-miR-1224-5p | 5 |
| Central carbon metabolism | hsa-miR-484 | 10 |

| Pathways | Differentially expressed miRNA | Number target genes |
|----------|-------------------------------|---------------------|
| Steroid biosynthesis | hsa-miR-484 | 4 |
| Glucose amino glycan | hsa-miR-484 | 4 |
| 2'-Oxycarboxyl | hsa-miR-484 | 4 |

| Pathways | Differentially expressed miRNA | Number target genes |
|----------|-------------------------------|---------------------|
| Steroid biosynthesis | hsa-miR-484 | 4 |
| Glucose amino glycan | hsa-miR-484 | 4 |
| 2'-Oxycarboxyl | hsa-miR-484 | 4 |
of lipids in caseous pulmonary granulomas from TB patients [61]. Transcriptome analysis of these granulomas demonstrated a significant up-regulation of genes involved in the sequestration, catabolism, and synthesis of host lipids [61]. Moreover, an increased level of TNF-α in response to exposure to M.tb cell wall components, upregulates the activation of genes related to lipid metabolism in infected cells [61]. MicroR-1224 has a negative regulatory effect on TNF-α gene expression [35] suggesting an interaction between these pathways. Overall, it is evident that alteration of the host’s lipid metabolism plays a crucial role in the survival of intracellular M.tb [61–63]. M.tb uses the host's fatty acids as a source of carbon and to limit the effect of propionyl-CoA, a potentially toxic intermediate, has on its own survival. The metabolism of host lipids could lead to the expansion of the acetyl CoA pool in infected cells [64].

The dysregulated microRNAs reported here also target central carbon metabolism (CCM) pathways. CCM transforms carbon to energy via glycolysis, gluconeogenesis, the pentose phosphate pathway and the tricarboxylic acid cycle (TCA) pathways [65]. The pathogenicity of M.tb depends on the reprogramming of the host cells' metabolic pathways [62, 66–68]. Moreover, CCM plasticity determines pathogen adaptation to the intercellular milieu of the macrophage [58]. The macrophage glycolytic flux is disturbed by M.tb infection which results in increased glucose uptake by the pathogen-infected cell [57]. Metabolomic profiling using H-NMR of mycobacterial-infected lung tissue showed significant changes in the host cell's metabolic pathways [69, 70]. Mtb infection decreases the intracellular levels of glucose, glycogen, NAD and NADP whilst increasing the levels of lactate. This reduced level of TCA cycle intermediates in infected tissues is associated with enhanced aerobic glycolysis resulting from a pentose-phosphate shunt and enhanced uptake of glucose [70].

Dysregulated miRNAs released into exosomes from BCG-infected macrophages also affected amino acid synthesis and metabolism pathways (Table 3). Metabolic profiling demonstrated increased levels of amino acids and activation of pyrimidine and purine nucleotide biosynthesis within M.tb-infected lung tissue [71].

Another group of differentially expressed miRNAs in exosomes from infected macrophages were associated with cell membrane and communication pathways such as adherens junction, gap junction, glycosaminoglycan biosynthesis and heparan sulfate/keratin sulfate metabolism. MicroR-1293 for example targets the tissue inhibitors of metalloproteinases (TIMPs) [72]. TIMP-1 is an inhibitor of matrix metalloproteinases and is involved in the invasion and spreading of bacteria through the epithelial cell [73]. M.tb infection up-regulates the expression of matrix metalloproteinases (MMP) and perturbs the MMP/TIMP balance in human monocytes [74]. The up-regulation of miR-1293 in exosomes from BCG-infected macrophages may reflect the ability of mycobacterium antigens to alter the host cell membrane structure and subsequently affect macrophage survival.

miR-484 and miR-425 were preferentially found in exosomes from BCG-infected macrophages. miR-484 regulates intermediate metabolic pathways by targeting the mitochondrial fission protein 1 (Fis1) [75] and altered miR-425 expression is linked to insulin resistance. The role of these miRNAs in infected macrophages remains unclear at this point although it is evident that miR-425 regulates several metabolic pathways and has been associated with metabolic disorders [76].

miRs-1224, -1293, -4467, -4732, -5094, -6848 and -6849 are human mirtrons which are produced via splicing of introns from mRNA coding genes rather than by the formation of hairpin loops by Drosha [77]. The expression of these mirtrons were significantly higher in the exosomes released from infected macrophages. This suggests that mycobacteria may recognize, at least in part, the pattern of miRNA production within the host cell and program over-expression of these mirtrons in order to recruit host metabolic pathways that favour M.tb infection.

| Pathways            | Differentially expressed miRNA | Number target genes |
|---------------------|-------------------------------|---------------------|
| Val/leu/isoleu synthesis | hsa-miR-484 | 5                   |
|                     | hsa-miR-4732-1 | 2                   |
|                     | hsa-miR-5094 | 3                   |
|                     | hsa-miR-425-5p | 1                   |
|                     | hsa-miR-4467-1 | 1                   |
|                     | hsa-miR-1293-1 | 1                   |
| Proteoglycan        | hsa-miR-5094 | 5                   |
|                     | hsa-miR-484 | 27                  |
|                     | hsa-miR-425-5p | 17                  |
|                     | hsa-miR-1293 | 2                   |
|                     | hsa-miR-1224-5p | 4                   |
|                     | hsa-miR-4732-2 | 2                   |
|                     | hsa-miR-6849-4 | 3                   |
|                     | hsa-miR-6848-4 | 4                   |
| Lysine degradation  | hsa-miR-484 | 11                  |
|                     | hsa-miR-5094 | 3                   |
|                     | hsa-miR-1293 | 4                   |
|                     | hsa-miR-425-5p | 4                   |
|                     | hsa-miR-6848-1 | 1                   |
|                     | hsa-miR-1224-5p | 1                   |
Conclusion
This pilot study demonstrated the differential expression of many miRNAs within exosomes released from BCG-infected macrophages. These miRNAs indicate that metabolic reprogramming may occur to favour M. tuberculosis survival. Further studies are needed in large cohorts of patients to test for the presence of these 11 miRNAs in blood exosomes to determine their true value as a possible diagnostic biomarker for TB infection. The profiling of miRNAs upon BCG infection may shed additional light on the host-pathogen interaction and changes in cellular function. Future studies on miRNA expression and function in TB may provide greater understanding of M. tuberculosis pathogenesis.

Abbreviations
BCG: Bovis Bacillus Calmette–Guerin; CCM: central carbon metabolism; FBS: fetal bovine serum; LPS: lipopolysaccharide; MDM: monocyte-derived macrophages; MMP: matrix metalloproteinases; miRNA: microRNAs; QuantiFERON-TB Gold (QFT-G); interferon-gamma (IFN-γ) release assay; PPD: purified protein derivative; TEM: transmission electron microscopy; TB: tuberculosis.

Authors' contributions
SDA performed the majority of the experiments and wrote the first draft of the manuscript. All authors participated in experimental conception and design and with the revision of the manuscript and data interpretation. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All data is available from the authors upon request.

Consent for publication
All authors have consented to the publication of this manuscript.

Ethics approval and consent to participate
PBMCs were isolated from healthy subjects. Ethical approval was granted by the Institutional Review Board (IRB) of the Dr. Mash Danyeshvari Hospital (Tehran) and all volunteers consented to the use of their blood samples for this study.

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